UNIVERSITAT POLITÈCNICA DE VALÈNCIA

INSTITUTO UNIVERSITARIO DE INGENIERÍA DE ALIMENTOS - FoodUPV

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Desarrollo y caracterización de nuevas harinas de lenteja y quinoa fermentadas con *Pleurotus ostreatus*

TESIS DOCTORAL

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Dra. Ana María Andrés Grau, Catedrática de Universidad perteneciente al Departamento de Tecnología de Alimentos de la Universidad Politécnica de València,

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CONSIDERAN: que la memoria titulada "Desarrollo y caracterización de nuevas harinas de lenteja y quinoa fermentadas con *Pleurotus ostreatus*" que presenta Dª Janaina Madelein Sánchez García, para aspirar al grado Doctor de la Universitat Politècnica de València, y que ha sido realizada bajo su dirección en el Instituto Universitario de Ingeniería de Alimentos - FoodUPV de la Universitat Politècnica de València, reúne las condiciones adecuadas para constituir su tesis doctoral, por lo que AUTORIZAN a la interesada para su presentación.

Valencia, noviembre de 2023

Fdo.: Ana María Andrés Grau

Fdo.: Ana Belén Heredia Gutiérrez

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PRÓLOGO

Justificación del estudio

La población mundial está creciendo y se espera que siga aumentando, por lo que alimentar a la población mundial supone un gran desafío. Se estima que para el año 2050 las necesidades de alimentos de origen animal aumentarán en un 68% para satisfacer los requerimientos nutricionales de proteína, lo que resulta insostenible desde el punto de vista medioambiental. Por lo tanto, es necesario incrementar la producción sostenible de alimentos con alto valor nutricional y ricos en proteínas. Los productos de origen animal han sido identificados como generadores de altas emisiones de gases de efecto invernadero, de ahí la intensificación en la búsqueda de fuentes alternativas, y más concretamente el aumento de la incorporación de proteínas vegetales a la dieta, todo ello impulsado por la sostenibilidad ambiental y el menor coste de producción de estas. Legumbres como la lenteja, o pseudocereales como la quinoa, son ampliamente consumidas en todo el mundo, y son una fuente de proteínas, micronutrientes esenciales y compuestos antioxidantes. Sin embargo, a pesar de que la lenteja y la quinoa tienen un perfil nutricional de buena calidad, también contienen antinutrientes que limitan la digestibilidad y absorción adecuada de proteínas y minerales. En este contexto, el estudio que se plantea en esta tesis consiste en aplicar la fermentación en estado sólido (SSF) como bioproceso para mejorar el valor nutricional de la lenteja y la quinoa, y obtener harinas con propiedades de digestibilidad mejoradas y un perfil sensorial más atractivo.

Por otro lado, el aumento de la población mundial va acompañado de un envejecimiento de la misma, y de ahí la necesidad de desarrollar nuevos alimentos adaptados a las necesidades de la población mayor. La presente tesis se ha desarrollado en el marco del proyecto “Conceptualización para la creación de

alimentos con procesamiento oral eficiente basados en proteínas sostenibles con digestibilidad mejorada para el adulto mayor **(My Best Elderly Food-MyBEEF)**" (PID2019-107723RB-C22), financiado por el Ministerio de Ciencia e Innovación (MCIN/AEI/10.1309/501100011033), España. MyBEEF es un proyecto coordinado entre el Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) y el Instituto Universitario de Ingeniería de Alimentos – FoodUPV (IIA-FoodUPV), el cual consta de dos subproyectos:

- Conceptualización en la creación de alimentos sostenibles enriquecidos con proteínas. Creación de alimentos con textura para mejorar la calidad y el confort alimentario de la población senior. Ejecutado por el IATA-CSIC.
- Conceptualización en la creación de alimentos ricos en proteínas sostenibles. Obtención de ingredientes ricos en proteínas vegetales con mejor digestibilidad y funcionalidad para la población sénior. Ejecutado por el IIA-FoodUPV.

La Figura A muestra la relación y organización de los paquetes de trabajo (PT) de los dos subproyectos previamente mencionados. Las actividades bajo la responsabilidad del IATA están identificadas en color azul, mientras que las actividades designadas para el IIA se presentan en color verde. El presente trabajo de tesis de desarrolla en el contexto de los paquetes de trabajo 4 y 5, los cuales se enfocan en la obtención de nuevas harinas mejoradas ricas en proteínas a partir de lenteja y quinoa, y el posterior estudio de digestibilidad *in vitro* de estas harinas. Los resultados obtenidos en estas etapas proporcionarán retroalimentación esencial para orientar la ejecución de los siguientes paquetes de trabajo dentro del proyecto.

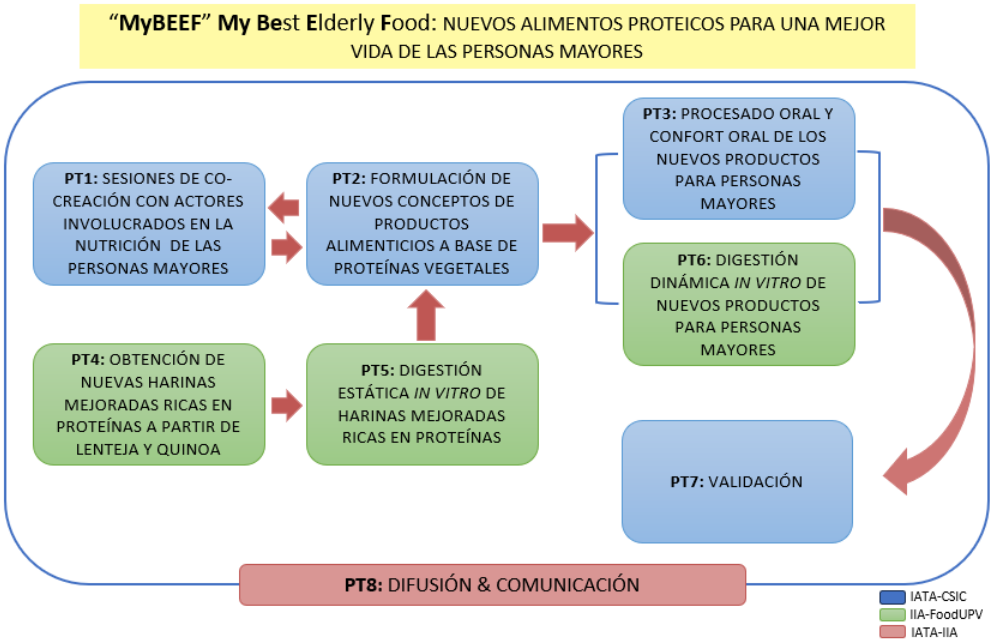

Figura A. Relación y organización de los paquetes de trabajo (PT) del proyecto MyBEEF.

RESUMEN

La población mundial está en constante crecimiento, por lo que la soberanía alimentaria se ha convertido en un desafío crucial. Se estima que la demanda de alimentos de origen animal aumentará en un 68% para el año 2050, lo cual resulta insostenible a nivel medioambiental. Así, impulsar un mayor consumo de proteína vegetal se plantea como una de las estrategias dirigidas a promover la sostenibilidad ambiental, asegurando la disponibilidad de proteína dietética para toda la población. Si bien ciertas las legumbres y pseudocereales son una excelente fuente de nutrientes y en particular de proteína, también contienen ciertos antinutrientes que pueden limitar su digestibilidad. Este aspecto es especialmente relevante en aquellos grupos poblaciones con alteraciones gastrointestinales, como las que pueden aparecer con la edad en población sénior.

En este contexto, el objetivo general de esta tesis doctoral es aplicar la fermentación en estado sólido (FES) como bioestrategia para la obtención de harinas de lenteja y quinoa con digestibilidad y bioaccesibilidad mejoradas. Para alcanzar este objetivo, se llevó a cabo la fermentación con el hongo *Pleurotus ostreatus*, en dos variedades de lentejas y quinoa, y se estabilizaron posteriormente mediante secado por aire caliente a diferentes temperaturas, además de la liofilización como método de referencia. Posteriormente, las harinas fermentadas fueron digeridas *in vitro* simulando el proceso digestivo de un adulto sano (estándar de referencia), así como en condiciones alteradas del adulto mayor. Los resultados obtenidos evidenciaron un incremento de proteína total, así como de la actividad inhibidora de la enzima convertidora de angiotensina (ECA), conjuntamente con una disminución del contenido en ácido fítico, todo ello como resultado de la actividad metabólica del hongo sobre el sustrato. A pesar de que la FES también ocasionó una reducción de la

actividad antioxidante, el posterior secado por aire caliente, especialmente a 70 °C incrementó este parámetro. Asimismo, la FES y el secado por aire caliente promovieron cambios en el perfil fenólico, disminuyendo algunos compuestos e incrementando otros como el ácido gálico hasta 5 veces su contenido inicial. En cuanto al perfil volátil de las harinas fermentadas, este se caracterizó por un aroma dulce, afrutado y con matices a cacao, acompañado de notas de setas y sustratos cocidos, debido a las concentraciones de benzaldehído, hexanal, nonanal, furfural y 1-octen-3-ol que se generaron durante la fermentación. Por otro lado, con respecto a la digestibilidad de las harinas fermentadas en condiciones estándar de adulto sano, la FES y el secado a 70 °C incrementó la hidrólisis de las proteínas, así como la liberación de aminoácidos hidrófobos y aminoácidos cargados negativamente. La FES también disminuyó la actividad inhibidora de la ECA en los digeridos, sin embargo, ésta aumentó después del secado a 70 °C debido a las melanoidinas generadas durante el secado. Además, las propiedades antioxidantes y la bioaccesibilidad de minerales también se vieron incrementados con la FES y el posterior secado a 70 °C. Finalmente, la simulación de las alteraciones gastrointestinales que comúnmente se dan en el adulto mayor, indicaron que estas impactaban negativamente en la mayoría de los parámetros evaluados, a excepción de la bioaccesibilidad del magnesio, hierro y calcio en comparación con el modelo estándar.

En conclusión, se ha logrado mejorar el perfil nutricional y funcional de las nuevas harinas obtenidas por fermentación con el hongo *P. ostreatus*, y posterior secado por aire caliente en comparación con las harinas obtenidas a partir de sustrato no fermentado, conduciendo esto a una mejora significativa en la digestibilidad y la bioaccesibilidad de los nutrientes, lo que puede ser especialmente relevante para el diseño de alimentos orientados a grupos de población con alta demanda de proteína de fácil digestión.

ABSTRACT

The world's population is constantly growing, making food sovereignty a crucial challenge. It is estimated that the demand for animal-based food will increase by 68% by 2050, which is environmentally unsustainable. Thus, encouraging greater consumption of plant protein is one of the strategies aimed to promote environmental sustainability by ensuring the availability of dietary protein for the entire population. Although certain legumes and pseudocereals are an excellent source of nutrients and in particular protein, they also contain certain anti-nutrients that can limit their digestibility. This aspect is especially relevant in those population groups with gastrointestinal disorders, such as those that may appear with age in the elderly population.

In this context, the general objective of this doctoral thesis is to apply solid-state fermentation (SSF) as a biostrategy to obtain lentil and quinoa flours with improved digestibility and bioaccessibility. To achieve this objective, fermentation with the fungus *Pleurotus ostreatus* was carried out on two varieties of lentils and quinoa, and subsequently stabilised by hot air drying at different temperatures, in addition by freeze-drying as a reference method. Subsequently, the fermented flours were digested *in vitro* simulating the digestive process of a healthy adult (reference standard), as well as under altered conditions of the elderly. The results obtained evidenced an increase in total protein, as well as in angiotensin-converting enzyme (ACE) inhibitory activity, together with a decrease in phytic acid content, all as a result of the metabolic activity of the fungus on the substrate. Although SSF also caused a reduction in antioxidant activity, subsequent hot air drying, especially at 70 °C, increased this parameter. Similarly, SSF and hot air-drying promoted changes in the phenolic profile, decreasing some compounds and increasing others such as gallic

acid up to 5 times its initial content. The volatile profile of the fermented flours was characterised by a sweet, fruity aroma with hints of cocoa, accompanied by notes of mushrooms and cooked substrates, due to the concentrations of benzaldehyde, hexanal, nonanal, furfural and 1-octen-3-ol that were generated during fermentation. On the other hand, regarding the digestibility of the fermented flours under standard healthy adult conditions, SSF and drying at 70 °C increased the hydrolysis of proteins, as well as the release of hydrophobic and negatively charged amino acids. SSF also decreased the ACE inhibitory activity of the digests, however, it increased after drying at 70 °C due to melanoidins generated during drying. Furthermore, antioxidant properties and mineral bioaccessibility were also increased with SSF and subsequent drying at 70 °C. Finally, simulation of gastrointestinal disturbances commonly found in the older adult indicated that these impacted negatively on most of the parameters evaluated, with the exception of the bioaccessibility of magnesium, iron and calcium compared to the standard model.

In conclusion, the nutritional and functional profile of the new flours obtained by fermentation with the fungus *P. ostreatus,* and subsequent hot air drying has been improved compared to flours obtained from unfermented substrate, leading to a significant improvement in the digestibility and bioaccessibility of nutrients, which may be particularly relevant for the design of foods oriented to population groups with a high demand of easily digestible protein.

RESUM

La població mundial està en constant creixement, per la qual cosa la sobirania alimentària ha esdevingut un desafiament crucial. S'estima que la demanda d'aliments d'origen animal augmentarà un 68% per a l'any 2050, cosa que resulta insostenible a nivell mediambiental. Així, impulsar un consum més gran de proteïna vegetal es planteja com una de les estratègies dirigides a promoure la sostenibilitat ambiental, assegurant la disponibilitat de proteïna dietètica per a tota la població. Si bé certs els llegums i pseudocereals són una excel·lent font de nutrients i en particular de proteïna, també contenen certs antinutrients que poden limitar-ne la digestibilitat. Aquest aspecte és especialment rellevant en aquells grups de poblacions amb alteracions gastrointestinals, com les que poden aparèixer amb l'edat en població sènior.

En aquest context, l'objectiu general d'aquesta tesi doctoral és aplicar la fermentació en estat sòlid (FES) com a bioestratègia per obtenir farines de llentia i quinoa amb digestibilitat i bioaccessibilitat millorades. Per assolir aquest objectiu, es va dur a terme la fermentació amb el fong *Pleurotus ostreatus*, en dues varietats de llenties i quinoa, i es van estabilitzar posteriorment mitjançant assecat per aire calent a diferents temperatures, a més de la liofilització com a mètode de referència. Posteriorment, les farines fermentades van ser digerides *in vitro* simulant el procés digestiu d'un adult sa (estàndard de referència), així com en condicions alterades de l'adult més gran. Els resultats obtinguts van evidenciar un increment de proteïna total, així com de l'activitat inhibidora de l'enzim convertidor d'angiotensina (ECA), conjuntament amb una disminució del contingut en àcid fític, com a resultat de l'activitat metabòlica del fong sobre el substrat. Tot i que la FES també va ocasionar una reducció de l'activitat antioxidant, el posterior assecat per aire calent,

especialment a 70 °C va incrementar aquest paràmetre. Així mateix, la FES i l'assecatge per aire calent van promoure canvis en el perfil fenòlic, disminuint alguns compostos i incrementant-ne d'altres com l'àcid gàlic fins a 5 vegades el contingut inicial. Quant al perfil volàtil de les farines fermentades, aquest es va caracteritzar per una aroma dolça, afruitada i amb matisos a cacau, acompanyat de notes de bolets i substrats cuits, a causa de les concentracions de benzaldehid, hexanal, nonanal, furfural i 1-octen -3-ol que es van generar durant la fermentació. D'altra banda, pel que fa a la digestibilitat de les farines fermentades en condicions estàndard d'adult sa, la FES i l'assecatge a 70 °C va incrementar la hidròlisi de les proteïnes, així com l'alliberament d'aminoàcids hidròfobs i aminoàcids carregats negativament. La FES també va disminuir l'activitat inhibidora de l'ACA en els digerits, però aquesta va augmentar després de l'assecat a 70 °C a causa de les melanoïdines generades durant l'assecat. A més, les propietats antioxidants i la bioaccessibilitat de minerals també es van veure incrementats amb la FES i el posterior assecat a 70 °C. Finalment, la simulació de les alteracions gastrointestinals que comunament es donen a l'adult major, van indicar que aquestes impactaven negativament a la majoria dels paràmetres avaluats, a excepció de la bioaccessibilitat del magnesi, ferro i calci en comparació del model estàndard.

En conclusió, s'ha aconseguit millorar el perfil nutricional i funcional de les noves farines obtingudes per fermentació amb el fong *P. ostreatus*, i posterior assecat per aire calent en comparació amb les farines obtingudes a partir de substrat no fermentat, conduint-ho a una millora significativa en la digestibilitat i la bioaccessibilitat dels nutrients, cosa que pot ser especialment rellevant per al disseny d'aliments orientats a grups de població amb alta demanda de proteïna de fàcil digestió.

TABLA DE CONTENIDO

LISTA DE TABLAS

LISTA DE FIGURAS

Figure 5.19. ACE inhibitory activity (%) in Pardina (**a**) and Castellana (**b**) lentil and white (**c**) and black (**d**) quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flours obtained with a standard or older adult *in vitro* digestion model. [a,b,c,d] Different lowercase letters indicate significant differences ($p < 0.05$) between samples. [A,B] Different capital letters indicate significant differences ($p < 0.05$) between digestion models. 375

1. INTRODUCCIÓN

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1.1. Contextualización

1.1.1. Crecimiento de la población mundial

La población mundial sigue aumentando, y actualmente, es más de tres veces superior que a mediados del siglo XX. En el año 1950 existía una población estimada de 2.5 billones de personas, y para el 2022 la población mundial alcanzó los 8 billones. Se estima que para el año 2050 la población será de 9.7 billones personas, pudiendo llegar a un pico de 10.4 billones en el 2080. Entre los países más poblados del mundo se encuentran China e India con 1.4 billones de personas cada uno, representando el 35% de la población mundial (Roser, Ritchie, et al., 2013; UN, 2022b). También, se ha previsto que África subsahariana doble su población para el 2050 debido a que tiene la tasa de crecimiento demográfico más alta (>3%) que la máxima registrada en el mundo a principios de la década de 1960 (UN, 2022b).

Existen factores que influyen en el crecimiento de la población, entre ellos: la tasa de fertilidad, el aumento de la longevidad (esperanza de vida) y la migración internacional. En la era premoderna, las tasas de fertilidad de 4.5 a 7 hijos por mujer eran comunes (Roser, 2014). Sin embargo, una alta tasa de mortalidad mantuvo bajo el crecimiento de la población. Posteriormente, las condiciones de salud mejoraron disminuyendo la mortalidad de la población (Roser, Ritchie, et al., 2013), incrementando la esperanza de vida desde alrededor de 30 años en la era premoderna a 73 años en 2019 (Roser, Ortiz-Ospina, et al., 2013), y se prevé un aumento superior a 77 años para el 2050 (UN, 2022b). Por otro lado, la migración internacional es uno de los factores menos influyentes en el crecimiento de la población, pero no menos importante, ya que las personas al migrar a otros lugares

pueden tener una mayor esperanza de vida que la que les puede ofrecer su propia región. Todos estos factores son los que han venido desencadenando un crecimiento acelerado de la población. Sin embargo, en la actualidad, la tasa de fecundidad se ha visto reducida a un promedio de 2.3 hijos por mujer, y se espera que en el 2050 disminuya a 2.1, y con esto, una ralentización del incremento de la población a largo plazo (Roser, 2014; UN, 2022b).

1.1.2. Soberanía alimentaria y nutrición

La alimentación de una población cada vez más numerosa es uno de los desafíos a los que se enfrenta la sociedad para acabar con el hambre y la malnutrición. En el 2015, la Asamblea General de la ONU adoptó la Agenda 2030 para el desarrollo sostenible, que plantea la consecución de un conjunto de objetivos globales para erradicar la pobreza, proteger el planeta y asegurar la prosperidad (UN, 2015).

La soberanía alimentaria mundial se mantuvo relativamente estable desde el 2015, sin embargo, la prevalencia de la subalimentación incrementó del 8% en 2019 a 9.3% en 2020, y siguió aumentando en el 2021 como consecuencia de la pandemia de la COVID-19. Aproximadamente entre 702 y 828 millones de personas, es decir, entre el 8.9% y 10.5% de la población sufrieron hambre en 2021 (FAO et al., 2022). Además, la guerra entre Rusia y Ucrania amenaza con incrementar el número de personas malnutridas, debido a que la interrupción de las cadenas de suministro ha provocado incrementos en los precios de los alimentos, y, por lo tanto, un difícil acceso a dietas saludables y nutritivas (FAO et al., 2022; The World Bank, 2023). Todo esto, ha afectado la consecución del objetivo 2 de los Objetivos de Desarrollo Sostenible (ODS) que busca erradicar el hambre, por lo cual es importante bregar por una producción agrícola y alimentaria sostenible.

Una dieta saludable implica el consumo de macronutrientes en proporciones adecuadas para satisfacer las necesidades energéticas y fisiológicas, al mismo tiempo que se garantiza la ingesta de micronutrientes e hidratación para satisfacer las demandas del cuerpo. Dentro del grupo de macronutrientes las proteínas juegan un papel fundamental, ya que también intervienen como fuente de energía (4 kcal/g) y aminoácidos, incluidos los esenciales que el cuerpo humano requiere, pero no puede producir por sí mismo (Cena & Calder, 2020). Basado en esto, la ingesta de alimentos proteicos como fuente de aminoácidos son imprescindibles para la salud, el crecimiento, el desarrollo, la reproducción, la lactancia, etc. (G. Wu, 2016).

1.1.3. Envejecimiento de la población

El número de personas adultos mayores en la población está incrementando a un ritmo acelerado. El descenso de la tasa de fertilidad y el aumento de la longevidad son la causa principal del envejecimiento de la población mundial. Sin embargo, también es importante tener en cuenta que la migración internacional ha desempeñado un papel clave en el cambio de la composición de las edades en diferentes países y áreas geográficas, haciendo que en los lugares con grandes flujos migratorios se observe un aparente retraso del envejecimiento global de la población; no obstante, si estas personas se establecen en el lugar de migración acabarán formando parte del grupo de población de mayor edad (UN, 2022a). En el 2020, la población >60 años fue de un billón y se espera un incremento para el 2030 a 1.4 billones y para el 2050 a 2.1 billones, es decir, el 22% de la población. Además, se ha previsto que entre el 2020 y el 2050 el número de personas >80 años se triplicará de 143 millones a 426 millones (UN, 2022a; WHO, 2022).

Tabla 1.1. Factores que afectan a la ingesta de nutrientes en las personas mayores.

1. Factores sociales
- Pobreza, aislamiento social, soledad, ignorancia - Disminución de la independencia - Incapacidad para hacer la compra o preparar comida - Falta de conocimiento sobre comida, cocina y nutrición - Monotonía de la comida institucionalizada
2. Factores psicológicos
- Depresión, duelo, alcoholismo, demencia, paranoia, manía, anorexia, sociopatía, confusión, ansiedad
3. Factores fisiológicos
- Disfunción gastrointestinal, por ejemplo, malabsorción - Poco apetito y mala alimentación - Problemas orales (dientes flojos y disfagia) - Pérdida del gusto y el olfato - Trastornos respiratorios - Trastornos endocrinos (ejemplo: diabetes mellitus tipo 2) - Trastornos neurológicos (ejemplo: enfermedad de Parkinson) - Infecciones, por ejemplo, infecciones del tracto urinario - Incapacidad física para alimentarse por sí mismo - Interacciones con los medicamentos - Náuseas y vómitos - Demanda metabólica alterada/aumentada - Enfermedades crónicas como el cáncer, artritis, osteoporosis…

Fuente: (Brownie, 2006; Pathy et al., 2006; Rémond et al., 2015)

Desde una perspectiva biológica, el proceso de envejecimiento se origina debido a la acumulación progresiva de diversos tipos de daños a nivel molecular y celular a lo largo del tiempo. Esta acumulación de daños conduce a una disminución gradual de las habilidades físicas y mentales, aumentando el riesgo de enfermedades y, en última instancia, llevando al fallecimiento (WHO, 2022). Las personas de edad avanzada son en general susceptibles de sufrir una alimentación deficiente en

comparación con los adultos más jóvenes, lo que las hace más propensas a padecer deficiencias nutricionales tanto de macro como micronutrientes (Brownie, 2006). Los factores que afectan a la ingesta de nutrientes en las personas mayores se encuentran divididos en tres categorías como se muestra en la Tabla 1.1.

El envejecimiento suele implicar una serie de alteraciones digestivas con cambios en la cavidad oral, como la pérdida de piezas dentales, el uso de prótesis dentales, la gingivitis y la disminución de la producción de saliva. El sentido del gusto y del olfato también puede verse alterado, lo que disminuye la palatabilidad y aumenta la inapetencia, produciendo cambios en los hábitos alimentarios en cuanto a cantidad y tipo de alimentos consumidos (Brownie, 2006). Las funciones del tracto gastrointestinal tienden a ser subóptimas, con una disminución del peristaltismo y del vaciado gástrico y alteraciones en la motilidad esofágica, dando lugar a un aumento del reflujo gastroesofágico y de la dispepsia funcional (Grassi et al., 2011). La presencia de gastritis atrófica e hipoclorhidria es otra alteración que puede producirse dando lugar a malabsorción de vitamina B12, ácido fólico, hierro, calcio y betacaroteno que son pH-dependientes para una correcta absorción (Brownie, 2006). Además, la reducción o alteración de la secreción de enzimas, la motilidad del tracto gastrointestinal y el pH pueden conducir a una mala digestión y malabsorción de proteínas, lo que aumenta la predisposición a padecer enfermedades como la sarcopenia e incrementa el riesgo de caídas (S. Lee et al., 2023). La Ingesta Diaria Recomendada (IDR) de proteína es de 0.8 g por kg de peso corporal por día para personas adultas independientemente de la edad, siendo este valor la cantidad más baja de proteína requerida para prevenir la disminución continua de masa muscular en la mayoría de las personas, pero podría ser inadecuada para mantener la salud muscular en los adultos mayores. Existe evidencia de que una ingesta por encima de la IDR puede mejorar la masa muscular, la fuerza y la función en las personas adultas

mayores. Además, otros factores, como el estado inmunitario, la cicatrización de heridas, la presión arterial y la salud ósea, pueden mejorar al aumentar la ingesta de proteínas por encima de la IDR (Landi et al., 2016; Wolfe et al., 2008). Como se mencionó en párrafos anteriores, las personas mayores pierden el apetito y en especial las que sufren de enfermedades crónicas, lo que provoca un insuficiente consumo proteico. Por lo tanto, es importante que las personas de este grupo poblacional incluyan dentro de su dieta proteínas de alta calidad (Wolfe et al., 2008).

1.2. Sostenibilidad ambiental en la producción de alimentos

Con la proyección del crecimiento de la población para el 2050, se espera que la demanda mundial de alimentos se duplique, al mismo tiempo que los recursos naturales necesarios para la agricultura se volverán más limitados, degradados y susceptibles a los efectos del cambio climático (The World Bank, 2007). Como consecuencia de esta demanda de alimentos, las necesidades de alimentos de origen animal aumentarán en un 68% hasta el 2050. La cría de animales para la producción de carne, especialmente de rumiantes, requiere de un elevado consumo hídrico y de disponibilidad de tierra, y emiten una cantidad significativamente mayor de gases de efecto invernadero, en comparación con los alimentos de origen vegetal (Searchinger et al., 2018). Entre las estrategias para aumentar la producción de alimentos sostenibles se encuentran: (1) la expansión del área cultivada aprovechando las tierras de cultivo inactivas poniéndolas a producir; (2) la intensificación del área cultivada maximizando su rendimiento y eficiencia mediante técnicas avanzadas y prácticas agrícolas sostenibles; (3) sustituir de forma parcial el consumo de alimentos de origen animal por fuentes vegetales, como legumbres o cereales (Goodland, 1997).

En el siglo XXI, la investigación se ha centrado en la transición hacia dietas más sostenibles y la búsqueda de fuentes alternativas de proteínas debido a las preocupaciones ambientales (Lonnie & Johnstone, 2020). Las dietas sostenibles son aquellas dietas con bajo impacto ambiental que contribuyen a la soberanía alimentaria y a una vida sana para las generaciones presentes y futuras. Además, protegen y respetan la biodiversidad y los ecosistemas, son culturalmente aceptables, accesibles, económicamente justas y asequibles; nutricionalmente adecuadas, seguras y saludables; al tiempo que optimizan los recursos naturales y humanos (FAO, 2012).

Las dietas basadas en plantas que incluyen cereales, semillas, frutos secos, legumbres y vegetales son fuentes sostenibles de proteínas que además de aportar nitrógeno, son fuente de fitonutrientes, vitaminas, minerales y fibra que son esenciales para el organismo. Estas fuentes sostenibles, se producen de manera más eficiente y requieren menos agua, tierra, nitrógeno y energía fósil para producir una cantidad de proteína en relación con la proteína de origen animal (Nadathur et al., 2017).

La producción de granos requiere aproximadamente 4.6 m^2 de tierra/100 g de proteína, mientras que para la producción de carne de res se necesitan 163.6 m^2/100 g de proteína. Desde el punto de vista de las emisiones de gases de efecto invernadero, para la producción de 100 g de proteína se liberan 2.7 y 49.9 kg de CO_2-equivalentes para la producción de granos y carne de res respectivamente (Poutanen et al., 2022; Ritchie et al., 2022)l., 2022). Sustituir las proteínas de origen animal por proteínas de vegetales, podría disminuir las emisiones de gases de efecto invernadero, optimizar el aprovechamiento de la tierra y garantizar la alimentación de más personas (Nadathur et al., 2017; Poutanen et al., 2022).

1.3. Producción y consumo de alimentos vegetales ricos en proteínas

Las legumbres, los cereales y los pseudocereales son fuentes importantes de nutrientes y constituyen una buena base para una dieta saludable para la creciente población y demanda de alimentos.

1.3.1. Legumbres

Las legumbres son semillas maduras de la familia de plantas *Fabaceae*. En el conjunto de las leguminosas se incluyen las judías, habas, guisantes, garbanzos, lentejas, soja y cacahuetes (Belitz et al., 2009c), los cuales poseen un alto valor nutricional (Tabla 1.2). Destaca su contenido de proteínas, que oscila entre el 20% y el 45%. Los guisantes y las judías tienen un contenido de proteína que varía del 17% al 20%, ubicándose en el extremo inferior del rango. Por otro lado, los lupinos y la soja tienen un contenido de proteína del 38% al 45%, situándose en el extremo superior del rango. Las legumbres son ricas en el aminoácido esencial lisina. No obstante, tienen una baja concentración de aminoácidos esenciales que contienen azufre, tales como la metionina, cistina, cisteína y triptófano. Por lo tanto, al complementar la dieta con fuentes vegetales adicionales, como los cereales que son ricos en aminoácidos que contienen azufre y bajos en lisina, se logra mejorar la calidad de la proteína mediante su consumo combinado (Maphosa & Jideani, 2017). Las legumbres contienen entre un 60% y un 80% de carbohidratos, y su contenido de fibra dietética puede variar del 3% al 30%, e incluso llegar al 55%, dependiendo del tipo de cultivar. Así mismo, el contenido de grasa es generalmente inferior al 3% en la mayoría de las legumbres, a excepción del lupino, la soja y el cacahuete que pueden tener un contenido entre el 15% y el 50%. El contenido de cenizas varía del 2% al 5%, y representan una fuente importante de minerales como el calcio,

magnesio, potasio, fósforo, hierro y zinc como se muestra en algunas legumbres en la Tabla 1.3 (Belitz et al., 2009c; Hall et al., 2017).

Tabla 1.2. Composición química de algunas legumbres expresada en g/100 g de materia seca.

	Proteína cruda	Carbohidratos	Fibra dietaria	Grasa	Cenizas
Garbanzo	19-27	52-71	6-15	2-7	1.8-3.5
Lenteja	23-31	42-72	7-23	1-3	2.1-3.2
Lenteja verde	24.6	63.4	10.7	1.06	2.71
Lenteja roja	22.5-23.9	63.1	10.8	2.2-3	3
Guisante	14-31	55-72	3-20	1-4	2.3-3.7
Lupino	32-44	47	14-55	5-15	2.6-3.9
Soja	41	32	24	20	5.5
Cacahuete	31	20	12	51	2.7
Haba	23-36	57-76	15-24	2	3.6
Judía riñón	17-27	63-74	18-30	1-5	3.2-5.2
Judía blanca	19-27	67-75	14-25	2	4.0-4.9
Judía pinta	18-25	70-76	14-26	1-2	2.5-4.7
Judía negra	21-23	56-72	18-26	2-3	4.6-5.4
Judía roja	22	71-72	21-22	1	3.9-5.6
Judía verde	24	73	19	2	4.4

N.I.: no informado. Adaptado de: (Belitz et al., 2009c; Bressani et al., 1961; Hall et al., 2017; Labba et al., 2021; Mlyneková et al., 2014; Mohammad et al., 2012; Pal et al., 2017).

Las legumbres también constituyen una fuente de vitaminas del grupo B (Tabla 1.4), como la tiamina (B1), riboflavina (B2), niacina (B3) y ácido fólico (B9), mientras que su contenido de vitamina C es relativamente bajo (Hall et al., 2017; Martín-Cabrejas, 2019). Además, contienen principalmente ácidos fenólicos, flavonoides y taninos condensados entre varios compuestos fenólicos conocidos (Tabla 1.5). Estos compuestos se distribuyen de manera diferente en la cubierta de la semilla (flavonoides) y el cotiledón (ácidos fenólicos) (Singh et al., 2017).

Tabla 1.3. Contenido de minerales en algunas legumbres expresado en mg/100g.

	Calcio	Hierro	Magnesio	Potasio	Fósforo	Zinc
Garbanzo	82-272	4.6-7.5	147-195	994-1264	394-452	3.4-4.4
Lenteja	59-463	6.3-9.2	99-726	285-943	57-407	2.6-3.8
Lenteja verde	29-76	5.3-8	36-115	540-849	257-525	2.5-4
Lenteja roja	42-65	4.8-12	48-114	564-841	270-583	2.7-4.5
Guisante	60-111	1.9-8.0	130-172	876-1463	279-291	3.0-3.4
Lupino	139-300	1.7-6.2	138-308	760-1424	382-846	4.3-7.6
Soja	210	7.1	210	1400	490	4.2
Cacahuete	59	2.1	160	710	370	3.1
Judía riñón	76-163	5.2-7.8	128-170	1308-1709	385-566	2.8-3.8
Judía blanca	138-330	4.2-7.8	170-208	1335-1946	398-621	2.1-3.0
Judía pinta	63-173	4.4-13	144-180	1164-1864	375-6019	2.2-3.8
Judía negra	123-225	4.6-6.9	171-184	1468-1843	504-614	1.9-2.9
Judía roja	62-103	3.4-8.2	143-168	1250-1731	362-573	1.9-3.4
Judía verde	110	6.0	130	1300	430	2.6

Adaptado de: (Belitz et al., 2009c; Benayad & Aboussaleh, 2021; Hall et al., 2017).

Tabla 1.4. Contenido de vitaminas en algunas legumbres expresado en mg/100 g.

	Tiamina (B1)	Riboflavina (B2)	Niacina (B3)	Ácido fólico (B9)
Judía común	0.56	0.18	2.3	0.17-0.18
Garbanzo	0.45	0.17	1.6	0.04-0.53
Lenteja	0.56	0.28	2.5	0.15-0.29
Guisante	0.12	0.06	N.I.	0.03-0.20
Altramuz	0.23-0.39	0.23-0.65	3.9	N.I.
Soja	0.76-0.82	0.3-0.43	3.3	N.I.

N.I.: no informado. Adaptado de: (Belitz et al., 2009c; Erbas et al., 2005; Hall et al., 2017).

Tabla 1.5. Contenido de compuestos fenólicos identificados en algunas legumbres expresado en mg/100 g.

	Garbanzo	Lenteja	Guisante	Judía Great Northern	Judía blanca	Judía pinta	Judía negra	Judía riñón
Ácidos fenólicos								
Cafeico	0.3-2.3	0-1.1	0.2-0.3	N.I.	N.I.	1.1	10.9	N.I.
Clorogénico	108-175	15.5-39.1	8.3-16.1	N.I.	N.I.	46.5	22.6	N.I.
Cinámico	N.I.	N.I.	N.I.	N.I.	7.8	2.2-7.2	0.3-7.5	7.0
Cumárico	7.0-10.0	0.3-12.2	3.8-4.2	6.3-17.0	4.3-12.2	0.9-5.6	1.2-11.6	1.8-7.0
Ferúlico	106	N.I.	N.I.	9.4-17.2	5.5-26.6	7.6-23.0	6.1-25.5	7.6-15.3
Gálico	3.2-7.6	9.1-15.0	8.1-9.1	N.I.	N.I.	11.2	10.6	N.I.
Hidroxibenzóico	1.8-6.0	0.1-4.5	1.8-6.0	N.I.	N.I.	3.0	2.4	N.I.
Sinápico	0.3	0.2-227	0.3	9.0-30.4	2.1-9.0	3.8-26.4	4.5-14.0	2.6-3.8
Vanílico	0.07	0.1	0.2-0.3	N.I.	N.I.	13.5	5.8	N.I.
Flavonoides								
Kaempferol-3-glucósido	N.D.	0.4-0.8	0-0.4	N.I.	N.I.	7.5-49.2	2.7	N.I.
Luteolina	N.D.	0.2-9.7	N.D.	N.I.	N.I.	N.I.	N.D.	N.I.
Catequina	1.6-151	0.1-190	20.5-28.3	N.I.	N.I.	N.I.	24.7	N.I.
Epicatequina	0-14.6	0.1-495	N.D.	N.I.	N.I.	N.I.	21.4	N.I.
Procianidinas	N.I.	0.2-4.1	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.

N.I.: no informado; N.D.: no detectado. Adaptado de: (Hall et al., 2017).

INTRODUCCIÓN

Las legumbres son una rica fuente de nutrientes saludables y compuestos bioactivos beneficiosos. Sin embargo, también producen metabolitos secundarios conocidos como antinutrientes, que son parte de su mecanismo de defensa contra depredadores. Estos compuestos pueden reducir la digestibilidad y biodisponibilidad de los nutrientes en los alimentos y, en grandes cantidades, pueden resultar tóxicos. No obstante, en dosis adecuadas, algunos antinutrientes pueden tener efectos positivos en la salud y contribuir a la prevención de enfermedades crónicas (Das et al., 2022; A. Sharma, 2021). A continuación, se describen algunos de los antinutrientes más comunes en las legumbres:

Ácido fítico: También llamado mioinositol ácido hexafosfórico o fosfato de dihidrógeno hexakis de mioinositol, es la principal forma de almacenamiento de fósforo en muchas semillas de leguminosas, y cuando está en forma de sal, se le conoce como fitato (Oatway et al., 2001; A. Sharma, 2021). Su fuente principal es la judía común, la lenteja, el cacahuete, el guisante verde y la soja (Das et al., 2022). La propiedad antinutricional del ácido fítico radica en su capacidad para formar complejos insolubles con minerales como el hierro, zinc, calcio y magnesio, lo que dificulta su absorción y puede resultar en deficiencias de estos minerales (Das et al., 2022; A. Sharma, 2021). Además, puede generar complejos insolubles con las proteínas, alterando su estructura y, como consecuencia, dificultando la actividad enzimática, la solubilidad y la digestibilidad de estas proteínas. Los carbohidratos también pueden formar complejos con el ácido fítico, lo que disminuye su solubilidad y, como resultado, afecta la digestibilidad y absorción de la glucosa, llevando a un índice glucémico bajo. Además, el fitato, al unirse al ion Ca^{++}, inhibe la actividad de la amilasa, contribuyendo a esta respuesta glucémica más baja (Kumar et al., 2010).

Taninos: Son biomoléculas polifenólicas de origen vegetal, solubles en agua, termoestables, de alto peso molecular (≥ 500 Da) y astringentes por naturaleza. Se dividen en dos categorías: taninos hidrolizables y condensados. En las legumbres, los taninos condensados son los polifenoles predominantes y se encuentran en la cubierta de la semilla. Las principales fuentes de estos taninos son la judía adzuki (Vigna angularis), el gandul, la lenteja, la judía común, la judía mungo y el garbanzo (Das et al., 2022; A. Thakur et al., 2019). Los taninos exhiben propiedades anticancerígenas, antimutagénicas, antimicrobianas y antioxidantes. Sin embargo, son considerados antinutrientes ya que forman fuertes complejos con minerales como el fósforo, calcio y magnesio, y macromoléculas como carbohidratos y proteínas, lo que dificulta su absorción en el intestino. Inhiben el funcionamiento adecuado de las enzimas digestivas como la amilasa, la lipasa y las proteasas afectando la digestión gastrointestinal, lo que conlleva a una reducción de la digestibilidad y absorción de nutrientes (Das et al., 2022; A. Sharma, 2021).

Lectinas: Son un grupo de proteínas que se unen a los carbohidratos y de forma selectiva y reversible a los monosacáridos presentes en la superficie de las células (Das et al., 2022; Popova & Mihaylova, 2019). Las lectinas tienen actividad hemaglutinante, que se usa para tipificar la sangre, y también tienen otros roles importantes en el sistema inmunológico, crecimiento celular y regulación de la grasa corporal, entre otros (A. Sharma, 2021). Las fuentes principales de lectinas son la judía, la lenteja, el guisante, la soja y el cacahuete (Das et al., 2022). Las lectinas, debido a su estructura terciaria globular, no son degradadas por las enzimas digestivas, lo que puede causar problemas de salud. Estas proteínas reducen el área de superficie de las vellosidades en el intestino, afectando la absorción de nutrientes y la flora bacteriana (Das et al., 2022; A. Sharma, 2021).

Saponinas: Son compuestos de bajo peso molecular que tienen al menos un enlace glucosídico en C-3 entre la aglicona y una cadena de azúcar. Son capaces de formar espumas estables similares al jabón en soluciones acuosas. Los glucósidos triterpenoides son más comunes en las leguminosas y las principales fuentes de estos compuestos son la judía canavalia, la judía lablab, la judía espada, la judía común, la judía terciopelo, el guandú, el cacahuete bambara y la judía lima (Das et al., 2022). Las saponinas interactúan con el colesterol en las membranas de los eritrocitos, causando hemólisis (A. Sharma, 2021). También se unen a las células del intestino delgado, reduciendo la absorción de nutrientes y actuando como inhibidores de enzimas clave en la digestión de carbohidratos, lípidos y proteínas. Además, forman complejos con esteroles que tienen estructuras similares a las vitaminas liposolubles, inhibiendo su absorción y actividad (Das et al., 2022; Samtiya et al., 2020).

Inhibidores de enzimas: Los inhibidores de enzimas (proteasa y α-amilasa) presentes en las leguminosas provocan una deficiente bioaccesibilidad de los minerales y una reducción en la digestibilidad de nutrientes (Das et al., 2022; Samtiya et al., 2020). Las principales fuentes de estos compuestos son la soja, el guandú, la judía común, el caupí, la judía mungo, el haba, la judía alado y el garbanzo (Das et al., 2022). Los inhibidores de tripsina de Kunitz y el inhibidor de Bowman-Birk, encontrados en las legumbres, son capaces de inhibir las enzimas tripsina y quimotripsina, limitando su actividad mediante interacciones proteína-proteína (Das et al., 2022; Pedrosa et al., 2021). Por otro lado, los inhibidores de la α-amilasa aumentan el tiempo de absorción de carbohidratos al retrasar su digestión, disminuyendo la absorción de glucosa y afectando el nivel normal de glucosa posprandial en plasma (Samtiya et al., 2020).

La Tabla 1.6 presenta los niveles de antinutrientes encontrados en algunos tipos de legumbres.

Tabla 1.6. Contenido de antinutrientes en algunas legumbres.

	Ácido fítico (mg/g)	**Taninos (mg/g)**	**Lectinas (HU/mg [a]; mg/g [b])**	**Saponinas (mg/g)**	**Act. inhibidora de tripsina (U/mg [a]; mg/g [b])**
Garbanzo	1.2-6.9	2.2-4.9	N.I.	0.9-1.2	8.1-15.7[a]
Lenteja	3-17	3.9	0.5-0.6[a]	0.6-3.5	7.4[a]
Lenteja roja	3-8.4	5-6.9	N.I.	N.I.	2.6-3[b]
Soja	1.2-8.4	1.8-1.9	0.11-8[b]	6	94.1[a]
Cacahuete	2.6-14.7	8.9	0.14[b]	N.I.	5.6[a]
Haba	8.4	6.5	N.I.	4	4.6[a]
Judía riñón	6.3-24.1	5.4-28.8	N.I.	1.06-13	3.1[a]

N.I.: no informado. Adaptado de: (Pal et al., 2017; Rudra et al., 2023; Samtiya et al., 2020; A. Sharma, 2021; Vidal-Valverde et al., 1994; N. Wang, 2008).

Las lentejas ocupan el segundo lugar en cuanto a consumo de legumbres en España, después de los garbanzos. En el año 2019, el consumo per cápita de lentejas fue de 0.98 kilos por persona al año, como parte de un total de 3.34 kilos por persona al año de legumbres en general (MAPA, 2019). Destacan por su excelente valor nutricional, ya que son una fuente excepcional de proteínas, contienen una cantidad significativa de fibra alimentaria y su contenido de grasa es bajo. Además, son una rica fuente de minerales esenciales como el calcio, hierro y magnesio, así como de vitaminas del grupo B. Asimismo, presentan compuestos fenólicos notables, incluyendo el ácido gálico.

1.3.2. Cereales y Pseudocereales

Los cereales pertenecen al grupo de plantas monocotiledóneas y los pseudocereales al grupo de las dicotiledóneas, ambos producen semillas ricas en almidón y tienen un excelente valor nutricional. Dentro de la categoría de los

cereales se encuentran el trigo, arroz, maíz, cebada, avena, centeno, mijo y sorgo, entre otros; mientras que en la categoría de los pseudocereales se puede mencionar la quinoa, el amaranto y el trigo sarraceno. En la actualidad, se ha observado un aumento en la demanda de los pseudocereales debido a su idoneidad en las dietas exentas de gluten (McKevith, 2004; Schoenlechner et al., 2008).

Centrándonos en la composición nutricional de estos dos grupos (Tabla 1.7), ambos se clasifican como alimentos ricos en carbohidratos representando del 50-75% de su composición (McKevith, 2004; P. Thakur et al., 2021). Los cereales tienen un contenido de proteínas que oscila entre el 6%–15% (McKevith, 2004) y en los pseudocereales del 10%–20%. Los pseudocereales presentan una composición de aminoácidos excepcional, ya que contienen niveles más altos de lisina, metionina y cisteína en comparación con los cereales convencionales, los cuales suelen ser deficientes principalmente en lisina y secundariamente deficientes en treonina y triptófano (Martínez-Villaluenga et al., 2020). Tanto los cereales como los pseudocereales presentan un rango de contenido de grasa total que oscila entre el 1%–10%, así como un contenido de cenizas que varía entre el 2%–4%.

Los cereales integrales presentan cantidades significativas minerales (Tabla 1.8) como el hierro, magnesio y zinc (McKevith, 2004), mientras que los pseudocereales se caracterizan por ser ricos en potasio, fósforo y magnesio (Martínez-Villaluenga et al., 2020). En términos de contenido vitamínico (Tabla 1.9), los cereales y pseudocereales representan una fuente importante de algunas vitaminas del grupo B, como la tiamina, riboflavina y niacina, además de contener cantidades apreciables de vitamina E (Martínez-Villaluenga et al., 2020; McKevith, 2004). Además, los granos de cereales y pseudocereales contienen compuestos bioactivos como ácidos fenólicos y flavonoides (Tabla 1.10), con mayores concentraciones en la capa

de la semilla y menores en el endospermo (Stuper-Szablewska & Perkowski, 2019). Los cereales se caracterizan por ácidos ferúlico, vanílico, cumárico y el flavonoide quercetina, mientras que los pseudocereales tienen ácidos gálico, hidroxibenzóico, vanílico, cumárico y ferúlico, junto con los flavonoides quercetina y kaempferol.

Tabla 1.7. Composición química de algunos cereales y pseudocereales expresada en g/100 g de materia seca.

	Proteína	**Carbohidratos**	**Fibra**	**Grasa**	**Cenizas**
Malta	13.1	77	5.7	1.9	2.4
Arroz	7.4-7.5	74-77	0.9-2.2	1.9-2.4	1.2
Maíz	8.9-9.2	64-72	2-9.7	3.8-3.9	1.2-1.3
Trigo	11.7-13.3	60-71	2.3-13.3	2-2.2	1.5-1.7
Sorgo	11	73	1.7	3.3	1.7
Mijo	9.9-10.6	69-73	3.2-3.8	2.9-4.1	1.6-2.5
Centeno	9.5-13.4	61-80	2.6-13.2	1.7-1.8	1.9-2.1
Cebada	10.6-10.8	63-81	4.4-9.8	1.9-2.1	2.2-2.3
Avena	11.6-12.6	56-70	9.7-10.4	5.2-7.1	2.8-2.9
Quinoa	10-18	48-77	7-27*	4.5-8.8	2.4-3.8
Amaranto	13-21	63-70	3-17*	1.9-10.6	3.2-4
Trigo sarraceno	5.7-19	63-82	8.6-18*	1-6.9	1.6-3.9

* Fibra dietaria. Adaptado de: (Belitz et al., 2009b; Charalampopoulos et al., 2002; Martínez-Villaluenga et al., 2020; Mlyneková et al., 2014; Schoenlechner et al., 2008; P. Thakur et al., 2021).

Los cereales y pseudocereales, al igual que las legumbres, contienen compuestos antinutricionales como el ácido fítico, saponinas, taninos e inhibidores de enzimas, que tienen la capacidad de unirse a diversos nutrientes presentes en los alimentos. Esta unión dificulta la digestión, absorción y utilización de dichos nutrientes, como se explica detalladamente en el aparatado 1.3. Si bien estos compuestos pueden ser beneficiosos en ciertas cantidades moderadas, un consumo excesivo de los mismos puede ser perjudicial para la salud humana (McKevith, 2004; P. Thakur et al., 2021). En la Tabla 1.11 se presentan contenidos de antinutrientes presentes en algunos cereales y pseudocereales.

Tabla 1.8. Contenido de minerales en algunos cereales y pseudocereales expresados en mg/100 g.

	Calcio	Hierro	Magnesio	Potasio	Fósforo	Zinc
Arroz integral	10	1.4	110	250	N.I.	1.8
Trigo duro	16	1.3	30	83	350	3.1
Sorgo	3	1.1	19	24	35	0.3
Mijo	51	20	149	280	288	6.6
Centeno	35	4.4	133	357	362	3.1
Cebada	74	12.8	197	457	457	7.4
Avena	52	3.8	110	350	N.I.	3.3
Quinoa	27-149	1.1-16.7	207-502	656-1475	140-530	0.8-4.8
Amaranto	175-206	12-17	254-266	290-434	441-455	3.7-5.2
Trigo sarraceno	46-50	12-15	390	450	330-395	2.1-2.4

N.I.: No informado. Adaptado de: (Martínez-Villaluenga et al., 2020; McKevith, 2004; Ragaee et al., 2006).

Tabla 1.9. Contenido de vitaminas en algunos cereales y pseudocereales expresado en mg/100 g.

	Tiamina (B1)	Riboflavina (B2)	Niacina (B3)	Vitamina E
Arroz integral	0.59	0.07	4.36	0.80
Trigo integral	0.38	0.11	4.38	1.40
Mijo	0.31	0.08	4.29	Trazas
Centeno	0.40	0.22	4.17	1.60
Cebada	0.36	0.14	4.07	0.40
Avena	0.90	0.09	1.03	1.5
Quinoa	0.3-0.4	0.3-0.4	1.1-1.5	2.5
Amaranto	0.01-0.1	0.04-0.41	< 0.01-8.04	1.5
Trigo sarraceno	0.1-3.3	0.06-10.6	2.1-18.0	0.9-1.6

Adaptado de: (Belitz et al., 2009c; Çatak, 2019; Erbas et al., 2005; Garg et al., 2021; Hall et al., 2017; Lebiedzińska & Szefer, 2006).

Tabla 1.10. Contenido de compuestos fenólicos identificados en algunas legumbres expresado en mg/100 g.

	Arroz	Trigo	Centeno	Avena	Quinoa	Amaranto
Ácidos fenólicos						
Gálico	N.I.	0.1-3.7	N.I.	0.2-4.7	32	40-44
Hidroxibenzóico	0.7-1.4	0.1-8.2	N.I.	N.I.	1.9-7.7	0.8-3.7
Vanílico	0.1-0.3	3-7	2-3	0.1-1	4.3-14.6	1.5-6.7
Siríngico	-	0.1-6.2	2-3.9	N.I.	N.D.	N.D.
Cafeico	0.1-0.5	0.2-9	N.I.	1-2.7	0.25-4	0.7-1.1
Cumárico	N.I.	0.1-6.3	2.8-5.1	2-2.8	2.3-27.5	0.4-0.99
Ferúlico	0.1-2.6	27-145	72-104	1-2.8	12-20	6.2-8.3
Cinámico	N.I.	0.3-8.3	N.I.	N.I.	1	N.D.
Flavonoides						
Catequina	0-1.39	0.83-1.79	Trazas	0.56	N.I.	N.I.
Epicatequina	0.34-1.41	Trazas	Trazas	Trazas	N.I.	N.I.
Hesperidina	Trazas	< 0.01-0.02	N.I.	N.I.	0.19	N.D.
Vitexina	1863-1965	0.89-2.66	N.I.	Trazas	70	41
Isovitexina	0-1320	Trazas	N.I.	Trazas	N.D	26.6
Quercetina	0-1.87	1.96-10.48	Trazas	10.18	11.6-53.2	21-84
kaempferol	0-0.38	1.04-2.27	Trazas	0.97	0.5-54.2	2.24-5.97
Rutina	0.24-0.38	0.63-1.45	Trazas	0.3	36	N.D.

N.I.: no informado; N.D.: no detectado. Adaptado de: (Paśko et al., 2008; Repo-Carrasco-Valencia et al., 2010a; Stuper-Szablewska & Perkowski, 2019; Tiozon et al., 2022).

Tabla 1.11. Contenido de antinutrientes en algunos cereales y pseudocereales.

	Ácido fítico (mg/g)	**Taninos (mg/g)**	**Saponinas (mg/g)**	**Act. inhibidora de tripsina (U/mg; U/mL*; mg/g**)**
Maíz	0.87-6.83	N.I.	N.I.	N.I.
Trigo	7.95-8	1.43-1.84	N.I.	0.11**
Arroz	0.93	N.I.	N.I.	N.I.
Mijo	0.05-0.08	0.003-0.005	N.I.	N.I.
Sorgo	0.03	0.006	N.I.	N.I.
Quinoa	10.5-13.5	0-0.5	0.3-20.5	1.36-5.04*
Amaranto	2.9-7.9	0.4-5.2	0.001-0.9	3.05-4.34
Trigo sarraceno	35-38	4-16	N.I.	N.I.

N.I.: no informado. Adaptado de: (L'Hocine et al., 2023; Samtiya et al., 2020; Schoenlechner et al., 2008; P. Thakur et al., 2021).

La quinoa destaca de forma significativa en comparación con otros cereales y pseudocereales debido a sus diversas bondades nutricionales y beneficios para la salud. En primer lugar, la quinoa posee una proteína que se acerca al patrón recomendado por la FAO en términos de aminoácidos esenciales (Schoenlechner et al., 2008). También, es una fuente importante de fibra dietaria, lo que favorece a la digestión y a mantener un sistema digestivo saludable. Además, su ausencia de gluten la convierte en una opción ideal para personas que padecen celiaquía (P. Thakur et al., 2021). Por otro lado, tiene un contenido importante de minerales como el magnesio, potasio, calcio y fósforo, además de ser una excelente fuente de vitaminas del grupo B y vitamina E.

1.3.3. Beneficios y desventajas nutricionales derivados de la ingesta de proteínas vegetales

Beneficios

A continuación, se presentan algunos de los beneficios para la salud asociados a la ingesta de alimentos provenientes de fuentes vegetales (Ewy et al., 2022; Hertzler et al., 2020; Martín-Cabrejas, 2019):

- La inclusión de proteínas vegetales en la dieta reduce los riesgos de enfermedades cardiovasculares en adultos.
- Consumir una cantidad adecuada de fibra en la dieta ayuda a mantener una salud metabólica óptima, ya que aumenta la saciedad y previene la obesidad en personas jóvenes y adultas. Además, mejora la salud del tracto gastrointestinal y promueve la diversidad del microbioma.
- Una alimentación basada en vegetales proporciona nitratos que se transforman en óxido nítrico dentro del organismo. Esto contribuye a regular la presión arterial, relajar los vasos sanguíneos y mejorar el flujo de sangre.
- Las propiedades antioxidantes y antiinflamatorias de los productos vegetales protegen al cuerpo contra la isquemia y la lesión por reperfusión.
- Mejora de los niveles de colesterol a través del alto contenido de esteroles y ácidos grasos poliinsaturados en alimentos vegetales.
- La interacción sinérgica de la fibra, los fitoquímicos y las proteínas presentes en las dietas ricas en alimentos vegetales integrales contribuye a la protección y mantenimiento de la función renal.
- El consumo de proteínas vegetales se ha relacionado con una reducción en la mortalidad. Reemplazar tan solo un 3% de proteínas de origen animal por

proteínas vegetales se asocia con una reducción del 10% en la mortalidad general (Huang et al., 2020).

Desventajas

Entre las posibles desventajas para la salud relacionadas con el consumo de proteínas de origen vegetal, cabe mencionar:

- Las fuentes vegetales de proteínas tienen una composición de aminoácidos diferente a las fuentes animales en las dietas a base de vegetales. Es importante incluir una variedad de alimentos vegetales ricos en proteínas para obtener los aminoácidos esenciales necesarios para la síntesis de proteínas en el cuerpo. De esta manera, se garantiza una cantidad suficiente de aminoácidos necesarios para la síntesis de proteínas en el organismo (Ewy et al., 2022).
- La digestibilidad de las proteínas provenientes de fuentes vegetales se reduce debido a la existencia de factores antinutricionales. Sin embargo, una vez que las fuentes de proteínas vegetales son libres de estos compuestos antinutricionales, pueden presentar una tasa de digestibilidad comparable a las proteínas de origen animal (Ewy et al., 2022; van Vliet et al., 2015).
- El consumo exclusivo de fuentes vegetales puede llevar a deficiencias de vitaminas B12 y D, así como de calcio y zinc si la dieta no es variada y complementada. Esto puede aumentar el riesgo de osteoporosis, pero se puede reducir ajustando la dieta para incluir más minerales. Además, se recomienda el consumo de alimentos vegetales fortificados y suplementos multivitamínicos/minerales para asegurar una ingesta adecuada de estos nutrientes esenciales (Craig, 2010; Ewy et al., 2022).

1.3.4. Desarrollo de alimentos basados en proteínas vegetales

En la actualidad, existe un creciente interés por la investigación y desarrollo de alimentos que se derivan de proteínas vegetales, motivado por su destacado valor nutricional y su carácter sostenible en términos de producción. Se ha utilizado legumbres, cereales y pseudocereales tanto de forma individual como en combinaciones para la creación de una variedad de productos de panadería, pastas, bebidas, entre otros. En las Tablas 1.12 y 1.13 se presenta una breve revisión de estudios en los que se ha evaluado la incorporación de harinas de legumbres (Tabla 1.12) o de cereales/pseudocereales (Tabla 1.13) en su formulación.

Aunque hay diversas propuestas, es importante destacar que la industrialización de estos productos enfrenta limitaciones debido a las barreras tecnológicas que surgen al reemplazar los productos vegetales convencionales, como el trigo, por alternativas no convencionales basadas en legumbres, cereales y pseudocereales. Primero, la presencia de factores antinutricionales, que como se mencionó anteriormente, pueden unirse a otros nutrientes dificultando su digestión y biodisponibilidad. No obstante, los componentes antinutricionales pueden reducirse utilizando diferentes métodos como se describe en la Tabla 1.14. Además de los componentes antinutricionales, el sabor amargo distintivo que tienen las legumbres, que puede variar según el tipo, tiene un impacto considerable en su aceptación y, en consecuencia, su consumo. Por esta razón, tradicionalmente se han utilizado métodos como la fermentación y la germinación para mejorar tanto las características nutricionales como sensoriales de las legumbres, ya que estos procesos generan compuestos aromáticos y azúcares que mejoran su olor y sabor (Bresciani & Marti, 2019). Finalmente, otro problema importante que surge de la incorporación de legumbres o cereales no convencionales son los de carácter

tecnológico. La sensación en la boca, la textura de la miga, el volumen de la hogaza, la apariencia del pan y la vida útil se ven afectadas por el reemplazo de un 20% de harina de trigo por harinas no convencionales. La formación de la red de gluten disminuye cuando se incorporan sustitutos del trigo, ya que el gluten no solo se diluye, sino que se produce una competencia por el agua entre las proteínas del trigo (gliadina y glutenina) y las de las legumbres (albúmina y globulina), lo que impide que se forme adecuadamente esta red de gluten. Además, la fibra de legumbres también afecta la formación de las hebras de gluten-gliadina, ya que también compite por el agua, dificultando así la formación de la red elástica (Bresciani & Marti, 2019; Siddiqui et al., 2022). Se han realizado varias investigaciones para mitigar los aspectos funcionales y sensoriales de los sustitutos del trigo con altos niveles de adición. Se ha encontrado que la solución consiste en agregar gluten vital o agentes texturizantes (aditivos químicos, enzimas, emulsionantes e hidrocoloides) que imiten las características del gluten, creando una masa cohesiva y viscoelástica (Siddiqui et al., 2022).

Tabla 1.12. Desarrollos de productos con incorporación de harinas de legumbres en su formulación.

Alimento	Material vegetal	Referencia
Galleta	100% harina de garbanzo, lenteja verde y roja, guisante amarillo, fríjol pinto y blanco, y 100% aislado de proteína, almidón y fibra de guisante	(J. Han et al., 2010)
	25, 50, 75, 100% harina de guisante, lenteja verde, judía blanca y judía pinta en sustitución de harina de trigo	(Zucco et al., 2011)
	5, 10, 15% harina de soja, garbanzo y lupino en sustitución de harina de trigo	(Hegazy & Faheid, 1990)
Pan	10, 20, 30% harina de garbanzo en sustitución por harina de trigo	(Mohammed et al., 2012)

	10, 20, 30% harina de lenteja y judía en sustitución de harina de trigo	(Kohajdová et al., 2013)
	18-54% harina de garbanzo, guisante y soja en sustitución de harina de trigo	(Angioloni, 2012)
Bizcocho	25 y 50% harina y fracciones (almidón, proteína) de guisantes en sustitución de harina de trigo	(Gómez et al., 2012)
	50 y 100% harina de garbanzo en sustitución de harina de trigo	(Gómez et al., 2008)
Pasta	35% harina de guisante y haba en sustitución por sémola de trigo, y 100% harina de guisante y haba	(Petitot et al., 2010)
	Proporción 70:30 harina de maíz: haba	(Giménez et al., 2013)
	100% harina y almidón de haba	(Rosa-Sibakov et al., 2016)
	100% okara (residuo del extracto hidrosoluble de soja)	(Guimarães et al., 2018)
Bebidas	10% (p/v) granos secos de garbanzo y lupino en agua	(Lopes et al., 2020)
	Bebida de soja en polvo preconcentrado (22% sólidos totales) y secado por aspersión	(Giri et al., 2017)
	12.5% (p/v) granos de soja en agua	(Achouri et al., 2007)
Aceite	Lípidos de soja (21.3%) y maní (49.7%)	(Pattee et al., 1983)
Hamburguesa	Texturizado con proteína de guisante enriquecida	(Peñaranda et al., 2023)
Tofu	Soja coagulada y deshidratada	(Das et al., 2022)
Tempeh	Soja fermentada de forma natural con forma de pastel	(Das et al., 2022)
Leblebi	Garbanzos asados	(Coşkuner & Karababa, 2007)

Tabla 1.13. Desarrollos de productos con incorporación de harinas de cereales y pseudocereales en su formulación.

Alimento	Material vegetal	Referencia
Magdalenas	25, 50, 75 y 100% harina de quinoa en sustitución de harina de arroz	(Bhaduri, 2013)
	0–50% harina de amaranto en sustitución de harina de arroz negro	(Bhatt et al., 2021)
Galleta	50:50 harina de trigo sarraceno-maíz y trigo sarraceno-arroz, y 50:25:25 harina de trigo sarraceno-maíz-arroz	(Altindag et al., 2015)
	30% harina de quinoa, 25% hojuelas de quinoa y 45% almidón de maíz	(Brito et al., 2015)
	10, 20 y 30% de trigo sarraceno en sustitución de harina de arroz	(Torbica et al., 2012)
Pan	100% harina de trigo sarraceno, avena, quinoa, sorgo, teff	(Wolter et al., 2013)
	60–70% harina de amaranto reventada y 30–40% harina de amaranto cruda	(María et al., 2010)
	40–100% de harina blanca de quinoa en sustitución de harina de arroz y maíz	(Elgeti et al., 2014)
Pasta	10% harina de amaranto, 40% harina de quinoa y 50% harina de arroz	(Makdoud & Rosentrater, 2017)
	60% harinas integrales de diferentes cereales (trigo, avena, centeno, cebada y arroz) a sémola de trigo duro	(Durazzo et al., 2013)
	100% harina de avena y teff	(Hager et al., 2012)
Bebidas	Kunu elaborado con uno o más cereales (mijo, sorgo, maíz, arroz y acha) en proporción 1:2	(Gaffa et al., 2002)
	25–35% p/p suspensión de leche de avena	(Deswal et al., 2014)
Helado	Helado tipo yogurt proporción 4:2:1 harina de arroz, lenteja y garbanzos	(Pontonio et al., 2022)
Hamburguesa	Quinoa, lupino y maíz en diferentes proporciones	(Chilón-Llico et al., 2022)

Tabla 1.14. Métodos para la eliminación de factores antinutricionales.

Método	Breve descripción
Procesamiento físico:	
Descascarillado	Eliminación de la cubierta de la semilla.
Remojo	Exposición a agua, solución salina y soluciones ácidas y alcalinas.
Tratamiento térmico	Cocción ordinaria, cocción a presión (esterilización en autoclave, olla a presión) >100 °C. El remojo y la cocción sucesivos son más efectivos para reducir los antinutrientes que la cocción sola.
Extrusión	Procesado a alta temperatura y corta duración.
Tostado	Calentamiento en seco a 120 °C-250 °C.
Irradiación	Un nivel de hasta 10 kGy es eficaz para inactivar o descomponer los inhibidores de la proteasa, la lectina, el ácido fítico y los oligosacáridos.
Bioprocesamiento:	
Germinación	Activación de enzimas que reducen el contenido de antinutrientes. Durante la germinación se liberan nutrientes de reserva y aumentan los contenidos de vitaminas y minerales.
Fermentación	Modificación bioquímica de los productos alimenticios primarios provocada por las actividades de los microorganismos y sus enzimas. La fermentación, proporciona una mejor digestibilidad, disponibilidad de micronutrientes, vitaminas, aminoácidos esenciales y antinutrientes reducidos.

Adaptado de: (A. Sharma, 2021; A. Thakur et al., 2019)

1.4. Fermentación

La fermentación es un proceso biológico en el que microorganismos como levaduras, bacterias y hongos convierten sustratos complejos en compuestos simples que son útiles para los humanos a escala industrial. Los principales resultantes de la biotransformación son ácidos orgánicos y alcohol que se generan por los cambios bioquímicos de los sustratos fermentados (Adebo et al., 2017; R. Joshi et al., 2018). Además, se producen metabolitos secundarios que son conocidos como compuestos bioactivos y que tienen propiedades biológicas. Estos compuestos incluyen compuestos fenólicos, compuestos aromáticos, pigmentos alimentarios, antibióticos y alcaloides, entre otros (R. Joshi et al., 2018; Kumar et al., 2021). Como consecuencia de la fermentación, se obtienen fermentados que presentan un mayor valor nutricional y/o mejor digestibilidad, además de poseer una textura, aroma y sabor distintos a los sustratos originales (Garrido-Galand et al., 2021).

Los procesos de fermentación se clasifican principalmente en:

- ***Fermentación en estado sólido (FES):*** La FES es un proceso que ocurre en un medio sólido natural con baja humedad, permitiendo reutilizar sustratos ricos en nutrientes. Aunque es un proceso lento, permite una liberación controlada de nutrientes. Es una tecnología de fermentación ideal para microrganismos como hongos y levaduras, pero no es adecuada para bacterias que requieren mayor contenido de agua (Fonseca-Hernández et al., 2022; R. Joshi et al., 2018).

- ***Fermentación sumergida (FSm):*** En la FSm los microorganismos se cultivan en un medio líquido utilizando caldos como sustratos. Durante este proceso, los compuestos bioactivos producidos se liberan en el medio de cultivo, lo

que facilita su separación y purificación. Este método es particularmente adecuado para microorganismos que requieren altos niveles de humedad, como las bacterias. La FSm se emplea ampliamente para mejorar el valor nutricional de alimentos específicos, incluyendo jugos y bebidas derivadas de legumbres y leguminosas (Fonseca-Hernández et al., 2022).

1.4.1. Fermentación en estado sólido (FES)

La fermentación en estado sólido (SSF) es un proceso biotecnológico en el que los organismos crecen en materiales no solubles o sustratos sólidos con muy poca o ninguna presencia de agua libre (Sadh et al., 2018). La FES se diferencia de la FSm principalmente por presentar una baja actividad de agua, lo que conlleva a notables diferencias en las características de este sistema en comparación con la fermentación que se realiza en medios líquidos (Tabla 1.15). Además, la FES presenta una serie de ventajas frente a la FSm tales como (Garrido-Galand et al., 2021; Paulová et al., 2013):

- El uso de un medio concentrado, lo que implica un reactor más pequeño y menores costos de inversión.
- Existe un menor riesgo de contaminación debido a los bajos niveles de humedad y la complejidad del sustrato.
- La simplicidad de la tecnología, bajo consumo de agua y baja generación de aguas residuales.
- Mayor rendimiento del producto y una recuperación más fácil del producto.
- La posibilidad de utilizar desechos agrícolas como sustratos para aplicaciones específicas.

Tabla 1.15. Principales diferencias entre la fermentación en estado sólido (FES) y la fermentación sumergida (FSm).

Fermentación en estado sólido	Fermentación sumergida
Bajo contenido de agua del medio de cultivo (40%–80%).	Medio de cultivo líquido (∼95% de contenido de agua)
Sistema trifásico: gas-líquido-sólido	Sistema bifásico: gas-líquido
Sustrato complejo insoluble en agua, alta concentración local de nutrientes	Los nutrientes se disuelven en agua, la concentración de nutrientes en menor
Sistema no homogéneo, gradiente de nutrientes	Sistema homogéneo
Los microorganismos se cultivan en la superficie del sustrato sólido	Los microorganismos se cultivan en el medio líquido
Transferencia de oxígeno gas-líquido y líquido-sólido	Transferencia de oxígeno gas-líquido
Limitaciones en la transferencia de calor, oxígeno y nutrientes	Los procesos de transporte generalmente no están limitados (la excepción puede ser la transferencia de oxígeno)
El calor se elimina utilizando una corriente de aire o colocando el biorreactor en una cámara de temperatura controlada	El enfriamiento se logra mediante el sistema de enfriamiento de la camisa del biorreactor
El monitoreo y el control del proceso son difíciles	La supervisión y el control en línea del proceso son habituales
Alta concentración del producto	El producto se disuelve en la fase líquida

Adaptado de: (R. Joshi et al., 2018; Paulová et al., 2013).

1.4.2. Microorganismos utilizados en la FES

Los microorganismos más utilizados en la fermentación son hongos y bacterias. Los hongos filamentosos son especialmente importantes, ideales y altamente adaptados para la FES. Su modo de crecimiento en forma de hifas y su capacidad para tolerar condiciones de baja actividad de agua y alta presión osmótica les brindan ventajas significativas sobre los microorganismos unicelulares al colonizar sustratos sólidos y aprovechar los nutrientes disponibles (Krishna, 2005). Sin embargo, las bacterias y las levaduras, a pesar de requerir un contenido de humedad relativamente más elevado para llevar a cabo una fermentación eficiente, también pueden emplearse en la FES, pero con menor rendimiento. Los microorganismos utilizados en la FES pueden presentarse como cultivos puros individuales, cultivos mixtos identificables o un consorcio de microorganismos autóctonos mixtos (Sadh et al., 2018). La Tabla 1.16. muestra diferentes microorganismos tales como hongos, levaduras y bacterias utilizados en los procesos de FES y los principales resultados obtenidos a nivel nutricional y funcional.

Los hongos son considerados una fuente de alimento con un valor incalculable por su calidad nutricional (Espinosa-Páez et al., 2017). El hongo *P. ostreatus* en particular, se considera que es rico en proteínas, fibra, carbohidratos, minerales y vitaminas, y bajo en grasas. Además, este hongo posee un sabor y propiedades aromáticas únicas que lo hacen especialmente atractivo (Deepalakshmi & Sankaran, 2014).

Tabla 1.16. Tipos de microrganismos utilizados en la fermentación en estado sólido (FES).

Microorganismo	Sustrato	Principales resultados
	LEGUMBRES	
Hongos *Aspergillus oryzae* *Pleurotus ostreatus* *Rhizopus oligosporus*	Harina de semilla de judía ojo negro (*Vigna unguiculata*) Judía riñón (*Phaselous vulgaris*) Judía negra (*Phaselous vulgaris*) Lenteja (*Lens culinaris*) Judía térapi (*Phaseolus acutifolius*)	↑ proteína, fibra dietética en judía riñón, ↓ lípidos, fibra dietética en judía negra, ↓ carbohidratos en lenteja. ↑ AAE, AAL, CFT, isoflavonas, contenido de minerales. ↑ digestibilidad de proteínas, biodisponibilidad mineral, AAox, ↓ taninos. ↑ CRA, CUA, propiedades emulsionantes, ↓ densidad aparente.
Bacterias *Lacticaseibacillus casei*	Harina de soja integral	↑ proteína, grasa y fibra cruda, ácidos grasos ω-3. ↑ AAE, AAL, ácidos fenólicos, isoflavonas. ↑ AAox, ↓AIT y actividad lipoxigenasa.
Co-cultivo *Aspergillus sojae + Aspergillus ficuum* *Pediococcus pentosaceus + Pediococcus acidilactici + Pediococcus lolii*	Harina de lupino Harina de garbanzos	↑ contenido mineral, CFT, ↓ pH. ↑ DPIV, ↓ rafinosa y estaquiosa, ↓ ácido fítico. ↑ CRA, ↓ capacidad espumante, ↑ olores más suaves y ácidos, ↓ olor a judía.
	CEREALES	
Levaduras *Saccharomyces cerevisiae*	Harina mixta de arroz y judía mungo negra	↑ contenido de proteínas, ↓ azúcares fermentables. ↑ CFT, acidez titulable, ↓ contenido de humedad, pH.

		↑ AAox. ↑ propiedades de textura y sensación en boca, optimización de las condiciones de procesamiento.
Hongos *Helvella lacunosa X1* *Agaricus bisporus AS2796* *Fomitiporia yanbeiensis G1* *Aspergillus oryzae* *Cordyceps militaris* *Rhizopus oligosporus* *Lentinula edodes*	Avena Quinoa Trigo Arroz Maíz Mijo Trigo sarraceno Sorgo Arroz integral	↑ contenido de proteínas, contenido de grasas, azúcares reductores, ↓ fibra dietética. ↑ TFC, ácidos fenólicos, avenatramidas, ↓ compuestos fenólicos conjugados. ↑ digestibilidad de proteínas, AAox, actividad de fitasa, endocelulasa y polifenol oxidasa, ↓ taninos y ácido fítico, actividad de lipasa. ↑ CRA, luminosidad en arroz y trigo, actividad antiobesidad *in vitro* en trigo, actividad de amilasa, xilanasa y β-glucosidasa en arroz, protección contra daños en el ADN en avena
Bacteria *Bifidobacterium spp. B. animalis, B. breve and B. longum*	Harinas de quinoa y trigo	↑ %DH, péptidos de pequeño tamaño (<6 kDa), CFT, ↓ pH. ↑ AAox, actividad inhibidora de la ECA, inhibición de la actividad de la α-glucosidasa y la α-amilasa.
Co-cultivo *Rhizopusoryzae + Lactiplantibacillus plantarum*	Cebada descascarillada Avena integral	↑ proteína soluble, péptidos de pequeño tamaño, azúcares reductores. ↑ nitrógeno aminoácido, TFC, FPC, ↓ pH. ↑ DPPH, actividad de eliminación de radicales ABTS, actividad de amilasa y proteasa. ↑ solubilidad de proteínas, formación de aromas, actividad

	inhibidora de la ECA, enriquecimiento de microorganismos probióticos.

AAE: Aminoácidos esenciales; **AAL:** Aminoácidos libres; **CFT:** Contenido fenólico total; **CRA:** Capacidad de retención de agua; **CUA:** Capacidad de unión de aceite; **AAox:** Actividad antioxidante; **AIT:** Actividad inhibidora de la tripsina; **DPIV:** Digestibilidad de proteínas *in vitro*.
Adaptado de: (Garrido-Galand et al., 2021)

1.4.3. Cambios en las propiedades nutricionales durante la FES y su potencial beneficio para la salud

Durante la fermentación, los microorganismos realizan una serie de transformaciones bioquímicas mejorando el valor nutricional, las propiedades funcionales y la digestibilidad de nutrientes, mientras que disminuyen compuestos antinutrientes como los inhibidores de la proteasa, lectinas, oligosacáridos, fitatos, taninos, entre otros. Se pueden obtener diferentes compuestos con actividad biológica y beneficios para la salud, dependiendo del tipo de microorganismo y material vegetal utilizado en dicha fermentación. Algunos de los compuestos bioactivos más comunes incluyen péptidos, polifenoles, ácidos grasos, vitaminas, carotenoides, fitoesteroles, entre otros (Fonseca-Hernández et al., 2022).

La influencia de la fermentación en el contenido de proteínas ha generado resultados inconsistentes debido a la variabilidad en los diseños experimentales y la combinación sustrato-cepa. Algunos estudios han observado aumentos en proteínas y aminoácidos, mientras que otros han reportado disminuciones. El aumento del contenido proteico es mayor en sustratos ricos en fibra, pero no es absoluto; mientras que las reducciones podrían atribuirse al hecho de que los microorganismos fermentadores utilizan aminoácidos como fuente de nitrógeno, lo que afecta negativamente la calidad de los alimentos fermentados. Por otro lado, la mejora en

la bioaccesibilidad de las proteínas inducida por la fermentación se atribuye principalmente a la digestión de factores antinutricionales y macromoléculas en pequeñas moléculas de proteínas, péptidos y aminoácidos libres (Nkhata et al., 2018; J. Wang et al., 2023).

Los péptidos bioactivos son producidos a través de la hidrólisis de proteínas mediante la acción de proteasas sintetizadas por microorganismos en procesos de fermentación. Estos péptidos son objeto de gran interés debido a sus múltiples beneficios para la salud. Hasta ahora, se han descrito péptidos con propiedades antihipertensivas, anticancerígenas, antiinflamatorias, antidiabéticas, inhibidoras de la ECA, antimicrobianas, antiadipogénicas, antimutagénicas, antitrombóticas y antiaterogénicas. Los péptidos bioactivos más estudiados son los péptidos inhibidores de la enzima convertidora de angiotensina-I (ECA) (Diez-Ozaeta & Astiazaran, 2022). La ECA en los pulmones convierte angiotensina I en angiotensina II, que es considerada el principal factor que aumenta la presión arterial (Diez-Ozaeta & Astiazaran, 2022; Niarchos et al., 1979). Al inhibir la ECA, se reduce la formación de angiotensina II, lo que ayuda a aliviar la presión arterial (Diez-Ozaeta & Astiazaran, 2022).

De manera similar a las proteínas, la fermentación ha demostrado variaciones en los contenidos de carbohidratos (como glucosa, almidón y fibra) y también en el contenido de grasa en fermentaciones que emplean diversos tipos de sustratos y cultivos iniciadores (Azeke et al., 2007; Olukomaiya et al., 2020). Esto sugiere que los microorganismos tienen la capacidad de utilizar diferentes nutrientes como fuentes de energía y que la elección adecuada del sustrato y la cepa es fundamental.

La biodisponibilidad de los minerales de origen vegetal es limitada debido a su unión con sustancias no digeribles, como los polisacáridos de la pared celular y el

ácido fítico. No obstante, mediante el proceso de fermentación, se liberan estos minerales complejos y los hace fácilmente biodisponibles (Nkhata et al., 2018).

Las propiedades antioxidantes de los alimentos fermentados se deben principalmente a la bioactividad de los compuestos fenólicos. La actividad de enzima β-glucosidasa de las cepas de fermentación transforman los compuestos fenólicos complejos o conjugados en compuestos activos más simples o libres, más bioaccesibles y biodisponibles (Diez-Ozaeta & Astiazaran, 2022; Gahlawat et al., 2017; Y. S. Zhao et al., 2021). Los compuestos fenólicos bioactivos post-fermentación han mostrado actividades anticancerígenas, antidiabéticas, antiinflamatorias y contra la obesidad (Leonard et al., 2021). Randhir & Shetty (2007) encontraron que la FES de judía mungo resultó en un aumento significativo del contenido fenólico, lo que mejoró su actividad antioxidante, lo que contribuyó a la inhibición de la α-amilasa (que es relevante para el control de la diabetes), así como a la inhibición del crecimiento de Helicobacter pylori (vinculado al tratamiento de la úlcera péptica) (Martins et al., 2011). También, ciertos estudios indican que no todas las fermentaciones contribuyen de manera beneficiosa a la actividad antioxidante ya que tanto el material vegetal como la selectividad del microorganismo utilizado pueden afectar a los resultados de la fermentación (Y. S. Zhao et al., 2021).

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2. OBJETIVOS

2. OBJETIVOS

El objetivo principal de la presente tesis es **aplicar la fermentación en estado sólido (FES) para la obtención de harinas de lenteja y quinoa fermentadas con digestibilidad y bioaccesibilidad mejoradas.**

Para el cumplimiento del objetivo principal, se plantean los siguientes objetivos específicos:

- Analizar el impacto del tamaño de partícula (grano/semilla y harina) durante la SSF con *P. ostreatus* sobre las propiedades nutricionales y funcionales de los sustratos fermentados de lenteja y quinoa.

- Determinar el efecto del tiempo de fermentación con *P. ostreatus* sobre las propiedades nutricionales y funcionales de los sustratos fermentados de lenteja y quinoa tanto en grano/semilla y harina.

- Evaluar el impacto de la FES sobre las propiedades nutricionales, funcionales y sensoriales de los granos de lenteja y las semillas de quinoa fermentadas con *P. ostreatus*.

- Determinar y modelizar la cinética de secado a distintas temperaturas de la lenteja y quinoa fermentadas con *P. ostreatus*.

- Evaluar el impacto de la temperatura de secado por aire caliente sobre las propiedades nutricionales, funcionales y sensoriales de la lenteja y la quinoa fermentadas con *P. ostreatus*.

OBJETIVOS

- Analizar el impacto de la FES sobre la digestibilidad de nutrientes, la bioaccesibilidad de compuestos bioactivos y las propiedades funcionales de los sustratos fermentados con *P. ostreatus*.

- Evaluar el impacto del secado y la molienda sobre la digestibilidad de nutrientes, la bioaccesibilidad de compuestos bioactivos y las propiedades funcionales de los sustratos fermentados con *P. ostreatus*.

- Determinar el efecto de las condiciones digestivas alteradas del adulto mayor, en comparación con las condiciones estándares (adulto sano), sobre la digestibilidad y propiedades funcionales de las harinas fermentadas con *P. ostreatus.*

3. PLAN DE TRABAJO

3. PLAN DE TRABAJO

El plan de trabajo propuesto para la consecución de los objetivos planteados se detalla a continuación:

3.1. Determinación del efecto del tiempo de fermentación y del tamaño de partícula del sustrato en lenteja y quinoa

- Análisis de la composición nutricional de la lenteja pardina y quinoa blanca tanto en grano/semilla y harinas.

- Fermentación en estado sólido (FES) sobre los sustratos en grano/semilla y harinas de lenteja pardina y quinoa blanca para evaluar el efecto del tamaño de partícula y del tiempo de fermentación sobre los cambios a nivel nutricional y funcional.

La Figura 1.1 muestra el proceso para la determinación del efecto del tiempo de la FES y del tamaño de partícula del sustrato.

3.2. Obtención y caracterización de harinas fermentadas de lenteja o quinoa

- Caracterización de la composición nutricional y compuestos bioactivos de los sustratos previamente a la fermentación. Se consideraron dos variedades de lentejas (Pardina y Castellana) en grano y dos variedades de quinoa (blanca y negra) en semilla.

- Fermentación en estado sólido (FES) de los sustratos seleccionados utilizando como cultivo iniciador el microorganismo *Pleurotus ostreatus*. La FES se realizó sobre las lentejas y quinoas en granos/semillas enteras.

- Acondicionamiento y estabilización de las lentejas y quinoas fermentadas aplicando operaciones unitarias de secado y molienda para la obtención de las harinas fermentadas. Se utilizaron dos métodos de secado, secado por

aire caliente a tres diferentes temperaturas (50, 60 y 70 °C) y la liofilización, este último reconocido como el mejor proceso para preservar la calidad de los alimentos. Así, las harinas liofilizadas obtenidas fueron consideradas como estándar de referencia.

La Figura 1.2 muestra el proceso para la obtención de las harinas fermentadas de lenteja (Pardina y Castellana) y quinoa (blanca y negra).

3.3. Digestión *in vitro* de las harinas fermentadas

- Selección de harinas fermentadas de lentejas y quinoas para llevar a cabo el proceso de simulación de la digestión *in vitro* basados en criterios de funcionalidad de la fracción proteica, el valor antioxidante y la reducción del contenido de antinutrientes.

- Digestión gastrointestinal de las harinas fermentadas seleccionadas para evaluar su digestibilidad y funcionalidad simulando el sistema de digestión *in vitro* de un adulto sano (estándar) y el de un adulto mayor, imitando las condiciones luminales gastrointestinales alteradas de las personas mayores.

La Figura 1.3 muestra el proceso para la digestión de muestras sin fermentar, fermentadas y fermentadas-secas de lenteja (Pardina y Castellana) y quinoa (blanca y negra).

Las siguientes determinaciones analíticas se realizaron para la caracterización de los sustratos no fermentados, fermentados y fermentados-deshidratados, y antes y después de la digestión gastrointestinal simulada en condiciones tanto de adulto sano (estándar) como de adulto mayor:

- *Composición proximal:* Humedad, proteína, grasa, cenizas, carbohidratos y fibra.
- *Contenido en azúcares reductores.*
- *Evaluación del crecimiento fúngico:* Biomasa fúngica.
- *Propiedades físicas y sensoriales:* ópticas, tamaño de partícula y perfil volátil.

- *Contenido y perfil proteico:* proteína soluble en TCA, péptidos bioactivos y perfil de aminoácidos.
- *Contenido en minerales de interés:* Fe, Ca y Mg.
- *Contenido en ácido fítico*.
- *Contenido y perfil fenólico:* perfil fenólico (libres y ligados), fenoles totales.
- *Propiedades funcionales:* Actividad antioxidante (ABTS, DPPH y FRAP) y actividad antihipertensiva (enzima convertidora de angiotensina (ECA)).

PLAN DE TRABAJO

Figura 1.1. Diagrama del plan de trabajo para la determinación del efecto del tiempo de fermentación y del tamaño de partícula del sustrato.

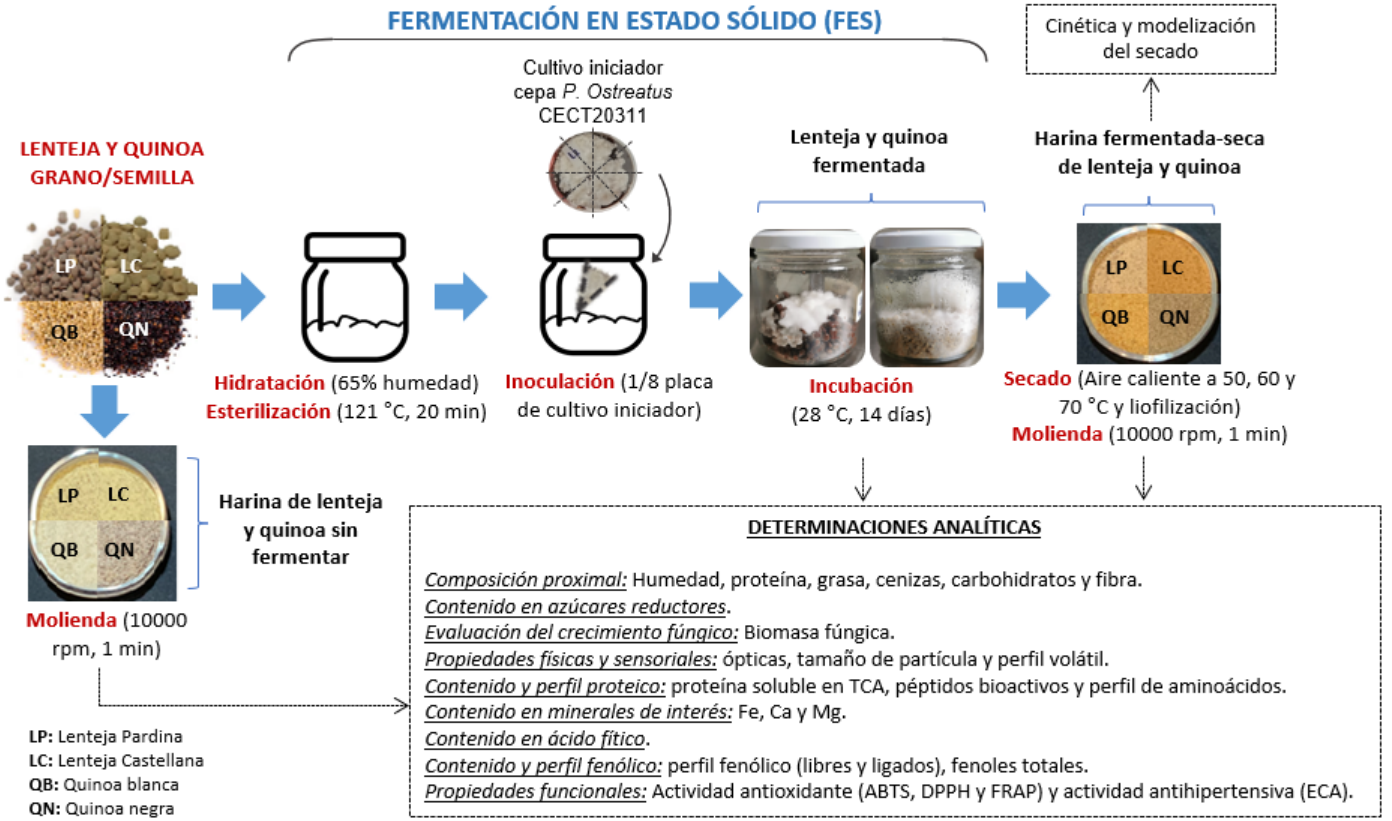

Figura 1.2. Diagrama del plan de trabajo para la obtención de harinas fermentadas de lenteja y quinoa, y evaluación del impacto del proceso sobre las propiedades nutricionales y funcionales.

PLAN DE TRABAJO

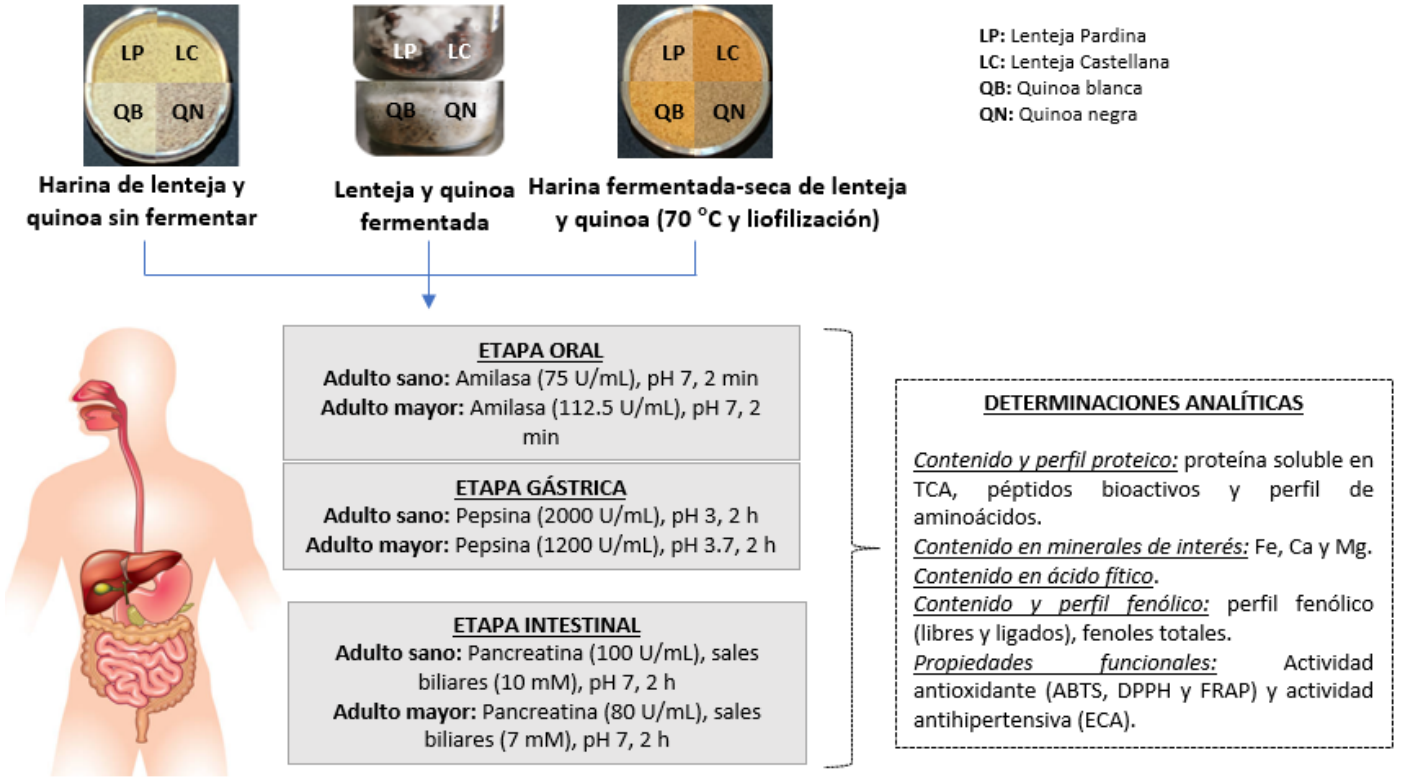

Figura 1.3. Diagrama del plan de trabajo para evaluar el impacto *in vitro* del proceso digestivo en condiciones simuladas de adulto sano (Brodkorb et al., 2019; Minekus et al., 2014) y mayor (Menard et al., 2023) en las muestras sin fermentar, fermentadas y fermentadas-secas de lenteja (Pardina y Castellana) y quinoa (blanca y negra).

4. METODOLOGÍA EXPERIMENTAL

4. METODOLOGÍA EXTERIMENTAL

4.1. Materias primas

Con la finalidad de evaluar el impacto del tiempo de fermentación y del tamaño de partícula de los sustratos iniciales, se adquirieron granos y harina de lenteja (*Lens culinaris*) de la variedad Pardina, además de semillas y harina de quinoa (*Chenopodium quinoa Wild*) blanca. Todos estos productos fueron suministrados por la empresa Molendum ingredients S.L.

Con el propósito de evaluar el efecto del secado a diferentes temperaturas (50, 60 y 70 °C) y la digestibilidad de las muestras fermentadas, se adquirieron granos de lenteja (*Lens culinaris*) de las variedades Pardina y Castellana de la marca Hacendado®, y semillas de quinoa (*Chenopodium quinoa Wild*) de las variedades blanca y negra de las marcas Hacendado® y Nut&me respectivamente. Estos productos fueron comprados en tiendas locales de Valencia (España).

La cepa *Pleurotus ostreatus* se obtuvo de la Colección Española de Cultivos Tipo (CECT20311).

4.2. Fermentación en estado sólido

Preparación del cultivo iniciador

El micelio de *Pleurotus ostreatus* procedente de un cultivo madre fue inoculado en placas Petri de agar malta elaborado con extracto de malta al 2%, glucosa al 2%, micopeptona al 0.1% y agar al 1.5%, y luego, se colocó en una incubadora (2001249, J.P. Selecta, Barcelona, España) a 28 °C durante 14 días. El micelio crecido se inoculó

en un caldo de cultivo elaborado con extracto de malta al 2%, glucosa al 2% y micopeptona al 0.1%, y se incubó a 28 °C durante 14 días.

Para la preparación del cultivo iniciador, 10 g de harina de lenteja (pardina o castellana) y quinoa (blanca o negra) fueron colocadas cada una en placas Petri, se hidrataron con agua hasta alcanzar un 65% de humedad y se esterilizaron en autoclave (4002136, J.P. Selecta, Barcelona, España) a 121 °C durante 20 min. Por último, se inoculó 1 mL del caldo de cultivo que contenía el micelio fúngico crecido y se incubó a 28 °C durante 14 días.

Proceso fermentativo

En primera instancia para evaluar el impacto del tiempo de fermentación y del tamaño de partícula de los sustratos iniciales, la FES se realizó colocando 35 g de lenteja pardina (grano o harina) y quinoa blanca (semilla o harina) hidratadas con agua hasta un 65% de humedad en frascos de vidrio, y esterilizados a 121 °C durante 20 min. Los sustratos se inocularon en los frascos de cristal añadiendo 1/8 del cultivo iniciador (placas Petri que contenían 10 g de sustrato colonizado dividido en 8 porciones) y luego se incubaron a 28 °C durante 14 días. Se tomaron tres frascos de vidrio (réplicas) a diferentes tiempos de fermentación (0, 2, 4, 6, 8, 10, 12 y 14 días) para realizar las determinaciones analíticas correspondientes.

En segunda instancia para evaluar el efecto del secado a diferentes temperaturas (50, 60 y 70 °C) y la digestibilidad de las muestras fermentadas, la FES se realizó colocando 35 g de granos de lenteja (Pardina o Castellana) y 35 g de semillas de quinoa (blanca o negra) y se siguieron los mismos pasos de hidratación, esterilización, inoculación e incubación como se indica en el párrafo anterior. Se inocularon varios frascos de vidrio para obtener suficiente sustrato fermentado para

realizar todos los ensayos incluyendo las curvas de secado. Los sustratos fermentados contenidos en los tarros de cristal se mezclaron para obtener una muestra homogénea para el posterior proceso de secado, digestión *in vitro* y determinaciones analíticas llevadas a cabo por triplicado.

4.3. Secado y modelización

4.3.1. Secado y molienda de los granos/semillas fermentadas

Las muestras se secaron por dos métodos de secado: aire caliente y liofilización. El secado por aire caliente se realizó utilizando un secador convectivo (Pol-Eko-Aparatura, CLW 750 TOP+, Kokoszycka, Polonia) a tres temperaturas de secado diferentes (50, 60 y 70 °C), la velocidad del aire fue de 10.5 ± 0.2 m/s y el porcentaje de humedad del aire fue de 23.2 ± 2.9, 14.2 ± 1.7 y 8.7 ± 1.2 para 50, 60 y 70 °C, respectivamente. La liofilización se llevó a cabo en un liofilizador (Telstar, Lyoquest-55, Terrassa, España) a -45 °C y 0.8 mBar durante 48 h.

La molienda tanto de las muestras sin fermentar como fermentadas-secas se realizó usando un robot de cocina (Thermomix®, TM6-1, Wuppertal, Alemania), aplicando 10000 rpm a intervalos de 15 s durante 1 min.

4.3.2. Cinética y modelización del secado

La cinética de secado se determinó midiendo la pérdida de masa a lo largo del secado a distintas temperaturas (50, 60 y 70 °C) con una balanza (Mettler Toledo, MS4002S). Se obtuvieron las curvas de secado de las muestras y se realizó un ajuste utilizando la ecuación del modelo de Lewis (Ecuación 4.1) para secado en bandejas, que es uno de los modelos matemáticos más utilizados en el proceso de secado de productos agrícolas (Chkir et al., 2015; Lewis, 1921).

$$\frac{X_t^w}{X_0^w} = e^{-k \cdot t} \qquad (4.1)$$

4.4. Análisis microbiológico

Se recogieron asépticamente muestras de las diferentes harinas para realizar los correspondientes análisis microbiológicos. Para ello, se diluyó 1 g de cada harina en 9 mL de agua destilada estéril y se realizaron diluciones decimales seriadas. Para determinar el recuento total de bacterias mesófilas aerobias, el recuento de mohos y levaduras y el recuento de *Escherichia coli*, se sembraron 0.1 mL de cada dilución seriada en agar Plate-count, agar dextrosa Sabouraud con 50 mg/L de Cloranfenicol y agar Triptona Bilis X-Glucurónido (medio cromogénico selectivo TBX) y se incubaron a 30 °C durante 72 h, 25 °C durante 5-7 días y 44 °C durante 24 h, respectivamente. Los análisis de detección de *Listeria monocytogenes* y *Salmonella spp.* se realizaron conforme a las normas ISO 6579-1:2017 e ISO 11290-1:2017.

4.5. Simulación de la digestión *in vitro*

Las muestras sin fermentar, fermentadas y fermentadas-secadas se digirieron bajo dos modelos estáticos de digestión *in vitro*: el modelo del adulto mayor (ancianos) (Menard et al., 2023) y el modelo del adulto sano (estándar) (Brodkorb et al., 2019; Minekus et al., 2014) como control (Tabla 4.1). Las actividades enzimáticas de las enzimas se determinaron antes de cada experimento de acuerdo a la información complementaria del protocolo publicado por Brodkorb et al. (2019), y se prepararon diariamente los fluidos salival (FSS), gástrico (FGS) e intestinal (FIS) simulados para el modelo de digestión estándar y de adulto mayor considerando las concentraciones de enzimas, sales biliares y pH de cada etapa digestiva.

Tabla 4.1. Condiciones gastrointestinales establecidas para un modelo de digestión *in vitro* para adulto sano (estándar) (Brodkorb et al., 2019; Minekus et al., 2014) y adulto mayor (Menard et al., 2023).

Etapa digestiva	Modelos de digestión	
	Adulto sano (estándar)	**Adulto mayor**
Etapa oral	Amilasa (75 U/mL) pH 7 2 min	**Amilasa (112.5 U/mL)** pH 7 2 min
Etapa gástrica	Pepsina (2000 U/mL) pH 3 2 h	**Pepsina (1200 U/mL)** **pH 3.7** 2h
Etapa intestinal	Pancreatina (100 U/mL) Sales biliares (10 mM) pH 7 2 h	**Pancreatina (80 U/mL)** **Sales biliares (7 mM)** pH 7 2 h

Las modificaciones realizadas en el modelo para adulto mayor en comparación con el adulto sano se destacan en negrita.

La digestión estática *in vitro* se llevó a cabo como se indica a continuación:

Etapa oral: Se mezclaron 5 g de muestra con 5 mL de FSS conteniendo la concentración de enzimas según el modelo de digestión (Tabla 4.1). Se ajustó el pH a 7 con NaOH 1M para ambos modelos, se mezcló a 25 rpm utilizando un Intelli-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Letonia) y se incubó en una cámara termostatada (JP Selecta SA, Barcelona) a 37 °C durante 2 min.

Etapa gástrica: Se añadieron 10 mL de FGS al bolo alimenticio según las condiciones simuladas en cada modelo (Tabla 4.1). Se ajustó el pH a 3 para el modelo estándar y a 3.7 para el modelo del adulto mayor con HCl 1M, se mezcló a 55 rpm y se incubó a 37 °C durante 2 h.

Etapa intestinal: Se añadieron 20 mL de FIS al quimo gástrico según la concentración de enzima y sales biliares (Tabla 4.1). Se ajustó el pH a 7 con NaOH 1M para ambos modelos, se mezcló a 55 rpm y se incubó a 37 °C durante 2 h.

Tras la digestión gastrointestinal, se inhibió la actividad enzimática ajustando el pH a 5 y manteniendo las muestras en un baño de hielo. Por último, las muestras se centrifugaron a 8000 ×*g* durante 10 min y se tomaron alícuotas de la fracción bioaccesible para las determinaciones analíticas.

4.6. Determinaciones analíticas

4.6.1. Composición proximal de los sustratos

La composición proximal de los sustratos se realizó según las metodologías normalizadas de la Association of Official Analytical Chemists (AOAC, 2000). Se analizaron los contenidos de humedad, proteínas, lípidos, cenizas e hidratos de carbono, este último por diferencia. Los contenidos de fibra total, soluble e insoluble se determinaron según los métodos AOAC Método 991.43 de la AOAC y método 32-07.01 de la AACC. Los resultados se expresaron en g/100 g de base seca.

4.6.2. Azúcares reductores

Para la determinación de azúcares reductores en la muestra sin digerir se utilizó la metodología propuesta por Miller (1959) y Sansano et al. (2015). Se preparó el reactivo DNS mezclando ácido 3.5-dinitrosalicílico 10 g/L, que contiene 300 g de tartrato de sodio y potasio tetrahidratado y 16 g de NaOH. Para elaborar el extracto, se mezclaron 0.3 g de muestra con 2 mL de etanol al 80%, se agitó en vórtex y se dejó reposar durante 30 min. Se centrifugó a 5000 ×*g* durante 5 min (5810R, Eppendorf, Hamburgo, Alemania). La extracción se repitió dos veces y los extractos se mezclaron.

Se tomó una alícuota de 500 µL y se mezcló con 1 mL de reactivo DNS. Se calentó en un baño de agua (J.P. Selecta, Barcelona, España) a 100 °C durante 5 min y luego se enfrió a temperatura ambiente. La muestra se diluyó con 6 mL de agua destilada y se midió la absorbancia a 546 nm (Helios Zeta UV-VIS Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). Los resultados se expresaron como g glucosa/100 g de base seca utilizando una curva estándar.

4.6.3. Biomasa fúngica

La biomasa producida por el hongo se determinó según el método publicado por Aidoo et al. (1981) y Tomaselli Scotti et al. (2001). El reactivo de Erhlick se preparó disolviendo 2.67 g de 4-dimetilamino benzaldehído en 100 mL de una mezcla 1:1 (v/v) de etanol grado reactivo y ácido clorhídrico concentrado. El reactivo de acetilacetona se preparó mezclando 1 mL de acetilacetona y 50 mL de carbonato de sodio 0.5 M. Para la determinación, se mezclaron 100 mg de muestra seca con 2.4 mL de ácido sulfúrico al 72% a 25 °C durante 24 h. Las muestras se diluyeron con 55 mL de agua destilada y se esterilizó la mezcla a 121 °C durante 2 h. A continuación, el hidrolizado se neutralizó a pH 7 con hidróxido de sodio 10 M y 0.5 M con un medidor de pH (Mettler-Toledo, SevenCompact S210). Se mezcló 1 mL del hidrolizado previamente neutralizado con 1 mL del reactivo acetilacetona en un tubo de vidrio con tapón, se calentó en un baño de agua hirviendo durante 20 min y luego, se dejó enfriar a temperatura ambiente. A continuación, se añadieron 6 mL de etanol seguidos de 1 mL del reactivo de Erhlick. La mezcla se incubó a 65 °C durante 10 min, se enfrió a temperatura ambiente y se midió la absorbancia a 530 nm en un espectrofotómetro (Beckman Coulter, DU 730). Los resultados se expresaron como mg de glucosamina/g de base seca.

4.6.4. Propiedades físicas y sensoriales

Propiedades ópticas

El color de las distintas harinas se realizó con un espectrocolorímetro (Minolta, CM-3600D), considerando un iluminante estándar D65 y un observador estándar de 10°. Se colocó la muestra en una cubeta plástica transparente de 20 mm de espesor y se utilizó un fondo negro para estandarizar las mediciones. Se midieron las coordenadas del espacio de color CIE-L*a*b*. El tono (h), el croma (C*) y las diferencias de color (ΔE) fueron calculadas de acuerdo a las ecuaciones 4.2, 4.3 y 4.4 respectivamente:

$$h_{ab} = arctg\left(\frac{b^*}{a^*}\right) \qquad (4.2)$$

$$C_{ab} = \sqrt{a^2 + b^2} \qquad (4.3)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (4.4)$$

Tamaño de partícula

El tamaño de las partículas se midió por vía seca utilizando un equipo de difracción láser (Mastersizer 2000, Malvern Instruments Limited). Los resultados se expresaron como la media ponderada del volumen D[4,3], la media ponderada de la superficie D[3,2] y el percentil del tamaño de las partículas d(0.5).

Perfil volátil (HS-SPME-GC-MS)

La determinación de compuestos volátiles se llevó a cabo en harinas de quinoa y lentejas sin fermentar, fermentadas y fermentadas-secas mediante una

microextracción en fase sólida (HS-SPME) y se analizó posteriormente mediante cromatografía de gases/espectrometría de masas (GC/MS) de acuerdo con la metodología descrita por Escriche et al. (2022).

Se añadieron 2.5 g de muestra y 7.5 mL de cloruro sódico al 20% a un vial de vidrio de 20 mL provisto de un tapón de rosca con un septo de PTFE-silicona y se homogeneizaron cuidadosamente con un mezclador de vórtice. La muestra se mantuvo caliente en una plataforma calefactora con agitación a 50 °C y 250 rpm durante 30 min para exponer los compuestos volátiles de la muestra en el espacio de cabeza del vial, y éstos se atraparon utilizando una fibra DVB/CAR/PDMS (divinilbenceno/carboxeno/polidimetilsiloxano, 50/30 µm). A continuación, la fibra se introdujo en el puerto de inyección del cromatógrafo GC/MS y los volátiles se desorbieron durante 30 min a 230 °C.

Los compuestos volátiles se analizaron utilizando un cromatógrafo de gases (Agilent, Intuvo 9000) acoplado a un detector de triple cuadrupolo (Agilent, Serie 7000 GC/TQ) equipado con una fuente de ionización de electrones a 70 eV. Para separar los compuestos volátiles se utilizó una columna capilar (Agilent, DB WAX, 30 m × 0.25 mm × 0.25 µm) con helio como gas portador a un caudal constante de 1 mL/min. El horno del GC se ajustó a una temperatura inicial de 35 °C durante 3 minutos, se elevó a 215 °C a una velocidad de 5 °C/min y, por último, se elevó a 250 °C a una velocidad de 30 °C/min, manteniéndose a esta temperatura durante 6 minutos. Los espectros de masas se obtuvieron en modo de cromatografía iónica total (TIC), explorando en el intervalo 40-280 m/z.

Para realizar el análisis de los datos y llevar a cabo la identificación de los compuestos volátiles se utilizó el software MassHunter Workstation (Unknown analysis). Los espectros de masas de cada compuesto se analizaron utilizando la

biblioteca espectral del NIST (NIST 17, National Institute of Standards and Technology), considerando siempre un factor de coincidencia ≥80%, y los índices de retención lineal (LRI). Los LRI de todos los compuestos se obtuvieron inyectando una mezcla de una serie homogénea de alcanos (C8-C20, Fluka Buchs, Schwiez, Suiza) bajo las mismas condiciones cromatográficas aplicadas a las muestras. La abundancia de cada compuesto se estimó considerando el área del pico base de deconvolución, correspondiente al valor medio de dos réplicas. Para facilitar la lectura de los datos, los resultados se presentan como área del pico/100000. La estimación de la cantidad total de compuestos volátiles se realizó sumando todas las áreas del cromatograma.

4.6.5. Caracterización de las proteínas

Proteína soluble en ácido tricloroacético (TCA)

La determinación de la proteína soluble en TCA de las muestras antes y después de la digestión *in vitro* se realizó siguiendo la metodología descrita por Hernández-Olivas et al. (2022) y Gallego et al. (2020). Para las muestras sin digerir, la extracción se realizó mezclando 100 mg de muestra con solución de TCA hasta una concentración final del 12% y se incubaron a 4 °C durante 15 min. A continuación, las muestras se centrifugaron (Eppendorf MiniSpin Plus) a 4200 ×*g* durante 10 min. Para las muestras digeridas, la determinación se realizó sobre la fracción bioaccesible.

El extracto/digerido se diluyó con tampón EDTA 50 mM y UREA 8M (pH 10) y la absorbancia se midió por espectrofotometría ultravioleta (Helios Zeta UV/Vis, Thermo Scientific) a 280 nm. Se utilizó una curva de calibración para la cuantificación utilizando tirosina como patrón. Los resultados se expresaron como g de proteína soluble en TCA/100 g de proteína.

Determinación de péptidos bioactivos

Las fracciones de péptidos bioactivos se determinaron por cromatografía por exclusión de tamaño (SEC) en las muestras antes y después de la digestión *in vitro*. Se realizó una doble extracción de la proteína de las muestras sin digerir mezclando 5 g de la muestra con 45 mL de agua destilada, se ajustó el pH a 11 y se centrifugó a 10000 ×*g* a 4 °C durante 20 min. Se mezclaron los sobrenadantes y se ajustó el pH al punto isoeléctrico (4.5), se mantuvo en agitación suave durante 1.5 h a 4 °C y se centrifugó a 10000 ×*g* a 4 °C durante 20 min. El sedimento se disolvió en tampón fosfato 50 mM, pH=7. Los extractos se analizaron inmediatamente; caso contrario, se almacenaron a -40 °C (Akillioğlu & Karakaya, 2009). Los extractos obtenidos y la fracción bioaccesible de las muestras digeridas fueron filtradas con un filtro hidrófilo de PVDF de 0.2 µm.

Los extractos/digeridos filtrados fueron analizados usando un HPLC-Alliance 2695, con un detector con arreglo de fotodiodos 2996 (Waters, EE.UU.) equipado con una columna Agilent Bio SEC-3 3 µm, 100A (7.8 x 300 mm) (Agilent, Palo Alto, CA, EE.UU.) de acuerdo a la metodología descrita por Zhuang et al. (2009). La fase móvil utilizada fue acetonitrilo (ACN): agua bidestilada en relación 1:1 con 1 mL/L de ácido trifluoracético. La temperatura de trabajo de la columna, la velocidad de flujo y el volumen de inyección fueron de 25 °C, 0.5 mL/min y 20 µL, respectivamente. Los compuestos fueron identificados a una longitud de onda de 220 nm. Se elaboró una curva de calibración del peso molecular de acuerdo a los tiempos de retención medios de los siguientes estándares de referencia: citocromo C (12.5 KDa), insulina (5.7 KDa), bacitracina (1.4 kDa), Gly-Gly-Tir-Arg (0.45 KDa) y triglicina (0.19 KDa). Los resultados se expresaron como g/100 g de proteína.

Perfil aminoacídico

La extracción de aminoácidos de las muestras sin digerir se llevó a cabo mediante tres diferentes hidrólisis (oxidación con ácido perfórmico, hidrólisis ácida e hidrólisis alcalina) de acuerdo a la metodología descrita por T. Lee et al. (2022) con algunas modificaciones. Para llevar a cabo la reacción de oxidación, se mezcló 0.2 g de muestra con 2 mL de ácido perfórmico (ácido fórmico y peróxido de hidrógeno en proporción 9:1 (v/v)) y se almacenó la mezcla a 0 °C durante 16 h. Se añadieron 0.4 mL de ácido bromhídrico y se almacenó a 4 °C durante 30 min. Las muestras fueron evaporadas utilizando un evaporador rotatorio (Heidolph) para eliminar el ácido perfórmico. A continuación, se llevó a cabo la hidrólisis ácida añadiendo 10 mL de HCl 6 M al mismo tubo, se selló y se calentó a 110 °C durante 24 h, y luego se dejó enfriar hasta temperatura ambiente. Para realizar la extracción alcalina, se mezcló 0.2 g de muestra con 10 mL de $LiOH.H_2O$ 4.3 M en un tubo sellado bajo atmósfera de nitrógeno a 120 °C durante 16 h, y luego, se dejó enfriar hasta temperatura ambiente. Los extractos obtenidos y la fracción bioaccesible de las muestras digeridas se filtraron con un filtro de acetato de celulosa de 0.2 µm. Se tomó una alícuota de 60 µL del extracto/digerido filtrado y se diluyó con 40 µL de estándar interno (ácido alfa aminobutírico) y 900 µL de agua bidestilada.

Para llevar a cabo el proceso de derivatización, se utilizó el kit AccQ-Tag de Waters. Se tomó una alícuota de 10 µL del extracto/digerido diluido que contiene el estándar interno en un tubo eppendorf y se le añadieron 70 µL de AccQ-Flour tampón borato y se mezcló en vortex brevemente. Posteriormente, se añadieron 20 µL del reactivo AccQ-Flour reconstituido y se mezcló inmediatamente en un vortex durante varios segundos. Se dejó reposar un minuto a temperatura ambiente y luego, se transfirió

el contenido a un inserto de volumen limitado para vial automuestreador. Se tapó el vial y se calentó en un bloque de calentamiento a 55 °C durante 10 min.

Las muestras derivatizadas se analizaron utilizando un HPLC 1200 Series Rapid Resolution acoplado a un detector de diodos Serie (Agilent, Palo Alto, CA, EE.UU.). La separación de aminoácidos se realizó usando una columna AccQ-Tag (3.9 x 150 mm) a 37 °C, con un caudal de 1 mL/min y volumen de inyección de 5 µL. La fase móvil A fue el Eluente A (90:10, agua bidestilada: AccQ-Tag Eluente A) y la fase móvil B fue Acetonitrilo (ACN) (60:40, ACN: agua bidestilada). Se estableció el siguiente programa de gradientes: 0 min, 100% A; 0.5 min, 98% A; 12 min, 95% A; 15 min, 93% A; 19 min, 90% A; 25 min, 67% A; 33 min, 67% A; 34 min, 0% A; 37 min, 0% A; 38 min, 100 % A; 42 min 100% A. La identificación de los compuestos desconocidos se realizó a una longitud de onda de 250 nm comparando los tiempos de retención cromatográficos resultantes con los estándares de referencia. Se utilizó una curva estándar para la cuantificación de los compuestos identificados y los resultados se expresaron como mg/g de proteína.

4.6.6. Determinación de minerales

Se realizó la determinación de minerales (Fe, Ca y Mg) antes y después de la digestión gastrointestinal mediante espectrometría de masas de plasma acoplado inductivamente (ICP-MS). El extracto de minerales se preparó según la metodología publicada por Barrera et al. (2009). Se pesaron 5 g de muestra para el alimento sin digerir y se tomó una alícuota de 3.5 mL de la fracción bioaccesible del alimento digerido. Se incineraron las muestras a 600 °C durante 10 h. Las cenizas se disolvieron con 1 mL de ácido nítrico al 69% y se volvieron a incinerar hasta obtener cenizas completamente blancas. Las cenizas blancas se suspendieron en 1.5 mL de ácido nítrico al 69% y 4 mL de agua bidestilada.

Las muestras se analizaron utilizando un ICP-MS equipado con un automuestreador (iCAP Q, Thermo, EE. UU.) de acuerdo a la metodología propuesta por Chen et al. (2020). Las condiciones de trabajo en el equipo fueron: Potencia de radiofrecuencia (1550 W), flujo de gas frío (14 L/min), flujo de gas auxiliar (0.8 L/min), flujo de gas nebulizador (1.08 L/min), velocidad de la bomba peristáltica (40 rpm), profundidad de muestreo (5 mm), temperatura de la cámara de pulverización (2.7 °C), tiempo de permanencia (20 ms). Los resultados se expresaron como µg/g.

4.6.7. Contenido en ácido fítico

El contenido de ácido fítico se midió en las muestras antes y después de la digestión *in vitro* siguiendo el protocolo descrito por (Haug & Lantzsch, 1983) y modificado por Peng et al. (2010). Se realizó una curva de calibración utilizando una solución de referencia de fitato (sal sódica hidratada de ácido fítico de arroz). Se preparó una solución madre con una concentración de ácido fítico de 1.3 mg/mL y se diluyó con HCl 0.2 M en un intervalo de 0.1-1 mL (3.16-31.6 µg/mL de fitato de fósforo). La solución férrica se preparó disolviendo 0.2 g sulfato de amonio y hierro(III) dodecahidrato en 100 mL de HCl 2 M y se completó hasta 1 L con agua destilada. La solución de 2,2'-bipiridina se preparó disolviendo 10 g de 2,2'-bipiridina y 10 mL de ácido tioglicólico en agua destilada y se completó hasta 1 L.

Para las muestras sin digerir, el extracto se preparó mezclando 50 mg de muestra con 10 mL de HCl 0.2 M y se dejó toda la noche a 4 °C. Para las muestras digeridas, la determinación se realizó en la fracción bioaccesible.

Se tomó una alícuota 500 µL del extracto/digerido en un tubo y se le añadió 1 mL de solución férrica. Se incubó en un baño de agua hirviendo durante 30 min y luego se enfrió a temperatura ambiente. El tubo se centrifugó durante 30 min a 3000 ×*g*,

se tomó 1 mL del sobrenadante y se mezcló con 1.5 mL de solución de 2,2'-bipiridina. La absorbancia se midió a 519 nm frente al agua destilada. Los resultados se expresaron como mg de ácido fítico/g de base seca.

4.6.8. Análisis de compuestos fenólicos

Contenido fenólico total (CFT)

La determinación de CFT de las muestras antes y después de la digestión *in vitro* se realizó mediante el método de Folin-Ciocalteu descrito por Chang et al. (2006). Para las muestras no digeridas, la extracción de compuestos fenólicos se realizó mezclando 7.5 mL del disolvente de extracción (mezcla de agua bidestilada y etanol 70:30) con 2.5 g de muestra. El pH se ajustó a 2 con HCl 2 M y se sometió a un baño ultrasónico durante 2 h a temperatura ambiente. Las muestras se centrifugaron a 8000 ×*g* durante 15 min. La extracción se repitió dos veces y ambos extractos se mezclaron. Para las muestras digeridas, la determinación se realizó sobre la fracción bioaccesible.

Se tomó una alícuota de 125 µL del extracto/digerido y se añadieron 500 µL de agua bidestilada, seguidos de 125 µL de reactivo de Folin-ciocalteu y se dejaron reaccionar durante 6 min. Se añadieron 1.25 mL de solución de carbonato de sodio al 7% y 1 mL de agua bidestilada para completar un volumen final de 3 mL. La mezcla se incubó a temperatura ambiente en la oscuridad durante 30 min, y la absorbancia se midió a 760 nm. Se utilizó una curva estándar para el ácido gálico y los resultados se expresaron como mg de ácido gálico/g de base seca.

Perfil polifenólico

Los compuestos fenólicos de las muestras sin digerir se extrajeron siguiendo la metodología propuesta por Caprioli et al. (2018) y Giusti et al. (2019). Para realizar la hidrólisis ácida, se pesaron 2.5 g de muestra y se añadieron 7.5 mL del disolvente de extracción (mezcla 70:30 de etanol y agua bidestilada). Se ajustó el pH con HCl 2 M hasta pH 2 y se sometieron a un baño de ultrasonidos (J.P. Selecta, 3000840) durante 2 h a temperatura ambiente. Las muestras se centrifugaron a 8000 ×*g* durante 15 min. La extracción se repitió dos veces. Los dos extractos obtenidos se mezclaron y filtraron con un filtro PTFE de 0.45 µm y, posteriormente, la fracción fenólica libre se analizó mediante HPLC.

Para llevar a cabo la hidrólisis alcalina, se añadieron 14 mL de una mezcla de NaOH 2 M con 0.01% de EDTA 10 mM y 0.1% de ácido ascórbico al residuo de la hidrólisis ácida (sedimento) y se dejó toda la noche para liberar los ésteres o éteres fenólicos unidos. El pH se ajustó a 2 con HCl 6 M y se centrifugó a 8000 ×*g* durante 15 min. A continuación, se añadieron 15 mL de una mezcla de acetato de etilo y éter dietílico en proporción 50:50 y se centrifugó a 5400 ×*g* durante 10 min y se repitió dos veces. Ambas fases orgánicas se juntaron y se concentraron en un evaporador rotatorio (Heidolph) a 25 °C. El concentrado se reconstituyó con 10 mL de metanol, se filtró con un filtro de PTFE de 0.45 µm y se analizó por HPLC.

Para las muestras digeridas, la determinación se realizó filtrando la fracción bioaccesible del digerido con un filtro PTFE de 0.45 µm y se analizaron por HPLC.

Los extractos/digeridos se analizaron utilizando un HPLC 1200 Series Rapid Resolution acoplado a un detector de diodos Serie (Agilent, Palo Alto, CA, EE.UU.) siguiendo la metodología descrita por Tanleque-Alberto et al. (2020). Los

compuestos fenólicos se separaron en una columna Brisa-LC 5 µm C18 (250 x 4.6 mm) (Teknokroma, España). La fase móvil A fue ácido fórmico al 1% y la fase móvil B fue acetonitrilo (ACN). Se estableció el siguiente programa de gradientes: 0 min, 90% A; 25 min, 40% A; 26 min, 20% A; manteniendo hasta 30 min; 35 min, 90% A; manteniendo hasta 40 min. La temperatura de trabajo de la columna, el caudal y el volumen de inyección fueron de 30 °C, 0.5 mL/min y 10 µL, respectivamente. Los compuestos desconocidos se identificaron comparando los tiempos de retención cromatográficos resultantes con los de los estándares de referencia a las siguientes longitudes de onda para cada compuesto: 250 nm para el ácido vanílico; 260 nm para el ácido 4-hidroxibenzoico, la rutina, el 3-glucósido de quercetina y la quercitrina; 280 nm para el ácido gálico, la epicatequina, la quercetina y el ácido trans-cinámico; 290 nm para la naringenina; 320 nm para el ácido 4-O-cafeoilquínico, el ácido cafeico, el ácido p-cumárico, el ácido sinápico, el ácido ferúlico y el 7-glucósido de apigenina; 380 nm para el kaempferol. La cuantificación de los compuestos identificados se llevó a cabo mediante una curva de calibración por análisis de regresión lineal del área bajo la curva frente a su concentración, y los resultados se calcularon en µg/g de base seca.

4.6.9. Propiedades funcionales

Actividad antioxidante

La actividad antioxidante de las muestras antes y después de la digestión *in vitro* se determinó mediante tres métodos diferentes: (1) ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), (2) DPPH: 2,2-diphenyl-1-picrylhydrazyl y (3) FRAP: Ferric reducing antioxidant power siguiendo la metodología descrita por Thaipong et al. (2006). Se utilizaron los mismos extractos empleados en la sección de contenido fenólico total (CFT) para las muestras sin digerir y digeridas.

METODOLOGÍA EXPERIMENTAL

Para el ensayo de ABTS, se prepararon soluciones stocks de ABTS 7.4 mM y de persulfato de potasio 2.6 mM, y después, se preparó la solución de trabajo ABTS mezclando las dos soluciones stocks en una proporción 1:1 y dejándolas reaccionar durante 12 h a temperatura ambiente en la oscuridad. Después de la reacción, se diluye 1 mL de la mezcla de soluciones stocks con 60 mL de metanol para obtener una absorbancia cercana a 1.1 a 734 nm. Una alícuota de 150 µL del extracto/digerido se hizo reaccionar con 2.85 mL de solución de trabajo ABTS durante 2 h en la oscuridad y se midió la absorbancia a 734 nm.

Para el ensayo de DPPH, se preparó una solución de trabajo fresca de 0.039 g/L de DPPH en metanol puro para obtener una absorbancia cercana a 1.1 a 515 nm. Una alícuota de 75 µL del extracto/digerido se hizo reaccionar con 2.925 mL de solución de trabajo de DPPH durante 30 min en la oscuridad y se midió la absorbancia a 515 nm.

Para el ensayo de FRAP, se prepararon soluciones madre de tampón acetato 300 mM (3.1 g de trihidrato de acetato de sodio y 16 mL de ácido acético glacial en 1 L de agua), pH 3.6, solución de TPTZ (2,4,6-tripiridil-s-triazina) 10 mM en ácido clorhídrico 40 mM, y solución de cloruro de hierro (III) hexahidratado 20 mM. Se preparó una solución de trabajo fresca mezclando tampón acetato, solución de TPTZ y solución de cloruro de hierro (III) hexahidratado en una proporción 10:1:1, respectivamente, y se incubó a 37 °C antes de su uso. Se hicieron reaccionar 150 µL del extracto con 2.85 mL de solución de trabajo FRAP durante 30 min en la oscuridad y se midió la absorbancia a 593 nm.

En los tres métodos para la determinación de la actividad antioxidante, se utilizó una curva estándar de trolox y los resultados se expresaron como mg trolox/g base seca.

Se calculó el índice antioxidante de cada muestra para todos los ensayos de actividad antioxidante (ABTS, DPPH y FRAP). Se asignó un valor de índice antioxidante de 100 a la puntuación más alta de la muestra en cada ensayo y, a continuación, se calculó el índice antioxidante para todo el grupo de muestras en cada ensayo de acuerdo con la ecuación 4.6 (S. Sharma et al., 2022):

$$Índice\ antioxidante\ (\%) = \left(\frac{puntuación\ de\ la\ muestra}{puntuación\ más\ alta\ de\ las\ muestras}\right) \times 100 \quad (4.6)$$

El índice compuesto de potencia antioxidante global (ICPA) se calculó promediando el índice antioxidante (%) de cada ensayo de actividad antioxidante de cada muestra.

Actividad inhibidora de la enzima convertidora de angiotensina (ECA ai (%))

La ECA ai (%) se determinó para las muestras antes y después de la digestión *in vitro* según la metodología descrita por Akillioğlu & Karakaya (2009) y Hernández-Olivas et al. (2022). Para las muestras sin digerir, se realizó una doble extracción de la proteína mezclando 5 g de la muestra con 45 mL de agua destilada, se ajustó el pH a 11 y se centrifugó a 10000 ×*g* a 4 °C durante 20 min. Se mezclaron los sobrenadantes y se ajustó el pH al punto isoeléctrico (4.5), se mantuvo en agitación suave durante 1.5 h a 4 °C y se centrifugó a 10000 ×*g* a 4 °C durante 20 min. El sedimento se disolvió en tampón fosfato 50 mM, pH=7. Los extractos se analizaron inmediatamente; caso contrario, se almacenaron a -40 °C. Para las muestras digeridas, la determinación se realizó sobre la fracción bioaccesible.

El reactivo ECA (25 mU/mL) y el sustrato Hip-His-Leu (5 mM), fueron ambos disueltos en tampón Tris base 0.15 M que contenía NaCl 0.3 M, y el pH se ajustó a 8.3. Se incluyeron tres controles: (i) 100 µL de ECA + 40 µL de agua destilada, (ii) 140

µL de agua destilada, (iii) 40 µL de extracto de muestra/digerido +100 µL de agua destilada, junto con las muestras (100 µL de ECA + 40 µL de extracto de muestra/digerido) y se incubaron a 37 °C durante 5 min. Se añadieron 100 µL de sustrato a cada tubo y se continuó la incubación durante 30 min a la misma temperatura. Se añadieron 150 µL de HCl 1 M para detener la reacción. Se añadió 1 mL de acetato de etilo y se mezcló vigorosamente en un mezclador de vortex. Las muestras se centrifugaron a 1200 ×g durante 10 min, y se recogieron 750 µL del sobrenadante y se colocaron en tubos limpios. El acetato de etilo contenido en el sobrenadante se evaporó mediante agitación suave a 80 °C. El ácido hipúrico sólido contenido en los tubos se disolvió en 1 mL de agua destilada y se midió la absorbancia a 228 nm. La ECA ai (%) se calculó de acuerdo con la ecuación 4.7:

$$ECA\ ai = 100 - \left\{100\ \times \frac{C - D}{A - B}\right\} \qquad (4.7)$$

Donde: A, B, C y D son la absorbancia de ECA + agua destilada, agua destilada, ECA + extracto de muestra o digerido y extracto de muestra o digerido + agua destilada, respectivamente.

4.6.10. Análisis estadístico

Los experimentos se realizaron por triplicado y los resultados se presentaron como media ± desviación estándar.

Análisis de varianza (ANOVA) unifactorial

Los resultados obtenidos se analizaron estadísticamente con el programa Statgraphics Centurion-XV. Se realizó un ANOVA unifactorial con un intervalo de confianza del 95% ($p < 0.05$). Se utilizó la prueba de múltiples rangos para determinar

diferencias significativas y analizar el efecto de la FES y de la temperatura de secado antes y después de la digestión gastrointestinal. Además, se determinó el efecto de las alteraciones gastrointestinales del modelo de digestión de un adulto mayor en comparación con la de un adulto sano.

Análisis de componentes principales (ACP)

Los resultados se sometieron a un análisis de componentes principales (ACP) para comprender el efecto de la FES de las dos variedades de lenteja y quinoa, y del secado posterior sobre los compuestos volátiles. Se utilizó el programa estadístico R versión 4.2.2 con un nivel de confianza del 95% ($p < 0.05$).

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5. RESULTADOS Y DISCUSIÓN

CAPÍTULO I

CAPÍTULO I: OBTENCIÓN DE NUEVAS HARINAS DE LENTEJA Y QUINOA FERMENTADAS CON DIGESTIBILIDAD Y FUNCIONALIDAD MEJORADA.

ARTÍCULO 1

Sánchez-García, J., Asensio-Grau, A., García-Hernández, J., Heredia, A., & Andrés, A. (2022). Nutritional and antioxidant changes in lentils and quinoa through fungal solid-state fermentation with Pleurotus ostreatus. *Bioresources and Bioprocessing*, *9*(1), 1-12.

ARTÍCULO 2

Sánchez-García, J., Muñoz-Pina, S., García-Hernández, J., Heredia, A., & Andrés, A. (2023). Impact of Air-Drying Temperature on Antioxidant Properties and ACE-Inhibiting Activity of Fungal Fermented Lentil Flour. *Foods*, *12*(5), 999.

ARTÍCULO 3

Sánchez-García, J., Muñoz-Pina, S., García-Hernández, J., Heredia, A., & Andrés, A. (2023). Fermented quinoa flour: Implications of fungal solid-state bioprocessing and drying on nutritional and antioxidant properties. *LWT*, *182*, 114885.

ARTÍCULO 4

Sánchez-García, J., Muñoz-Pina, S., García-Hernández, J., Heredia, A., & Andrés, A. (2024). Volatile profile of quinoa and lentil flour under fungal fermentation and drying. *Food Chemistry*, *430*, 137082.

RESUMEN DEL CAPÍTULO I

El **CAPÍTULO I** recoge los resultados obtenidos en el estudio de la obtención y caracterización de nuevas harinas de lenteja y quinoa fermentadas en estado sólido con *Pleurotus ostreatus*.

En primer lugar, se planteó determinar los cambios que se producen durante la fermentación en estado sólido (FES) resultantes de la actividad metabólica del hongo *Pleurotus ostreatus* sobre el perfil nutricional y funcional de la lenteja Pardina y la quinoa blanca. Además, se estableció evaluar si los resultados de la fermentación eran más favorables al utilizar el sustrato en forma de grano o harina. Para lo cual, se aplicó el proceso de FES en grano/semilla y harina de lenteja variedad Pardina y quinoa blanca utilizando la cepa de *Pleurotus ostreatus* como cultivo iniciador durante 14 días. Los sustratos se prepararon antes de la inoculación del microorganismo hidratándolos hasta una humedad del 65% y posterior esterilización a 121 °C en botes de vidrio. Se evaluó el impacto de la fermentación y del tamaño de partícula (grano/semilla o harina) sobre la producción de biomasa y proteína resultantes de la actividad metabólica del hongo, además de los cambios en las propiedades antioxidantes y compuestos antinutrientes.

Se pudo observar que la **biomasa** fúngica incrementó a través del tiempo de fermentación, siendo mayor en quinoa blanca que en lenteja Pardina. Concretamente, alcanzó valores de 30 y 32 mg glucosamina/g base seca en granos y harina de lenteja Pardina, y valores de 52 y 45 mg glucosamina/g base seca en semillas y harina de quinoa blanca, respectivamente. Por lo tanto, el crecimiento del hongo se ve afectado no solo por la cantidad de nutrientes, sino también por las características morfológicas (grano o harina) del sustrato. Asimismo, se ha

relacionado el crecimiento del micelio fúngico con el incremento de **proteína unicelular**. La mayor producción se dio en semillas de quinoa blanca (26%), seguido de harina de lenteja Pardina (21%). La proteína soluble disminuyó con el tiempo de fermentación y fue mayor en quinoa blanca que en lenteja Pardina. Por otro lado, el **ácido fítico** disminuyó entre un 27% y un 45% en granos/semillas y aproximadamente el 90% en harinas. Esta notable disminución puede atribuirse a la actividad de la fitasa exógena de *P. ostreatus*, en lugar de la acción de la fitasa endógena del sustrato. Con respecto a las propiedades antioxidantes, el tratamiento térmico (esterilización a 121 °C) produjo un incremento del **contenido fenólico total (CFT)** y de la **actividad antioxidante** (ABTS, DPPH y FRAP). Sin embargo, a pesar de lo contrariamente esperado, estos parámetros disminuyeron a medida que avanzaba el tiempo de fermentación. Las pérdidas fueron mayores en lenteja Pardina que en quinoa blanca después de los 14 días, con un promedio alrededor del 50% y 20%, respectivamente, indistintamente de tratarse de grano/semilla o harina. Solo en quinoa blanca se presentaron incrementos en semillas en el día 8 y en harina en el día 10 en el CFT. De acuerdo a estudios previos, los cambios en las propiedades antioxidantes vienen determinados por múltiples factores como el metabolismo del microorganismo implicado, el tipo de sustrato utilizado y condiciones de fermentación.

De acuerdo a este trabajo, se ha determinado que el hongo *P. ostreatus* crece de manera más efectiva en granos/semillas en comparación con la harina, dado que mostró una mayor producción de biomasa durante el proceso de fermentación. Esto debido al lecho del sustrato, que incluye espacios entre los granos/semillas, y una adecuada aireación, lograda manteniendo 3/4 de espacio de cabeza en el bote de fermentación, favoreciendo así al desarrollo óptimo de este microorganismo. En consecuencia, se tomó la decisión de que los estudios posteriores se enfoquen

únicamente en los sustratos en forma de grano/semilla. Además, considerando la importancia del tipo de sustrato para el desarrollo óptimo de microorganismos durante la fermentación, resulta interesante evaluar cómo responden otras variedades al proceso fermentativo, y de esta forma ampliar la implementación de la FES a otras variedades como la lenteja Castellana y la quinoa negra. Por lo tanto, se continuó con el estudio de la FES en dos variedades de lenteja (Pardina y Castellana) y dos variedades de quinoa (blanca y negra) en grano/semilla. Además, se evaluó el impacto del secado posterior para la estabilización de las muestras fermentadas, utilizaron dos métodos de secado: aire caliente a temperaturas de 50, 60 y 70 °C, y liofilización entendiendo esto como método de secado de referencia por esperarse la mayor preservación de las propiedades de las muestras de acuerdo con la bibliografía. Una vez aplicada la etapa de estabilización, las muestras fermentadas y secas se molieron hasta obtener una harina, y se evaluaron las propiedades nutricionales, antioxidantes y antihipertensivas, además de las propiedades físicas (color y tamaño de partícula), y sensoriales (perfil volátil).

La FES conllevó una mayor producción de **biomasa** en lenteja Castellana y quinoa blanca de 4 y 1.6 veces superior que en lenteja Pardina y quinoa negra, respectivamente. Este hecho se tradujo en un incremento porcentual del contenido de **proteína** en lenteja Castellana del 2% y quinoa blanca del 15%, con respecto a su contenido inicial. El **ácido fítico** se redujo aproximadamente el 90% en las dos variedades de quinoa y en lenteja Castellana, y el 20% en Pardina, sin reducción adicional en el secado posterior por aire caliente ni liofilización. Por otro lado, la FES tuvo un impacto negativo del CFT y la actividad antioxidante en los cuatro sustratos. Los **fenoles totales** disminuyeron en un 45% y un 30% en lenteja Pardina y Castellana, respectivamente, mientras que se presentó una reducción del 1% y el 38% en quinoa blanca y negra, respectivamente. Las muestras fermentadas presentaron índices de

actividad antioxidante (APCI) de 32% y 48% en lenteja Pardina y Castellana, y un APCI de 70% y 62% en quinoa blanca, respectivamente. Las disminuciones del CFT y la actividad antioxidante durante la FES podrían estar relacionadas a la unión de los fenoles libres con otras moléculas presentes en la matriz alimentaria, ser degradados por enzimas microbianas y/o hidrolizados por cepas microbianas específicas. Por otro lado, el secado por aire caliente a 50, 60 y 70 °C incrementó significativamente el CFT y la actividad antioxidante, especialmente a 70 °C, en las variedades de lenteja Castellana y quinoa blanca fermentadas, con incrementos superiores al 90% en el CFT e incrementos en el APCI con valores entre 80% y 90%. Estos incrementos en el CFT y la actividad antioxidante durante el secado por aire caliente podrían deberse a la formación de productos de reacción de Maillard. Además, el **perfil fenólico** experimentó cambios notables debido a la FES y el secado por aire caliente (50, 60 y 70 °C). Se presentaron aumentos significativos en el ácido vanílico en la lenteja Pardina, de hasta 2 veces su contenido inicial, así como en el ácido gálico en las lentejas Castellana y las variedades de quinoa blanca y negra, de 2 a 5 veces su contenido inicial, a expensas de la disminución de otros ácidos fenólicos y compuestos flavonoides. Por otro lado, la liofilización no produjo ventajas frente al secado por aire caliente dando lugar a una disminución aún más acusada del CFT, perfil fenólico y de la actividad antioxidante en comparación con el método de secado por aire caliente. Esto podría deberse a los largos tiempos de proceso asociado a la liofilización, superando las 24 h, y a las bajas temperaturas, con un impacto negativo en las propiedades antioxidantes. Las dos variedades de lentejas y quinoa fermentadas y secas mostraron **capacidad para inhibir la enzima conversora de angiotensina (ECA)**, debido a la FES y al secado por aire caliente con un incremento promedio del 70% y >100%, respectivamente, en comparación a sus análogos sin fermentar; el incremento en lenteja Castellana fue de un 28% tras el

secado. La generación de péptidos bioactivos durante la fermentación podría ser responsable de la actividad antihipertensiva observada.

En relación al tamaño de partícula, el secado por aire caliente indujo una disminución del 20% y 50% en las dos variedades fermentadas de quinoa y lenteja, respectivamente, con respecto a las harinas sin fermentar. La liofilización produjo tamaños de partícula aún más pequeños, con una reducción de hasta el 70%. Además, las **propiedades ópticas** también resultaron afectadas en todas las harinas, con una diferencia de color (ΔE) de 19 y 25 en lenteja Pardina y Castellana, respectivamente, y un ΔE de 15 y 23 para quinoa blanca y negra, respectivamente, en comparación con las muestras sin fermentar. Esto resultó en colores anaranjados y marrones intensos, posiblemente debido a las reacciones de Maillard y la presencia de enzimas polifenoloxidasas fúngicas. La liofilización produjo menores diferencias de color con un ΔE >10 en comparación con las muestras no fermentadas.

Con respecto al **perfil volátil**, se determinó que las harinas sin fermentar presentaron un perfil volátil pobre y poco variado en términos de compuestos volátiles participantes, con valores totales del área del pico de 160 y 199 en lenteja Castellana y Pardina, respectivamente, y de 32 y 96 en quinoa blanca y negra, respectivamente. La FES incrementó significativamente el perfil de volátiles (>570%). Durante la fermentación, se generaron compuestos volátiles comunes como el 1-octen-3-ol, benzaldehído, hexanal y 3-metoxibenzaldehído, proporcionando aromas dulces, a hierba/verde y a cacao. Además, la mezcla de compuestos alifáticos, oxigenados y de 8 carbonos, como por ejemplo el 1-octen-3ol, entre otros, están asociados con el olor a champiñón. El secado por aire caliente disminuyó el perfil volátil en promedio en un 50% el área total del pico en las harinas de quinoa (blanca y negra) y en lenteja Pardina, mientras que en lenteja Castellana la disminución fue

de un 70%, sin efecto significativo de la temperatura de secado. La liofilización retuvo mejor los compuestos volátiles en las dos variedades de lenteja y quinoa negra, excepto para la quinoa blanca donde las pérdidas fueron similares al secado por aire caliente. A pesar de las pérdidas de compuestos volátiles debido al secado por aire caliente, las harinas fermentadas mantuvieron valores elevados de los compuestos más relevantes. Estos confieren un aroma dulce, afrutado y a cacao con notas de setas y sustratos cocidos, debido a las concentraciones de benzaldehído, hexanal, nonanal, furfural y 1-octen-3-ol presentes en las harinas fermentadas. La liofilización preservó en mayor medida los compuestos volátiles mostrando pérdidas máximas del 40%.

A partir de los resultados obtenidos en esta investigación, se puede decir que, entre las diferentes variedades de granos de lenteja y semillas de quinoa estudiadas, la lenteja Castellana y la quinoa blanca exhibieron un mejor perfil nutricional y funcional después de haber sido sometidas a la FES seguida del proceso de secado por aire caliente a 70 °C.

ARTÍCULO 1

ARTÍCULO 1: Nutritional and antioxidant changes in lentils and quinoa through fungal solid-state fermentation with *Pleurotus ostreatus*.

ABSTRACT

Solid-state fermentation (SSF) may be a suitable bioprocess to produce protein-vegetal ingredients with increased nutritional and functional value. This study assessed changes in phenol content, antinutrient content, biomass production and protein production resulting from the metabolic activity of *Pleurotus ostreatus*, an edible fungus, in lentils and quinoa over 14 days of SSF. The impact of particle size on these parameters was also assessed because the process was conducted in both seeds and flours. Fungus biomass increased during fermentation, reaching 30.0 ± 1.4 mg/g dry basis and 32 ± 3 mg/g dry basis in lentil grain and flour and 52.01 ± 1.08 mg/g dry basis and 45 ± 2 mg/g dry basis in quinoa seeds and flour after 14 days of SSF. Total protein content also increased by 20% to 25% during fermentation, in all cases except lentil flour. However, the soluble protein fraction remained constant. Regarding phytic acid, SSF had a positive impact, with a progressive decrease being higher in flours than in seeds. Regarding antioxidant properties, autoclaving of the substrates promoted the release of polyphenols, together with antioxidant activity (ABTS, DPPH and FRAP), in all substrates. However, these parameters drastically decreased as fermentation progressed. These results provide scientific knowledge for producing lentil- or quinoa-based ingredients with low antinutrient content enriched with protein fungal biomass.

Keywords: seeds, flour, protein, polyphenols, antinutrients.

1. Introduction

The growing interest in seeking plant protein sources as an alternative to animal proteins is driven by environmental sustainability, cost and food security motivations. Both legumes and pseudocereals are relevant for agriculture and food security because of environmental and economic benefits associated with their ability to fix atmospheric nitrogen in soils (Khazaei et al., 2019). This fact contributes to mitigating greenhouse gas emissions and thus reducing the need for external nitrogen fertilisers (Nemecek et al., 2008; Sánchez-Navarro et al., 2020).

Legumes are one of the most consumed foods worldwide. They are an essential component not only of the Mediterranean diet but also of the diet in many developing countries (Clemente & Jimenez-Lopez, 2020). Lentils, chickpeas, beans and peas, amongst other legumes, are rich sources of protein and complex carbohydrates such as insoluble fibre, which has a low glycaemic index (Bouchenak & Lamri-Senhadji, 2013; Dhull et al., 2020). They also have a high content of bioactive compounds, such as B vitamins, minerals such as potassium and magnesium, and polyphenols (Becerra-Tomás et al., 2019; Khazaei et al., 2019). Lentils (*Lens culinaris*) in particular are frequently noted for their protein content, essential micronutrients and antioxidants (Khazaei et al., 2019). The presence of phenolic compounds and precursor proteins of bioactive peptides, known as bioactive molecules, provide them with antioxidant and antidiabetic activities of considerable interest (Alves Magro et al., 2019). Another interesting food group is that of pseudocereals, such as amaranth, buckwheat and quinoa, which differ from cereals in some morphological properties and their distinct chemical composition (e.g. they are high protein and gluten free). Specifically, quinoa (*Chenopodium quinoa Willd*), a crop from the Andean region, is one of the grains of the twenty-first century. Its cultivation has now

spread to European countries, the United States and Canada (Romano & Ferranti, 2019). It is a source of high-quality protein that contains the nine essential amino acids, with a high content of lysine, methionine and cysteine in comparison with common cereals (Motta et al., 2019). Despite the high-quality nutritional profile of lentils and quinoa, they also contain antinutrients (phytates, polyphenols, such as tannins, and gastric protease inhibitors), which hinder digestibility and the absorption of nutrients (Asensio-Grau et al., 2020; Nkhata et al., 2018; Schlemmer et al., 2009). Phytates mainly affect the bioavailability of minerals, as may also occur with tannins (Bouchenak & Lamri-Senhadji, 2013; Khazaei et al., 2019). Tannins react with amino acids, such as lysine and methionine, limiting their bioavailability (Samtiya et al., 2020; Sarwar Gilani et al., 2012). Protease inhibitors irreversibly alter gastric proteases, such as trypsin, leading to a decrease in protein digestion and amino acid absorption (Khazaei et al., 2019).

Cooking methods are known to reduce the negative impact of antinutrients and improve food digestibility (Muzquiz et al., 2012; Shi et al., 2017). Many of these molecules, such as protease inhibitors, are thermosensitive, whereas others, such as tannins, saponins and phytates, can be reduced by soaking, germination or even fermentation (Muzquiz et al., 2012). Fermentation is a biological process that entails the conversion of substrates into new added-value products through the metabolic actions of microorganisms. Compared with their non-fermented counterparts, the resulting fermented foods have improved nutritional composition and functionality thanks to the hydrolysis of complex macromolecules (fats, carbohydrates and proteins) into low molecular weight compounds that are likely to be easier to digest and can be further bioabsorbed (S. Gupta et al., 2018; Şanlier et al., 2019). This improved digestibility is especially relevant for some population groups suffering from

gastrointestinal disorders, such as pancreatic insufficiency and for vegans, whose main protein intake comes from vegetables.

Solid-state fermentation (SSF) offers an environmentally and economically sustainable alternative to classical liquid-state fermentation (submerged method, SmF). SSF occurs in the absence of free water, and the microorganism is in direct contact with gaseous oxygen (Raghavarao et al., 2003). In addition, SSF allows fermentation in a wide variety of substrates that may also be very cheap, such as agro-industrial waste. Furthermore, SSF reaches higher final product concentrations since enzymes inhibition is scarce; SSF would convert 20–30% of the substrate, whereas in SmF the maximum amount is around 5% (X. Liu & Kokare, 2017). The potential benefits of SSF have been described in relation to the revaluation of industrial by-products, such as the production of ethanol from lignocellulosic waste (S. Gupta et al., 2018; Raghavarao et al., 2003). SSF also represents a major advance in the production of protein-enriched foods from carbohydrate-rich substrates (Raghavarao et al., 2003). Moreover, scholars have reported the positive effect of SSF on the nutritional profile of legumes, such as chickpeas (Xiao et al., 2014), beans (Espinosa-Páez et al., 2017) and lentils in both grain (Dhull et al., 2020) and flour (Alves Magro et al., 2019). Temperature, humidity, available gases and pH, together with inoculum selection, are some of the key processing variables to optimise SSF processes (Pandey, 2003).

The employment of different microorganisms has been reported in the SSF of dietary substrates (Couto and Sanromán, 2006). In particular, mushrooms are considered a high nutritional value source due to their content of carbohydrates, essential amino acids, fibre, vitamins and minerals (Espinosa-Páez et al., 2017). Their potential medicinal and pharmacological benefits are also well documented (Atlı et

al., 2019). The genera *Ganoderma, Lentinula, Trametes, Cordyceps, Hericium* and *Pleurotus* are notable examples (Atlı et al., 2019). The edible species of the genus *Pleurotus*, catalogued as Generally Recognized As Safe (GRAS), have been highlighted by several authors for their ability to synthesise essential amino acids whilst developing characteristic organoleptic properties (Espinosa-Páez et al., 2017). The *Pleurotus ostreatus* species is one of the most commonly grown and produced species worldwide. This mushroom is capable of growing on lignocellulosic substrates, which makes it especially suitable for the degradation of substrates, such as legumes, seeds and grains.

The aim of this study was to analyse the impact of solid-state fermentation (SSF) with *P. ostreatus* on protein, phytate and polyphenol contents, as well as antioxidant activity, in lentil and quinoa substrates.

2. Materials and methods

2.1. Materials

Lentil (*Lens culinaris*) of "pardine" variety and quinoa (*Chenopodium quinoa Wild*) grains or seeds and flours were acquired from Molendum ingredients S.L. (batch: 19011573). The *Pleurotus ostreatus* strain was obtained from the Spanish Type Culture Collection (CECT) (20311; batch: 18-10-2016) at the Universitat de València (València, Spain). To formulate the culture media, malt extract, glucose, mycopeptone and agar powder were supplied by Scharlab (Barcelona, Spain).

The analytical determinations required the following reagents: sodium hydroxide (NaOH), acetylacetone ($Ce_5H_80_2$), ethanol (CH_3OH), methanol (CH_3CH_2OH), galic acid ($C_7H_6O_5$), trolox ($C_{14}H_{18}O_4$), DPPH reagent ($C_{33}H_{44}N_5O_6$), iron chloride hexahydrate

($FeCl_3$ (III) · $6H_2O$), TPTZ reagent ($C_{18}H_{12}N_6$), acetic acid ($C_2H_4O_2$), ABTS reagent ($C_{18}N_{24}N_6S_4$), Folin-Ciocalteu reagent, thioglycolic acid ($C_2H_4O_2S$), potassium persulfate ($K_2S_2O_8$), calcium chloride dihydrate (CaCl2 · 2H2O), p-dimethylamine benzaldehyde (C9H11NO) and glucose (C6H12O6). These reagents were acquired from Sigma-Aldrich (St Louis MO, USA). Glucosamine (TCI Chemicals, USA), acetylacetone ($C_5H_8O_2$), sulfuric acid (H_2SO_4), ammonium iron sulphate ($NH_4Fe\ (SO_4)_2 \cdot 12H_2O$) and hydrochloric acid (HCl) were acquired from AppliChem Panreac (USA). Sodium phytate was acquired from Biosynth Carbosynth (USA). Sodium carbonate (Na_2CO_3) was acquired from Scharlab (Barcelona, Spain).

2.2. Fungal solid-state fermentation (SSF)

Starter culture preparation

Pleurotus ostreatus colonies were isolated from the agar plate and cultured in agar petri dishes made with 2% glucose, 2% malt extract, 0.1% mycopeptone and 1.5% agar. They were then incubated for 14 days at 28 °C (Selecta J.P. 200207, Germany). The resulting mycelium was inoculated with a loop in the culture broth (2% glucose, 2% malt extract and 0.1% mycopeptone) and incubated again at 28 °C for 14 days. This broth was used as the starter culture for fermentation.

For the preparation of the starter culture, glass petri dishes containing 10 g of lentil or quinoa flour with 65% of moisture were sterilised (121 °C, 20 min), inoculated with 1 mL of *Pleurotus ostreatus* in the previously prepared liquid medium and incubated at 28 °C for 14 days until the lentil or quinoa surface was completely colonised by the mycelium.

Fermentation process

Lentil (grain and flour) and quinoa (seeds and flour) were subjected to fungal SSF as described by Asensio-Grau et al. (2020), with some modifications. Glass jars (250 mL) containing 35 g of grain or flour were moistened to 65% (for grain and flour, a distilled water proportion of 1:0.65 (w/v) was used) and sterilised at 121 °C for 20 min. Then, the glass jars were inoculated with one portion of the starter culture previously divided into eight portions. Finally, the glass jars were incubated at 28 °C for 14 days. Three glass jars were taken at each of the fermentation times 0, 2, 4, 6, 8, 10, 12 and 14 days to conduct the corresponding analytical determinations.

2.3. Analytical determinations

Substrate composition

Protein, lipid, ash, and moisture contents were determined by the AOAC methodologies in lentil and quinoa (AOAC, 2000). Carbohydrates were estimated by subtracting lipid, protein, and ash contents from the total solid content.

Fungus biomass

Glucosamine content was used to estimate fungus growth, considering glucosamine, such as a product of the chitin hydrolysis (Aidoo et al., 1981; Tomaselli Scotti et al., 2001). For fungal chitin hydrolysis into N-glucosamine, 100 mg of dried lentil and quinoa samples was incubated with 2.4 mL of 72% sulphuric acid (H_2SO_4) at 25 °C for 24 h. Then, samples were diluted with 55 mL of distilled water. The hydrolysis was carried out by sterilising the sample for 2 h at 121 °C. The hydrolysed products were neutralised to pH 7 using sodium hydroxide (NaOH) 10 M and 0.5 M. Next, 1 mL of hydrolysed product was added with 1 mL of acetylacetone reagent (1

mL of acetylacetone and 50 mL of sodium carbonate 0.5 M) in glass tubes and incubated in a boiling water bath for 20 min. After cooling the tubes, 6 mL of ethanol and 1 mL of Erhlick reagent (2.67 g *p*-dimethylamine benzaldehyde and ethanol:HCl solution 1:1 (v:v)) mixed into a 100-mL volumetric flask were added to the mixture. Then, the samples were incubated at 65 °C for 10 min, and absorbances were measured at 530 nm using a spectrophotometer (Thermo scientific, Helios Zeta UV/Vis). A calibration line taking glucosamine (0–0.5 mg/mL) as standard was used to quantify the fungus biomass. Results are expressed as mg glucosamine/g dry basis.

Protein content

Protein content was determined by the Kjeldahl method following AOAC methodologies (AOAC, 2000). Results are expressed as g protein/100 g dry basis.

Trichloroacetic acid (TCA) soluble protein

Amino acids released during fermentation were estimated as the amount of soluble protein in trichloroacetic acid (TCA) following the method described by Asensio-Grau et al. (2020) and Gallego et al. (2020). Samples (100 mg) were mixed with TCA solution to a final concentration of 12% and incubated at 4 °C for 15 min. Then, samples were centrifuged (Eppendorf MiniSpin Plus) at 4200 ×*g* for 10 min. The supernatant was diluted with 50 mM EDTA and 8 M UREA buffer (pH 10), and the absorbance was measured by ultraviolet spectrophotometry (Helios Zeta UV/Vis, Thermo Scientific) at 280 nm. A calibration line was used for quantification using tyrosine as standard. Results are expressed as g soluble protein fraction in TCA/100 g protein.

Phytate content

Phytate content was determined using the method published by (Haug & Lantzsch, 1983) and adapted from Peng et al. (2010). This method is based on the precipitation of phytic acid using an acidic iron solution. The decrease of iron in the supernatant is proportional to the amount of phytic acid in the sample. Ferric solution (0.2 g of NH4Fe (SO4)2·12H2O in 100 mL HCl 2 M, with the volume raised to 1000 mL with distilled water) and bipyridine solution (1 g 2,2-bipyridine and 1 mL of thioglycolic acid, with the volume raised to 100 mL with distilled water) were prepared in advance. For the analysis, 50 mg of the sample was extracted with 10 mL HCl 2 M overnight at 4 °C. Then, the samples were vortexed, and 0.5 mL of the extract was added to a capped glass tube with 1 mL of ferric solution. The samples were then placed in a boiling water bath for 30 min. After cooling the samples to 25 °C, 2 mL of bipyridine solution was added, and the samples were vortexed and immediately measured by spectrophotometry at 519 nm (Helios Zeta UV/Vis, Thermo Scientific). For quantification, a calibration line was produced using phytic acid as standard (0–0.15 mg/mL). Results are expressed as mg phytic acid (PA)/g dry basis.

Total polyphenols

Polyphenols were determined in samples using the Folin–Ciocalteu method following the indications of Espinosa-Páez et al. (2017) and Chang et al. (2006). An extraction with 80% methanol for 2 h in agitation (55 rpm, 25 °C, Intelli-Mixer RM-2) was performed to recover the hydrosoluble compounds from the samples. Methanol was added to the sample in a proportion of 1:20 (w:v). After agitation, samples were centrifuged (20 min, 8000 ×*g*, 20 °C), and the supernatant was used to quantify the

polyphenols by visible spectrophotometry (Helios Zeta UV/Vis, Thermo Scientific). A gallic acid line was used to quantify the total polyphenols (0–200 mg/L). Results are expressed as mg gallic acid (GA)/g dry basis.

Antioxidant activity

Three methods were used to measure antioxidant activity in fermented samples following the indications of Thaipong et al. (2006) and Espinosa-Páez et al. (2017): (1) ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); (2) DPPH: 2,2-diphenyl-1-picrylhydrazyl and (3) FRAP: Ferric reducing antioxidant power. An extraction with 80% methanol was conducted to determine antioxidant activity. After centrifugation, supernatants were used for quantification using a spectrophotometer (Helios Zeta UV/Vis, Thermo Scientific). In all methods, a calibration line was required using Trolox as standard (0–200 mg/L). Results are expressed mg trolox/g dry basis.

2.4. Statistical analysis

Simple factor analysis of variance (ANOVA) was performed with a confidence interval of 95% ($p < 0.05$) to study possible differences in structure (between grain/seeds and flour) and fermentation time (days). The statistical program Statgraphics Centurion-XV was used for this purpose. Fermentation and analyses were performed by triplicate.

3. Results and discussion

Lentils and quinoa can be considered good providers of nutrients for microorganism growth in fermentative processes. However, any modification (chemical or physical) of the starting substrate could affect the fermentative process, even when the same microbial species is used (Espinosa-Páez et al., 2017; Limón et

al., 2015; Michael et al., 2011). Table 5.1 shows the nutritional composition (in dry basis) of lentils and quinoa, before (grain/seed) and after milling and sieving (flour). Quinoa is richer in lipids, minerals, phytates and phenols than lentils. Regarding protein content, all substrates had more than 30 g per 100 g of dry basis, except quinoa grain, which had a lower protein content. The removal of some fibrous parts of the quinoa seeds during milling and after sieving may be responsible for the differences between seeds and flour in terms of protein content, as well as carbohydrate and lipid contents.

The antioxidant activity values (mg Trolox/g dry basis) of the substrates based on radical-based scavenging assays 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH)) and a non-radical redox potential-based scavenging assay (FRAP) also appear in Table 5.1. According to the results, higher values were observed in the ABTS assay than in the DPPH and FRAP assays, regardless of the substrate. Moreover, lentils had a slightly higher capacity to quench the ABTS and DPPH radicals than quinoa, despite the lower phenolic content of lentils. A positive relationship between total phenolic content and radical-based scavenging assays has been reported in vegetal foods (Devi et al., 2019; Marathe et al., 2011). In this study, this relationship seems to be related to the phenolic profile rather than the total content. Accordingly, phenolic compounds from lentils exhibited higher antioxidant activity than those from quinoa. Chemical species with hydrogen atom or electron donating ability exert antioxidant properties. In the case of phenols, these capabilities seem to be related to the position and number of hydroxyl groups attached to the aromatic rings. Catechin and proanthocyanidin compounds represent 69% of the identified phenols in pardina lentils (Aguilera et al., 2010). Phenolic acids together with flavanols comprise 60% of total compounds in white quinoa (Rocchetti et al., 2019). No statistically significant differences were

found amongst samples regarding the capacity to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+}–(TPTZ)_2]^{3+}$ from ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in acidic medium (Table 5.1).

Table 5.1. Proximate composition (g/100 g dry basis), phytic acid (mg PA/g dry basis), total phenolic content (mg GA/g dry basis), and antioxidant activity (mg trolox/g dry basis), of lentil grain, quinoa seeds and respective flours.

	Lentil grain (LG)	Lentil flour (LF)	Quinoa seed (QS)	Quinoa flour (QF)
Protein	31.9 ± 0.5^{A}	32.3 ± 0.3^{A}	25.2 ± 0.2^{A}	31.1 ± 0.3^{B}
Lipids	0.86 ± 0.08^{A}	1.19 ± 0.10^{B}	3.4 ± 0.3^{A}	8.6 ± 0.3^{B}
Ash	2.76 ± 0.09^{B}	2.58 ± 0.05^{A}	3.60 ± 0.01^{A}	4.09 ± 0.02^{B}
Carbohydrates	64.5 ± 0.7^{B}	63.9 ± 0.4^{A}	67.7 ± 0.6^{B}	56.2 ± 0.7^{A}
Moisture	10.15 ± 0.05^{B}	9.35 ± 0.02^{A}	10.34 ± 0.09^{B}	8.97 ± 0.05^{A}
Phytic acid content	4.5 ± 0.4^{A}	4.8 ± 0.4^{A}	15.2 ± 0.9^{A}	19.9 ± 0.2^{B}
Total phenolic content (TPC)	1.28 ± 0.05^{A}	1.19 ± 0.07^{A}	1.57 ± 0.06^{A}	2.00 ± 0.08^{B}
Antioxidant activity (ABTS)	3.8 ± 0.2^{A}	3.5 ± 0.2^{A}	2.4 ± 0.2^{A}	3.1 ± 0.2^{B}
Antioxidant activity (DPPH)	1.26 ± 0.09^{A}	1.10 ± 0.06^{A}	0.82 ± 0.05^{A}	0.94 ± 0.04^{B}
Antioxidant activity (FRAP)	2.09 ± 0.10^{A}	1.8 ± 0.2^{A}	1.8 ± 0.2^{A}	2.3 ± 0.2^{B}

Results represent the mean of three repetitions with their standard deviation. ^{A,B} Different capital letters indicate significant differences between grain/seeds and flour at the 95% ($p < 0.05$) significance level.

The evolution of the fermentation process was followed by estimation of the unicellular biomass generation in the medium. Fungal biomass is difficult to assess

because fungal cells do not easily separate from the solid substrate. The measurement of glucosamine (chitin monomer and a major constituent of the cell wall in fungi) is an acceptable indicator for the estimation of fungal mycelium development (Tomaselli Scotti et al., 2001). The biotransformation of the substrate using *P. ostreatus* depends on its ability to grow and secrete certain enzymes (mainly oxidative and hydrolytic) able to metabolise substrates rich in lignocelluloses (Rodrigues Da Luz et al., 2012), which are not directly fermentable. White rot fungi, such as *P. ostreatus*, have two types of extracellular enzyme systems: a hydrolytic system that produces hydrolases responsible for the degradation of polysaccharides and an extracellular and oxidative lignolytic system that degrades lignin (Ergun & Urek, 2017).

The growth of *P. ostreatus* (CECT 20311) observed by monitoring the evolution of glucosamine content is affected not only by the amount of nutrients but also by the morphological characteristics (i.e. grain or flour) of the substrate (Figure 5.1). The initial section of the curve, between day 0 and day 4, corresponds to the latency phase of the fungus. An exponential increase in the growth of the mycelium began on the 4th day of fermentation, without reaching a stationary phase during the observed period. Despite some observed differences in the growth rate between grains and flours, similar values were found after 14 days of incubation. However, there was higher biomass production in quinoa than in lentils. Substrates containing filamentous fungal biomass can be considered added-value ingredients for food and feed recipes because this biomass is rich not only in high biological value proteins but also in polyunsaturated fatty acids, minerals, vitamins, and pigments. In addition, scholars have noted the potential of using filamentous fungal biomass as a prebiotic because of the fungal cell wall polysaccharides (Karimi et al., 2021).

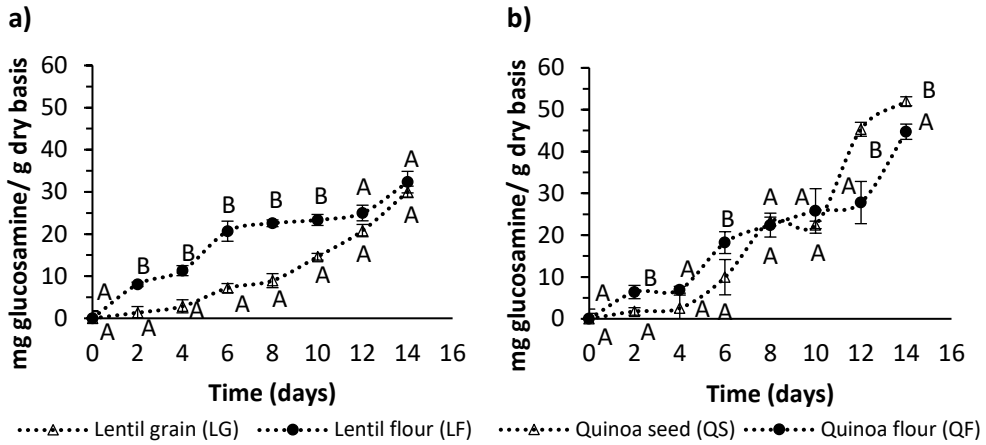


Figure 5.1. Biomass production (mg glucosamine/g dry basis) in lentil grain (**a**), quinoa seeds (**b**) and respective flours at different fermentation times. [A,B] Different capital letters indicate significant differences between grain/seeds and flour at the 95% ($p < 0.05$) significance level.

To evaluate the impact of biomass growth on the protein of the fermented samples, total protein content and the soluble protein fraction in TCA were evaluated at different times of the bioprocess (Table 5.2). Consistent with the biomass growth on the different substrates, a positive correlation between biomass and protein content was observed. Protein content increased by between 7% and 26% depending on the substrate. The highest production was found in quinoa seeds (26%), followed by lentil flour (21%). The impact of the particle size of the substrate on protein content is unclear and depends on the type of substrate because the increase of protein was higher in lentil flour than in grain, whereas, in quinoa, the opposite was observed. However, soluble protein decreased with fermentation time and was greater in quinoa seeds and flour than in lentils. An increase of total protein content has been reported in SSF with *P. ostreatus* in other pulses, such as kidney beans (13%), black beans (*Phaseolus vulgaris*; 6%; Espinosa-Páez et al., 2017) and *Lens culinaris* lentils (18.5%), a different lentil variety from the one used in this study. This

protein increase could be explained by the fact that, during fermentation, carbohydrates serve as an energy source for fungus growth, and some of them may be bioconverted into complex proteins, peptides, or even free amino acids (Asensio-Grau et al., 2020). Similar results were found by Mora-Uzeta et al. (2019), who observed that protein content increased in tepary beans (*Phaseolus acutifolius*) by more than 35% when fermented by *Rhizopus oligosporus*.

Table 5.2. Total protein content (g protein/100 g dry basis) and soluble protein fraction in TCA (g soluble protein/100 g protein) in lentil grain, quinoa seeds and respective flours at different fermentation times.

Fermentation time (days)	Total protein content and soluble protein fraction in TCA*			
	Lentil grain (LG)	**Lentil flour (LF)**	**Quinoa seed (QS)**	**Quinoa flour (QF)**
0	24.4 ± 1.5^{abc} (12.7 ± 0.7^{d})	27.5 ± 0.9^{a} (12.75 ± 0.15^{g})	20 ± 2^{a} (14.30 ± 1.15^{c})	25.5 ± 0.7^{a} (18.5 ± 0.5^{c})
2	24.5 ± 0.7^{abc} (12.1 ± 0.7^{cd})	29.0 ± 0.3^{b} (11.0 ± 0.2^{f})	20.4 ± 1.0^{ab} (13.1 ± 0.4^{b})	27.7 ± 0.9^{b} (20.0 ± 1.3^{d})
4	25.5 ± 1.3^{c} (11.3 ± 0.3^{abc})	30.8 ± 0.4^{c} (10.50 ± 0.04^{e})	21.7 ± 0.4^{bc} (11.1 ± 0.4^{a})	28.2 ± 0.6^{b} (16.67 ± 0.15^{b})
6	25.1 ± 0.6^{bc} (11.3 ± 0.5^{abc})	30.3 ± 0.3^{c} (10.34 ± 0.12^{e})	22.5 ± 0.2^{cd} (11.5 ± 0.2^{a})	30.8 ± 0.3^{d} (16.1 ± 0.3^{b})
8	24.7 ± 0.4^{bc} (10.6 ± 0.2^{a})	30.48 ± 0.15^{c} (10.04 ± 0.05^{d})	22.9 ± 0.3^{cd} (12.6 ± 0.4^{b})	29.36 ± 0.10^{c} (14.9 ± 0.2^{a})
10	24.5 ± 0.6^{abc} (11.8 ± 0.4^{bc})	30.4 ± 0.7^{c} (8.86 ± 0.02^{b})	23.8 ± 0.8^{de} (11.1 ± 0.2^{a})	29.3 ± 0.7^{c} (14.6 ± 0.4^{a})
12	23.1 ± 1.0^{a} (11.1 ± 0.6^{ab})	31.9 ± 0.5^{d} (8.26 ± 0.14^{a})	24.4 ± 0.4^{ef} (11.5 ± 0.5^{a})	29.36 ± 0.09^{c} (14.8 ± 0.3^{a})
14	23.8 ± 0.5^{ab} (11.5 ± 0.5^{bc})	33.3 ± 0.2^{e} (9.37 ± 0.09^{c})	25.2 ± 0.5^{f} (11.0 ± 0.3^{a})	27.4 ± 0.7^{b} (14.7 ± 0.3^{a})

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e,f} Different lowercase letters indicate significant differences between the different fermentation times with a 95% ($p < 0.05$) significance level. * Values in parenthesis correspond to soluble protein fraction in TCA.

Regarding the effect of fermentation on the soluble protein fraction, the scientific literature reports differing results depending on the substrate and/or inoculum employed. (Asensio-Grau et al., 2020) reported an increase of this fraction after SSF with *P. ostreatus* in *Lens culinaris* lentils, contributing to higher digestibility of the resulting flour. In contrast, SSF with *Aspergillus sojae* and *ficuum* resulted in a proteolysis reduction in lupin flour due to the entrapment of smaller protein fractions in the fibrous matrix (Olukomaiya et al., 2020).

An important aspect of the nutritional evaluation of a food or ingredient is the content of some antinutrient compounds. Phytates are known to contribute to decreasing the absorption of essential micronutrients, such as calcium, iron (Hurrell et al., 2003), zinc (Guttieri et al., 2006) and magnesium (Bohn et al., 2004; Peng et al., 2010). They also have a negative impact on protein digestibility because they can bond to dietary protein or digestive enzymes (proteases and amylases), inhibiting their hydrolytic activity (Espinosa-Páez et al., 2017; Muñoz-Llandes et al., 2019). Because fungal SSF is presented as a strategy to reduce the antinutrient content of certain substrates (Garrido-Galand et al., 2021), the evolution of phytic acid content was monitored during the fermentation process. The results are shown in Figure 5.2. According to the literature, quinoa seeds contain approximately 1% to 2% of phytic acid (Febles et al., 2002; Hídvégi & Lásztity, 2002), whereas lentils contain between 0.3% and 1.5%. These values are consistent with the initial values for the raw materials used in this study (Table 5.1).

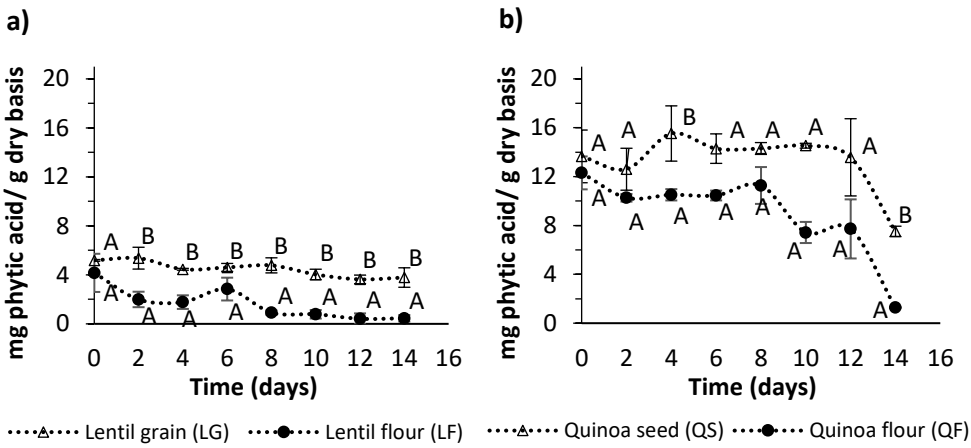


Figure 5.2. Phytic acid content (mg phytic acid/g dry basis) in lentil (**a**) grain, quinoa (**b**) seeds and respective flours at different fermentation times. [A,B] Different capital letters indicate significant differences between grain/seeds and flour at the 95% ($p < 0.05$) significance level.

The degradation of phytates as a consequence of fungal fermentation was observed. This degradation was more pronounced in flours than in grains. The percentage reduction was 27% and 89% in lentil grain and flour, respectively. In quinoa, the percentage reduction was 45% in seeds and 90% in flour. These changes began to be significant ($p < 0.05$) from the 10th day of fermentation and depended on the substrate characteristics. The degradation of phytates after fermentation was greater in flours than in grains. These results are in accordance with those of Castro-Alba et al. (2019), who reported different levels of phytate degradation in quinoa, canihua and amaranth according to their granulometry (seeds or flour). The degradation rate of phytates also seems to be moderated by the pH reduction during fermentation because of organic acid production, which depends on the inoculum employed. Castro-Alba et al. (2019) reported differences between spontaneous fermentation and fermentation with characterised species, such as L. plantarum.

Furthermore, they suggested that the greater degradation of phytates in flour depends on both the exogenous phytase production of the microorganism and the activation of endogenous phytase of the substrate. However, autoclaving prior to inoculation causes the inactivation of endogenous phytase (Brejnholt et al., 2011). Therefore, the notable decrease in phytates in the last few days of fermentation may be attributed mainly to the activity of the exogenous phytase from *P. ostreatus*, instead of the action of the endogenous phytase of the substrate. Similar results were found by (Liang et al., 2008), who reported that fermentation of brown rice was more effective in decreasing phytic acid than wet heating at 115 °C for 10 min.

Pleurotus ostreatus is also known to be an excellent producer of hydrolytic enzymes, which contribute to the release of conjugated phenolic compounds chelated into the cell walls by hydrolysis during fermentation. Phenolic compounds are the major contributors to antioxidant activity in fruit, vegetable, grain, and plant tissues. Changes in total phenol content (TPC) with SSF time are shown in Figure 5.3. First, the results show a positive impact of thermal treatment on bound phenolic compound release because TPC was much higher after autoclaving (time 0) than in the raw material (Table 5.1). The data agree with those reported by other authors (Bryngelsson et al., 2002; Madapathage Dona, 2011). Hence, thermal treatment could promote cell wall disruption with the release of structural phenols and/or the breakdown of insoluble polymeric phenols into smaller molecular weight compounds with enhanced extractability.

In contrast, a decreasing profile of TPC was observed as the fermentation progressed in lentil substrates, with a higher TPC in flour than in grain. Gebru & Sbhatu (2020) reported similar findings in white and brown teff subjected to SSF with *P. ostreatus*, with a negligible and even slight decrease of TPC after 6 days. However,

same authors reported a significant increase of teff phenols when *G. lucidum* was used as a starter and under the same SSF conditions. This result highlights the relevance of each fungal mycelium metabolism and enzyme synthesis in producing changes in bioactive compounds.

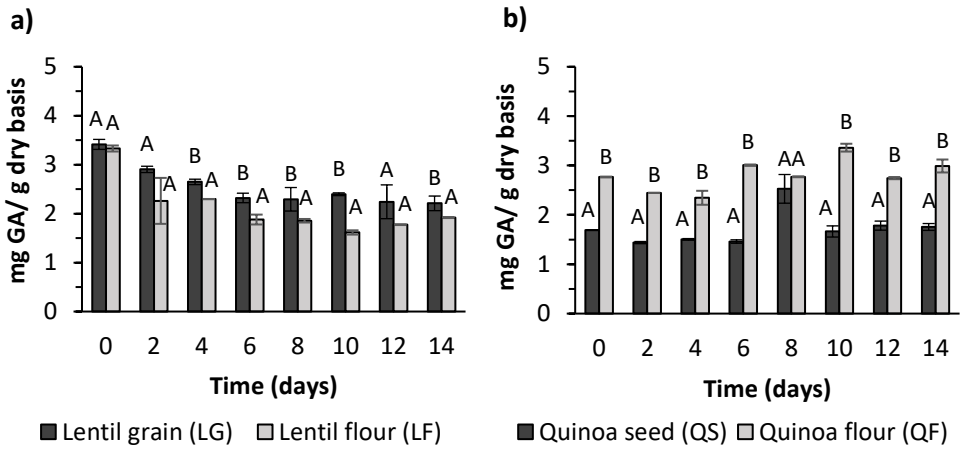


Figure 5.3. Total phenol content (mg GA/g dry basis) in lentil (**a**) grain and quinoa (**b**) seeds and respective flours at different fermentation times. ^{A,B} Different capital letters indicate significant differences between grain/seeds and flour at the 95% ($p < 0.05$) significance level.

Along these lines, a negative correlation between TPC and fermentation time was observed by Xu et al. (2018) in eight cereals and pseudocereals (wheat, corn, rice, millet, quinoa, oats, sorghum, and buckwheat) and two legumes (soybean and peas) fermented with three different fungi for 35 days. According to their results, an increase of TPC was only observed at 14 days in oats. For the other substrates, longer fermentation times were required to produce a significant increase of TPC content. For instance, Xu et al. (2018) reported a significant increase of TPC in fermented quinoa from 21 days of fermentation. This finding may explain the low TPC increase

observed in the present study. Enzyme production is known to change over time, affecting the transformation and production of particular compounds. Therefore, optimal enzyme production is obtained at a specific time in a given culture. The highest TPC value in quinoa seeds was detected after eight days of fermentation (2.5 ± 0.3 mg GA/g dry basis), whereas, in flour, the highest value was observed after 10 days (3.36 ± 0.08 mg GA/g dry basis).

The antioxidant activities of fermented lentils and quinoa appear in Table 5.3. Although SSF was indicated with the aim of obtaining new ingredients with enhanced antioxidant properties, the capacity of the fermented substrates to scavenge free radicals, such as ABTS and DPPH, and to reduce ferric ions in the FRAP assay decreased with fermentation time in this study. Greater losses of antioxidant activities were observed in lentils than in quinoa. In addition to total phenols, other metabolites, such as ergothioneine, that formed during the fermentation process may affect the antioxidant properties of the fermented products (Bei et al., 2017; Cai et al., 2012; Zhai et al., 2015). This fact may be responsible for the lack of correlation between TPC and antioxidant activity (Alves Magro et al., 2019; Torino et al., 2013). In addition, competitive reactions between prooxidant and antioxidant compounds can occur, resulting in an increase or reduction of a food's antioxidant capacity. The ability of phenolic compounds to promote or inhibit oxidative damage depends on the phenol concentration and pH, amongst other factors. Monohydroxylated phenols have been reported to exhibit low radical scavenging activity (Briante et al., 2003; Villaño et al., 2005). Fukumoto & Mazza (2000) found that benzoic and cinnamic acid derivatives behave like prooxidants. Accordingly, a higher release of prooxidant phenols occurred in lentils than in quinoa as fermentation time increased.

Table 5.3. Antioxidant activity (mg trolox/g dry basis) in lentil grain, quinoa seeds and respective flours at different fermentation times.

Substrate	Fermentation time (days)	Antioxidant activity		
		ABTS	DPPH	FRAP
Lentil grain (LG)	0	10.7 ± 0.5^{dA} (0 ± 5)	5.6 ± 0.5^{fB} (0 ± 9)	7.3 ± 0.3^{fA} (0 ± 5)
	2	9.7 ± 0.4^{bcA} (-9 ± 4)	4.4 ± 0.2^{eB} (-21 ± 3)	4.8648 ± 0.0012^{eA} (-33.30 ± 0.02)
	4	8.7 ± 0.2^{aA} (-19 ± 2)	3.92 ± 0.08^{dB} (-30.1 ± 1.5)	4.17 ± 0.06^{dB} (-42.9 ± 0.9)
	6	9.3 ± 0.2^{abA} (-13 ± 2)	3.1 ± 0.2^{cB} (-44 ± 3)	3.03 ± 0.11^{cB} (-58.5 ± 1.6)
	8	8.99 ± 0.12^{aA} (-16.3 ± 1.2)	2.5 ± 0.2^{abB} (-55 ± 3)	2.31 ± 0.08^{bB} (-68.3 ± 1.1)
	10	10.2 ± 0.2^{cdB} (-5 ± 2)	2.79 ± 0.12^{bcB} (-50 ± 2)	2.54 ± 0.03^{bB} (-65.2 ± 0.5)
	12	9.9 ± 0.9^{bcA} (-7 ± 8)	2.21 ± 0.11^{aB} (-61 ± 2)	1.8 ± 0.2^{aB} (-75 ± 3)
	14	10.0 ± 0.3^{bcA} (-7 ± 3)	2.15 ± 0.03^{aB} (-61.7 ± 0.6)	1.72 ± 0.14^{aB} (-76 ± 2)
Lentil flour (LF)	0	12.90 ± 0.02^{dB} (0.00 ± 0.13)	4.307 ± 0.006^{fA} (0.00 ± 0.14)	7.53 ± 0.06^{fA} (0.0 ± 0.8)
	2	11.1 ± 0.8^{cA} (-14 ± 6)	3.1 ± 0.3^{eA} (-29 ± 7)	4.5 ± 0.6^{eA} (-41 ± 8)
	4	10.90 ± 0.12^{cB} (-15.5 ± 0.9)	2.65 ± 0.08^{dA} (-39 ± 2)	3.68 ± 0.11^{dA} (-51 ± 2)
	6	10.3 ± 0.2^{abB} (-20.1 ± 1.5)	2.06 ± 0.02^{cA} (-52.2 ± 0.5)	2.52 ± 0.15^{cA} (-67 ± 2)
	8	10.06 ± 0.02^{aB} (-22.1 ± 0.2)	1.872 ± 0.014^{bA} (-56.5 ± 0.3)	1.92 ± 0.06^{bA} (-74.5 ± 0.7)
	10	9.81 ± 0.08^{aA} (-24.0 ± 0.6)	1.54 ± 0.02^{aA} (-64.3 ± 0.5)	1.19 ± 0.04^{aA} (-84.2 ± 0.6)
	12	9.99 ± 0.10^{aA} (-22.6 ± 0.8)	1.6087 ± 0.0008^{aA} (-62.65 ± 0.02)	1.43 ± 0.03^{aA} (-81.0 ± 0.5)
	14	10.67 ± 0.13^{bcB} (-17.3 ± 1.0)	1.66 ± 0.05^{aA} (-61.5 ± 1.1)	1.36 ± 0.12^{aA} (-82 ± 2)
Quinoa seed (QS)	0	11.54 ± 0.04^{bB} (0.0 ± 0.3)	3.08 ± 0.02^{eB} (0.0 ± 0.7)	2.74 ± 0.15^{dA} (0 ± 5)
	2	10.84 ± 0.04^{aB} (-6.1 ± 0.3)	2.76 ± 0.09^{dB} (-10 ± 3)	1.75 ± 0.03^{bA} (-36.2 ± 1.2)
	4	10.84 ± 0.04^{aB} (-6.1 ± 0.3)	2.576 ± 0.015^{cB} (-16.3 ± 0.5)	1.98 ± 0.05^{cA} (-28 ± 2)
	6	10.84 ± 0.03^{aB} (-6.1 ± 0.3)	2.48 ± 0.04^{bcB} (-19.3 ± 1.4)	1.55 ± 0.03^{aA} (-43.6 ± 1.0)
	8	12.83 ± 0.07^{cB} (11.1 ± 0.6)	2.30 ± 0.11^{aA} (-25 ± 4)	1.44 ± 0.07^{aA} (-47 ± 3)

	10	11.40 ± 0.08^{bB} (-1.3 ± 0.7)	2.28 ± 0.10^{aA} (-26 ± 3)	1.5 ± 0.2^{aA} (-45 ± 8)
	12	11.7 ± 0.4^{bB} (2 ± 3)	2.45 ± 0.02^{bB} (-20.3 ± 0.7)	1.42 ± 0.08^{aA} (-48 ± 3)
	14	10.7 ± 0.4^{aB} (-7 ± 4)	2.555 ± 0.002^{bcB} (-17.00 ± 0.06)	1.458 ± 0.004^{aA} (-46.7 ± 0.2)
Quinoa flour (QF)	0	9.37 ± 0.03^{cA} (-0.0 ± 0.4)	2.81 ± 0.04^{eA} (0.0 ± 1.4)	4.87 ± 0.06^{fB} (0.0 ± 1.3)
	2	8.908 ± 0.007^{bA} (-4.94 ± 0.07)	2.55 ± 0.03^{dA} (-9.2 ± 1.2)	3.75 ± 0.08^{eB} (-23 ± 2)
	4	8.5 ± 0.2^{aA} (-10 ± 2)	1.98 ± 0.06^{aA} (-30 ± 2)	2.4 ± 0.3^{cB} (-50 ± 5)
	6	9.36 ± 0.04^{cA} (-0.2 ± 0.5)	2.291 ± 0.008^{cA} (-18.4 ± 0.3)	2.77 ± 0.02^{dB} (-43.0 ± 0.3)
	8	9.60 ± 0.13^{deA} (2.4 ± 1.4)	2.39 ± 0.02^{cA} (-15.0 ± 0.8)	2.31 ± 0.08^{bcB} (-53 ± 2)
	10	9.44 ± 0.09^{cdA} (0.7 ± 1.0)	2.12 ± 0.05^{bA} (-24 ± 2)	1.97 ± 0.11^{aB} (-60 ± 2)
	12	9.516 ± 0.002^{cdeA} (1.55 ± 0.02)	2.09 ± 0.14^{bA} (-26 ± 5)	2.34 ± 0.02^{bcB} (-51.8 ± 0.4)
	14	9.7 ± 0.2^{eA} (3 ± 3)	2.16 ± 0.06^{bA} (-23 ± 2)	2.21 ± 0.05^{bB} (-54.5 ± 1.0)

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e} Different lowercase letters indicate significant differences between the different fermentation times at the 95% ($p < 0.05$) significance level. ^{A,B} Different capital letters indicate significant differences between grain and flour at the 95% ($p < 0.05$) significance level. Values in parenthesis correspond to the percentage of variation with respect to non-inoculated substrate (time 0).

Nevertheless, autoclaving was once again observed to have a positive effect in terms of radical scavenging and reducing power activity of both substrates because a notable increase in milligrams of Trolox per gram of dry basis at time 0 was observed compared to the values found in the raw materials (Table 5.1). These findings are in line with those of Rocchetti et al., (2019).

Despite the results, complementary analysis is needed to determine how down-streams unit operations, such as milling and drying, usually applied by the food industry to obtain stable flours can affect the studied parameters. Also, it would be of interest to analyse changes in the studied parameters with *in vitro* gastrointestinal digestion to establish the added value of SSF in terms of not only compositional

variation but also protein and carbohydrate digestibility and bioactive compound bioaccessibility.

4. Conclusions

Solid-state fermentation (SSF) with *P. ostreatus* has been proved to be an efficient way to enhance the nutritional profile of Pardina lentils and white quinoa in terms of increased protein and reduced phytates contents. Those nutritional changes along with the additional potential health benefits due to the presence of fungal biomass support the bioconversion of legumes and pseudocereals by SSF. Therefore, this bioprocess may be considered an environmentally sustainable biotechnological strategy to obtain gluten-free fermented lentil- and quinoa-based ingredients for novel food formulations that target specific population groups with high protein requirements, such as the elderly, athletes, vegans, or individuals with gastrointestinal disorders. It would be of interest to perform *in vitro* digestion studies that could help with decisions to establish optimal conditions for the production of fermented ingredients with enhanced digestibility. In conclusion, this study contributes to different twentyfirst century food technology challenges related to protein diversification and the environmentally sustainable bioproduction of food ingredients.

ARTÍCULO 2

ARTÍCULO 2: Impact of air-drying temperature on antioxidant properties and ACE-inhibiting activity of fungal fermented lentil flour.

ABSTRACT

Solid-state fermentation (SSF) with *Pleurotus ostreatus* enhance the nutritional value of legumes. However, drying can cause significant changes in physical and nutritional properties of the final products. Thus, this work studies the impact of air-drying temperature (50, 60, and 70 °C) on relevant properties (antioxidant properties, ACE-inhibitory capacity, phytic acid, colour, and particle size) of two fermented lentils flour (Pardina and Castellana) using freeze-drying as a reference method. Castellana variety is a better substrate for *Pleurotus*, generating 4 times more biomass. In addition, an almost total reduction of phytic acid from 7.3 to 0.9 mg/g dry basis is achieved in this variety. Air-drying significantly decreased the particle size and the final colour with ΔE >20; nonetheless, the temperature does not play a crucial role. SSF decreased the total phenolic content and the antioxidant capacity regardless the variety, however, drying at 70 °C increase total phenolic content (186%) in fermented Castellana flour. Comparing drying methods, freeze-drying implied a higher decrease in those parameters, reducing the TPC from 2.4 to 1.6 and from 7.7 to 3.4 mg gallic acid/g dry basis in Pardina and Castellana dried flours. Finally, the capacity of flours to inhibit the angiotensin I-converting-enzyme, fermentation, plus drying increased their potential cardiovascular benefits.

Keywords: fermentation, *Pleurotus*, drying, flours, lentil, phenols, antinutrients.

1. Introduction

Legumes were in the past a cornerstone in Mediterranean cuisine and gastronomy; they are rich in proteins (usually 20%, reaching 30% in some varieties), fibre, carbohydrates (around 60%), vitamins, minerals, healthy fats (5%), and dietary fibre but there has been a downward trend in their consumption (Maphosa & Jideani, 2017). Legumes consumption has been demonstrated a positive effect on health (Polak et al., 2015). Furthermore, environmentally, legumes cultivation promotes sustainable agriculture and contribute to climate change mitigation, and their ability to fix nitrogen can improve soil fertility and reduce the carbon footprint (Stagnari et al., 2017). Moreover, they are also a more sustainable source of proteins (CO_2 emissions of 1 kg of legume protein is 0.9 kg, while emissions of 1 kg of beef is 27 kg). However, its presence in the Mediterranean people diets has fallen to as low as 60-80 g/week, while the estimated recommendations are 200-250 g/week, according to FAO.

Solid-state fermentation has been recently explored as a strategy to preserve/enhance the nutritional value of legumes with promising results in obtaining new ingredients (Calvo-Lerma et al., 2022).

This kind of biotreatment has been recently applied in lentils, peas, soybeans, or quinoa, among others (Espinosa-Páez et al., 2017). Up-to-date results show increased protein (18%–23%) and antioxidant contents (30%–53%) in the fermented flours, along with increased protein digestibility (12%–17%) and a decrease in the carbohydrate content (6%–29%). A novel approach to do so relates to the use of edible fungi as starter cultures (which has been usually applied for agro-residual materials valorisation) (Hoogeveen & Hoogeveen, 2010; Lyons & Hoskins, 2014). In a previous study solid-state fermentation (SSF) with *Pleurotus ostreatus* was proved to

be an efficient way to enhance the nutritional profile of Pardina lentils and white quinoa (Sánchez-García et al., 2022). Additionally, the potential health benefits due to the presence of fungal biomass support the bioconversion of legumes and pseudo-cereals by SSF. In fact, mushrooms have been proved to perform good antioxidant capacity and demonstrated their inhibitory effect towards angiotensin I-converting enzyme (ACE) (Abdullah et al., 2012). The use of natural antioxidants in the production of new foods is critical to maintaining adequate levels of antioxidants to ensure balance with prevention of the pathologies. The inhibition of ACE enzyme would reduce blood pressure lowering the risk of hypertension complications (Piskov et al., 2020). This kind of bioprocessing could also be employed as a part of a multi-stage process, including stabilization unit operations (such as drying or milling, among others), to obtain promising flours from lentils. Lentils flours has been commonly used to nutritionally enhance food products as an ingredient used as a thickener, binder, gelling agent, and/or stabilizer (Marchini et al., 2021; Romano et al., 2021). Nowadays, there are different drying methods to obtain flour such as hot air recirculation, oven drying, tunnel drying, freeze-drying or spray drying and also different temperatures of drying. However, the drying process and temperature may have mixed influence on the final product due to the several composition and types of raw products. Drying processes can cause significant changes in the physical and nutritional properties of the final products (González et al., 2021; Patrón-Vázquez et al., 2019). Some drying methods might improve the quality, preservation, and value of raw materials while others may provoke a significant decline (Duan & Xu, 2015). Thus, the appropriate technology and conditions used for obtaining new dry products must be established separately for each type of food and based on the specific properties of the final product (Piskov et al., 2020).

To this day, according to authors' knowledge, there are no lentil fermented flours with *Pleurotus* in the bibliography, and no studies of the effect of drying on its physico-chemical properties. Thus, the aim of this work is to study the impact of fungal solid-fermentation and air-drying temperature (50, 60, and 70 °C) on the final properties of fermented lentils flours (Lens culinaris var. Pardina and Castellana) with *Pleurotus ostreatus*. Relevant properties such as antioxidant properties, ACE-inhibitory capacity, phytic acid, colour, and particle size have been analysed to provide recommendations for the usage of fermented lentil flours as perspective food ingredients.

2. Materials and methods

2.1. Materials

Lentils (*Lens culinaris*) of Pardina and Castellana varieties (Hacendado®) were purchased at local stores in Valencia (Spain). *Pleurotus ostreatus* strain was obtained from the Spanish Type Culture Collection (CECT20311).

Phytic acid sodium salt hydrated from rice ($C_6H_{18}O_{24}P_6 \cdot xNa^+ \cdot yH_2O$), 2,2'-bipyridine ($C_{10}H_8N_2$), thioglycolic acid ($C_2H_4O_2S$,), sulphuric acid ($H_2SO_4$), sodium hydroxide (NaOH), sodium chloride (NaCl), 4-dimethylamino benzaldehyde ($C_9H_{11}NO$), acetylacetone ($C_5H_8O_2$,), ascorbic acid ($C_6H_8O_6$), ethyl acetate ($C_4H_8O_2$), formic acid (CH_2O_2), 3,5-dinitrosalicylic acid (DNS) ($C_7H_4N_2O_7$), potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$), folin ciocalteu reagent (C_6H_6O), 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) ($C_{18}H_{16}N_4O_6S_4$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), 2,4,6-tripyridyl-s-triazine (TPTZ) ($C_{18}H_{12}N_6$), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($C_{14}H_{18}O_4$), gallic acid ($C_7H_6O_5$), Angiotensin Converting Enzyme (ACE) from rabbit lung (≥2.0 units/mg

protein) (A6778-25UN), N-Hippuric-His-Leu hydrate (HHL), glucose ($C_6H_{12}O6$), mycopeptone, chloramphenicol and tryptone bile X-glucuronide agar (TBX chromogenic selective medium) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). For HPLC analysis vanillic acid ($C_8H_8O_4$), 4-hydroxybezoic acid ($C_7H_6O_3$), rutin ($C_{27}H_{30}O_{16}$), quercetin 3-glucoside ($C_{21}H_{20}O_{12}$), quercitrin ($C_{21}H_{20}O_{11}$), epicatechin ($C_{15}H_{14}O_6$), quercetin ($C_{15}H_{10}O_7$), trans-cinnamic acid ($C_9H_8O_2$), naringenin ($C_{15}H_{12}O_5$), 4-O-caffeoylquinic ($C_{16}H_{18}O_9$), caffeic acid ($C_9H_8O_4$), p-coumaric acid ($C_9H_8O_3$), sinapic acid ($C_{11}H_{12}O_5$), ferulic acid ($C_{10}H_{10}O_4$), apigenin-7-glucoside ($C_{21}H_{20}O_{10}$) and kaempferol ($C_{15}H_{10}O_6$) were obtained also from Sigma-Aldrich Co. (St. Louis, MO, USA) all as analytical standard (HPLC grade).

Ethanol absolute (C_2H_6O), concentrated hydrochloric acid (HCl), acetic acid glacial ($C_2H_4O_2$), diethyl ether ($C_2H_5OC_2H_5$), ammonium iron (III) sulphate dodecahydrate ($NH_4Fe(SO_4)_2.12H_2O$), sodium carbonate (Na_2CO_3) and EDTA Calcium Disodium Salt ($C_{10}H_{12}CaN_2Na_2O_8$) were obtained from Panreac AppliChem (Barcelona, Spain). Methanol (CH_4O, HPLC grade), acetonitrile (C_2H_3N, HPLC grade), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), potassium persulphate ($K_2S_2O_8$) and sodium acetate trihydrate ($C_2H_3NaO_2{\cdot}3H_2O$) were obtained from Honeywell Fluka (Morris Plains, NJ, USA). Malt extract, agar, Plate-count agar and Sabouraud dextrose agar were obtained from Scharlau (Barcelona, Spain).

2.2. Fungal solid-state fermentation

Starter culture preparation

Pleurotus ostreatus mycelium from the stock culture was inoculated on malt agar petri dishes made with 2% malt extract, 2% glucose, 0.1% mycopeptone and 1.5% agar, and then placed in an incubator (2001249, J.P. Selecta, Barcelona, Spain) at 28

°C for 14 days. The grown mycelium was inoculated in a culture broth made with 2% malt extract, 2% glucose and 0.1% mycopeptone, and incubated at 28 °C for 14 days. A portion of the grown mycelium was taken and inoculated in a culture broth made with 2% malt extract, 2% glucose and 0.1% mycopeptone, and incubated at 28 °C for 14 days.

For starter culture preparation, 10 g of Pardina and Castellana flour each were placed in petri dishes, hydrated to 65% moisture, and sterilised in an autoclave (4002136, J.P. Selecta, Barcelona, Spain) at 121 °C for 20 minutes. Finally, 1 mL of the culture broth containing the grown fungal mycelium was inoculated and incubated at 28 °C for 14 days.

Fermentation process

Solid-state fermentation was performed by placing 35 g of lentils (Pardina and Castellana) humidified to 65% moisture in glass jars and then sterilizing them at 121 °C for 20 min. The substrates were inoculated into the glass jars by adding 1/8 of the starter culture (petri dishes containing 10 g of colonised substrate divided into 8 portions) and then incubated at 28°C for 14 days. Several glass jars were inoculated to obtain enough fermented substrate to perform all assays. Fermented substrates contained in glass jars were mixed to obtain a homogeneous sample for the subsequent drying process and analysis.

2.3. Drying and milling of fermented grains/seeds

Samples were dried by hot-air drying and lyophilisation methods using a load of 500 g. Hot-air drying was performed using a convective dryer (Pol-Eko-Aparatura, CLW 750 TOP+, Kokoszycka, Poland) at three different drying temperatures (50, 60

and 70 °C), air velocity was 10.5 ± 0.2 m/s and air humidity percentage was 23.2 ± 2.9, 14.2 ± 1.7 and 8.7 ± 1.2 for 50, 60 and 70 °C respectively. Lyophilisation was carried out in a freeze dryer (Telstar, Lyoquest-55, Terrassa, Spain) at -45 °C and 0.8 mBar for 48 h. Milling was carried out with a food processor (Thermomix®, TM6-1, Wuppertal, Germany), applying 10000 rpm at 15 s intervals for 1 min.

2.4. Drying kinetics and modelling

Drying kinetics was determined by measuring with a balance (Mettler Toledo, MS4002S, Greifensee, Zurich) the mass variation of the samples at each drying temperature (50, 60, and 70 °C) in a determined time interval, making several measurements along the time until a constant weight was obtained. Drying curves of the samples were obtained, and an adjustment was made using the Lewis model equation (Equation 5.1) for thin layer drying, which is one of the most common mathematical models used in the drying process of agricultural products (Chkir et al., 2015; Lewis, 1921).

$$\frac{X_t^w}{X_0^w} = e^{-k \cdot t} \qquad (5.1)$$

Where: X_t^w is the moisture at a determined time, X_0^w is the moisture at time 0, k is the model constant, and t is the time (min).

2.5. Analytical determinations

Proximal substrate composition

Proximate composition of the substrate was carried out according to standardised methodologies of the Association of Official Analytical Chemist (AOAC, 2000). Moisture, protein, lipid, ash, and carbohydrate content were analysed, the last one

by difference. Total fibre, soluble, and insoluble fibre content were determined according to the AOAC Method 991.43 and AACC Method 32-07.01. Results were expressed in g/100 g dry basis.

Reducing sugars

The methodology proposed by Miller (1959) and Sansano et al. (2015) for reducing sugars determination was used. An amount of 0.3 g of sample was mixed with 2 mL of 80% ethanol, vortexed and allowed to stand for 30 min. It was centrifuged at 5000 ×*g* for 5 min. The extraction was repeated twice, and the extracts were pooled. An aliquot of 500 µL was taken and mixed with 1 mL of DNS reagent (10 g/L of 3,5-dinitrosalicylic acid, containing 300 g potassium sodium tartrate tetrahydrate and 16 g sodium hydroxide). It was heated in a water bath at 100 °C for 5 min and then cooled to room temperature. Sample was diluted with 6 mL of distilled water and the absorbance was measured at 546 nm. Results were expressed as g glucose/100 g dry basis using a standard curve.

Fungus biomass

Biomass produced by the fungus was determined according to the method published by Aidoo et al. (1981) and Tomaselli Scotti et al. (2001). Briefly, 100 mg of dry sample was mixed with 2.4 mL of 72% sulphuric acid at 25 °C for 24 h. Samples were diluted with 55 mL of distilled water and sterilised the mixture at 121 °C for 2 h. Then, the hydrolysate was neutralised to pH 7 with 10 M and 0.5 M sodium hydroxide with a pH meter (Mettler-Toledo, SevenCompact S210, Greifensee, Zurich). Erhlick's reagent was prepared by dissolving 2.67 g of 4-dimethylamino benzaldehyde in 100 mL of a 1:1 mixture of ethanol reagent grade and concentrated hydrochloric acid. An aliquot of 1 mL of previously neutralised hydrolysate was mixed

with 1 mL of acetylacetone reagent (1 mL of acetylacetone and 50 mL of 0.5 M sodium carbonate) in a glass tube with cap and heated in a boiling water bath for 20 min, cooled to room temperature. Then 6 mL of ethanol was added followed by 1 mL of Erhlick's reagent. The mixture was incubated at 65 °C for 10 min, cooled to room temperature, and absorbance was measured at 530 nm in a spectrophotometer (Beckman Coulter, DU 730, Brea, USA). Results were expressed as mg glucosamine/g dry basis.

pH and water activity (aw)

A 10% dilution of the samples was prepared and measured for pH determination. Water activity was measured with a dew point water activity meter (Decagon Devices Inc, Aqualab 4TE, Cervera, Spain) at 25 °C.

Colour

Colour measurements of the different flours were carried out using a spectro-colourimeter (Minolta, CM-3600D, Tokyo, Japan), considering a standard illuminant D65 and a standard observer of 10°. The CIE-L*a*b*colour coordinates were measured. Tone (h) and chroma (C*) values were automatically calculated by the device with the a* and b* coordinates, and colour differences (ΔE) were calculated according to the following equation (Equation 5.2):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (5.2)$$

Particle size

Particle size was measured by the dry method using laser diffraction equipment (Mastersizer 2000, Malvern Instruments Limited, Malvern, UK). Results were

reported as the equivalent volume mean diameter D[4.3] and percentile particle size d(0.5).

Phytic acid content

Phytate content was determined by the method described by (Haug & Lantzsch, 1983) and modified by Peng et al. (2010). Stock solution with 1.3 mg/mL phytic acid concentration was prepared and diluted with 0.2 M hydrochloric acid in the range of 0.1-1 mL (3.16-31.6 µg/mL phytate phosphorus). Ferric solution was prepared by dissolving 0.2 g of ammonium iron (III) sulphate dodecahydrate in 100 mL of 2 M hydrochloric acid and made up to 1 L with distilled water. The 2,2'-bipyridine solution was prepared by dissolving 10 g of 2,2'-bipyridine and 10 mL of thioglycolic acid in distilled water and made up to 1 L. An amount of 50 mg of sample was mixed with 10 mL of 0.2 M hydrochloric acid and left overnight at 4 °C to prepare the extract. 1 mL of ferric solution and 500 µL of the extract were added in a tube. It was incubated in a boiling water bath for 30 min and then cooled to room temperature. The tube was centrifuged for 30 min at 3000 ×*g* and 1 mL of the supernatant was taken and mixed with 1.5 mL of 2,2'-bipyridine solution. The absorbance was measured at 519 nm against distilled water. A calibration curve was performed using a phytate reference solution. Assays were done in triplicate, and the results were expressed as mg phytic acid (PA)/g dry basis.

Phenolic compounds by HPLC analysis

Phenolic compounds were extracted according to the methodology proposed by Caprioli et al. (2018) and Giusti et al. (2019). To perform acid hydrolysis, 2.5 g of sample was weighed, and 7.5 mL of the extraction solvent (70:30 mixture of ethanol and bi-distilled water) was added. pH was adjusted with 2 M hydrochloric acid to pH

2 and subjected to an ultrasonic bath (J.P. Setecta, 3000840) for 2 h at room temperature. Samples were centrifuged at 8000 ×*g* for 15 min. The extraction was repeated twice. Both extracts obtained were pooled and filtered with a 0.45 µm PTFE filter, and subsequently, the free phenolic fraction was analysed by HPLC.

To carry out the alkaline hydrolysis, 14 mL of a mixture of 2 M sodium hydroxide with 0.01% 10 mM EDTA and 0.1% ascorbic acid was added to the acid hydrolysis residue (sediment) and left overnight to release bound phenolic esters or ethers. The pH was adjusted to 2 with 6 M hydrochloric acid and centrifuged at 8000 ×*g* for 15 min. Afterward, 15 mL of a mixture of ethyl acetate and diethyl ether in a 50:50 ratio and centrifuged at 5400 ×*g* for 10 min and repeated twice. Both organic phases were pooled and concentrated in a rotary evaporator (Heidolph, Kelheim, Germany) at 25 °C. The concentrate was reconstituted with 10 mL of methanol, filtered with a 0.45 µm PTFE filter, and analysed by HPLC.

The obtained extracts were analysed using an HPLC 1200 Series Rapid Resolution coupled to a diode detector Serie (Agilent, Palo Alto, USA) following the methodology explained by Tanleque-Alberto et al. (2020). Phenolic compounds were separated on Brisa-LC 5 µm C18 column (250 x 4.6 mm) (Teknokroma, Barcelona, Spain). Mobile phase A was 1% formic acid, and mobile phase B was acetonitrile (ACN). The following gradient program was established: 0 min, 90% A; 25 min, 40% A; 26 min, 20% A; holding until 30 min; 35 min, 90% A; holding until 40 min. The column working temperature, the flow rate and the injection volume were 30 °C, 0.5 mL/min, and 10 µL, respectively. Unknown compounds were identified by comparing the resulting chromatographic retention times with those of reference standards at the following wavelengths for each compound: 250 nm for vanillic acid; 260 nm for 4-hydroxybenzoic acid, rutin, quercitin 3-glucoside and quercitrin; 280 nm for gallic

acid, epicatechin, quercetin and trans-cinnamic acid; 290 nm for naringenin; 320 nm for 4-O-caffeoylquinic, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid and apigenin-7-glucoside; 380 nm for kaempferol. Quantification of the identified compounds was carried out using a calibration curve by linear regression analysis of the area under the curve versus their concentration, and the results were calculated in µg/g dry basis.

Total phenolic content

The total phenol content was determined with the Folin-Ciocalteu method described by Chang et al. (2006) using the same extract described above for acid extraction in the section of phenolic compounds by HPLC analysis. An aliquot of 125 µL of the extract was taken, and 500 µL of bi-distilled water was added, followed by 125 µL of folin ciocalteu reagent and left to react for 6 min. 1.25 mL of 7% sodium carbonate solution and 1 mL of bi-distilled water were added to complete a final volume of 3 mL. The mixture was incubated at room temperature in the dark for 30 min, and the absorbance was measured at 760 nm in a UV/Vis spectrophotometer. A standard curve for gallic acid was used, and the results were expressed as mg gallic acid (GA)/g dry basis.

Antioxidant activity

Antioxidant activity of the samples was determined by three different methods: ABTS, DPPH, and FRAP according to the methodology proposed by Thaipong et al. (2006) with some modifications. The same acid extract described above was used.

For ABTS assay, stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulphate were prepared, and then the working solution was prepared by mixing

the two stock solutions in a 1:1 ratio and leaving them to react for 12 h at room temperature in darkness. After reaction, 1 mL of fresh working solution is diluted with 60 mL of methanol to obtain an absorbance close to 1.1 at 734 nm in a UV/Vis spectrophotometer. An aliquot of 150 µL of the acidic extract was reacted with 2.85 mL of ABTS working solution for 2 h in the dark and the absorbance was measured at 734 nm.

For DPPH assay, a fresh working solution of 0.039 g/L DPPH in pure methanol was prepared to obtain an absorbance close to 1.1 at 515 nm in a UV/Vis spectrophotometer. An aliquot of 75 µL of the extract was reacted with 2.925 mL of DPPH working solution for 30 min in the dark and the absorbance was measured at 515 nm.

For FRAP assay, stock solutions of 300 mM acetate buffer (3.1 g sodium acetate trihydrate and 16 mL acetic acid glacial in 1 L water), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid, and 20 mM iron (III) chloride hexahydrate solution were prepared. Fresh working solution was prepared by mixing acetate buffer, TPTZ solution, and iron (III) chloride hexahydrate solution in a 10:1:1 ratio, respectively, and incubated at 37 °C before use. An amount of 150 µL of the extract was reacted with 2.85 mL of FRAP working solution for 30 min in the dark and the absorbance was measured at 593 nm.

In all three methods for antioxidant activity determination, a trolox standard curve was used, and the results were expressed as mg trolox/g dry basis.

The overall antioxidant potency composite index (APCI) was determined by assigning all antioxidant activity assays (ABTS, DPPH, and FRAP) an equal weight by assigning an antioxidant index value of 100 to the highest sample score in each assay,

and then calculating an antioxidant index for the other samples in each assay according to the following equation (Equation 5.3) (S. Sharma et al., 2022):

$$Antioxidant\ index\ (\%) = \left(\frac{sample\ score}{highest\ sample\ score}\right) \times 100 \qquad (5.3)$$

Finally, the APCI was calculated by averaging each antioxidant activity assay's antioxidant index (%) for each sample.

Angiotensin-converting enzyme inhibitory activity (ACE ia (%))

ACE ia *(%)* of the samples was determined according to the method described by (Akillioğlu & Karakaya (2009) and Hernández-Olivas et al. (2022). A double extraction of the protein was performed by mixing 5 g of the sample with 45 mL of distilled water, and the pH was adjusted to 11 and centrifuged at 10000 ×*g* at 4 °C for 20 min. Supernatants were pooled, and the pH was adjusted to the isoelectric point (4.5), kept in gentle agitation for 1.5 h at 4 °C, and centrifuged at 10000 ×*g* at 4 °C for 20 min. The sediment was dissolved in 50 mM phosphate buffer, pH=7. Extracts were analysed immediately, otherwise, they were stored at -40 °C.

ACE reactive (25 mU/mL) and the substrate Hip-His-Leu (5 mM) were dissolved in 0.15 M Tris base buffer, containing 0.3 M sodium chloride, and pH adjusted at 8.3. Three controls (100 µL ACE + 40 µL distilled water; 140 µL distilled water; 40 µL sample extract +100 µL distilled water) were included together with the samples (100 µL ACE + 40 µL sample extract) and then incubated at 37 °C for 5 min. 100 µL substrate to each tube was added, and the incubation was continued for 30 min at the same temperature. 150 µL of 1 M hydrochloric acid was added to stop the reaction. 1 mL ethyl acetate was added and mixed vigorously in a vortex mixer. Samples were centrifuged 1200 ×*g* for 10 min, and 750 µL of the supernatant were

collected and placed into clean tubes. Ethyl acetate contained in the supernatant was evaporated by gentle shaking at 80 °C. Solid hippuric acid contained in the tubes was dissolved in 1 mL of distilled water, and the absorbance was measured at 228 nm. ACE ia (%) was calculated according to the following equation (Equation 5.4):

$$ACE\ ia\ (\%) = 100 - \left\{100 \times \frac{C - D}{A - B}\right\} \qquad (5.4)$$

Where: A, B, C, and D are the absorbance of ACE + distilled water, distilled water, ACE + sample extract, and sample extract + distilled water, respectively.

Microbiological analysis

Samples of the different flours were collected aseptically to perform the corresponding microbiological analysis. For this, 1 g of each flour was diluted in 9 mL of sterile distilled water, and serial decimal dilutions were made. For the investigation of total aerobic mesophilic bacteria counts, mold and yeast count, and Escherichia coli count, 0.1 mL of each serial dilution were plated onto Plate-count agar, Sabouraud dextrose agar with 50 mg/L of Chloramphenicol and Tryptone Bile X-Glucuronide agar (TBX chromogenic selective medium) and incubated at 30 °C for 72h, 25 °C for 5-7 days, and 44 °C for 24h, respectively. Listeria monocytogenes and Salmonella spp. detection analysis were performed according to ISO 6579-1:2017 and ISO 11290-1:2017.

2.6. Statistical analysis

Experiments were carried out in triplicate and data were reported as mean ± standard deviation. One-Way ANOVA and Multiple Range Tests by the LSD procedure (least significant difference) of the Fisher test was performed to study possible

differences between different drying temperatures using the statistical program Statgraphics Centurion version XV (Rockville, MD, USA) with a confidence level of 95% (*p-value* < 0.05).

3. Results and discussion

3.1. Changes in proximal composition induced by fungal solid-state fermentation of lentils.

It is well known that legumes can be nutritionally modified by processing such as soaking, cooking, or dehydration among others. Processed legumes show increases in protein digestibility, available starch, and soluble fibre or important decreases in antinutritional factors such as phytic acid (Aguilera et al., 2010). Solid-state fermentation processing implies a sequence of unit operations such as soaking and sterilising the substrate prior to the fermentation itself, so the nutritional properties of fermented legumes are expected to be different. Fungal solid-state fermentation of lentils with *Pleurotus ostreatus* provokes different changes depending on the characteristics of the initial substrates, mainly the lentil cultivar, which implies significant differences in composition, structure, seed coat to cotyledon ratio or seed size (Chawla et al., 2017; Espinosa-Páez et al., 2017; Garrido-Galand et al., 2021; Mora-Uzeta et al., 2019). Table 5.4 shows the proximal composition of the substrates prior to and after the SSF process of both cultivars, as well as the biomass production, pH, and water activity. Since moisture content is the most affected component due to the conditioning process prior to inoculation and fermentation, the results are shown on a dry basis. Despite the slight differences between Pardina and Castellana cultivars in terms of initial composition, a clear difference in terms of biomass production was observed, being 14.1 and 61.8 mg of glucosamine/g dry basis, respectively, although the impact on the overall protein is not significant. These

results point out that Castellana lentil is a better substrate for SSF with *Pleurotus ostreatus,* and the explanation could be found in the morphometric characteristics of these two cultivars. Plaza et al. (2021) characterised the main Spanish lentil cult. They found that all of them, including the Castellana cultivar, showed a medium elliptic shape except for the cultivar Pardina which was classified as wide elliptic. The same authors reported the average weight (Pardina: 3.58 ± 0.24 and Castellana: 5.91 ± 0.15) and diameter (Pardina: 4.43 ± 0.09 and Castellana: 5.86 ± 0.09) that are morphometric properties affecting the density and porosity of the fermentation bed, and the growing ability of mycelium.

Pleurotus is a lignocellulosic fungus, which means that it can depolymerize a complex structure made of cellulose, hemicelluloses, and lignin. That explains the reduction of total fibre observed in fermented samples of both cultivars (Table 5.4), especially the insoluble fraction. In addition, this process is also reflected in the increase in reducing sugars in both samples. In the case of the Castellana lentil, this increase is much higher, probably due to the greater amount of biomass present. The increase in the soluble fraction could be attributed to a solubilization process provoked by soaking and heating the substrates before inoculation. Aguilera et al. (2010) observed similar results in Pardina lentils submitted to industrial dehydration processing, including previous soaking and cooking steps. The same authors observed lower ash values in processed flours than in raw lentils, coinciding with the ash reduction observed in fermented lentils, probably due to mineral losses during thermal processing in both cases.

Table 5.4. Proximate composition (g/100 g dry basis) in Pardina (P) and fermented Pardina (FP), and Castellana (C) and fermented Castellana (FC).

	Pardina (P)	Fermented Pardina (FP)	Castellana (C)	Fermented Castellana (FC)
Moisture	6.78 ± 0.06^{a}	118.25 ± 0.17^{b}	8.66 ± 0.05^{a}	156.4 ± 0.3^{b}
Protein	23.78 ± 0.4^{a}	24.1 ± 0.6^{a}	26.4 ± 0.2^{a}	26.9 ± 0.4^{a}
Lipids	1.06 ± 0.09^{a}	1.31 ± 0.11^{b}	1.38 ± 0.06^{a}	1.68 ± 0.13^{b}
Ashes	2.74 ± 0.02^{b}	2.481 ± 0.014^{a}	3.35 ± 0.02^{b}	2.596 ± 0.009^{a}
Total Carbohydrates*	72.32 ± 0.4^{a}	72.1 ± 0.7^{a}	68.9 ± 0.2^{a}	68.9 ± 0.5^{a}
Reducing sugars	0.22 ± 0.01^{a}	0.92 ± 0.08^{b}	0.22 ± 0.02^{a}	2.94 ± 0.18^{b}
Total fibre	16.0 ± 0.2^{b}	14.3 ± 0.2^{a}	17.3 ± 0.2^{b}	13.6 ± 0.3^{a}
Soluble fibre	1.39 ± 0.10^{a}	2.25 ± 0.10^{b}	1.94 ± 0.10^{a}	1.78 ± 0.10^{a}
Insoluble fibre	15.0 ± 0.3^{b}	12.26 ± 0.10^{a}	15.1 ± 0.2^{b}	11.5 ± 0.2^{a}
Biomass**	-	14.1 ± 1.5	-	61.8 ± 1.4
pH	6.457 ± 0.006^{a}	7.217 ± 0.006^{b}	6.603 ± 0.006^{b}	6.520 ± 0.010^{a}
a_w	0.367 ± 0.003^{a}	0.9798 ± 0.0011^{b}	0.5084 ± 0.0012^{a}	0.9736 ± 0.0008^{b}

Results represent the mean of three repetitions with their standard deviation. ^{a,b} Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level. *Carbohydrates calculated by difference; **(mg glucosamine/g dry basis).

3.2. Air drying kinetics of fermented lentils.

Fermented lentils were dried at different inlet air temperatures for modelling purposes. The drying curves obtained for both cultivars (Figure 5.4) revealed faster drying kinetics for Castellana lentils than Pardina. These results are in accordance with the results observed during soaking treatment before fermentation, on which water uptake was more rapid in Castellana lentils as reveals the higher moisture content of Castellana fermented lentils (Table 5.4) despite the same soaking time for both cultivars. Additionally, the impact of air-drying temperature is higher in Pardina lentil.

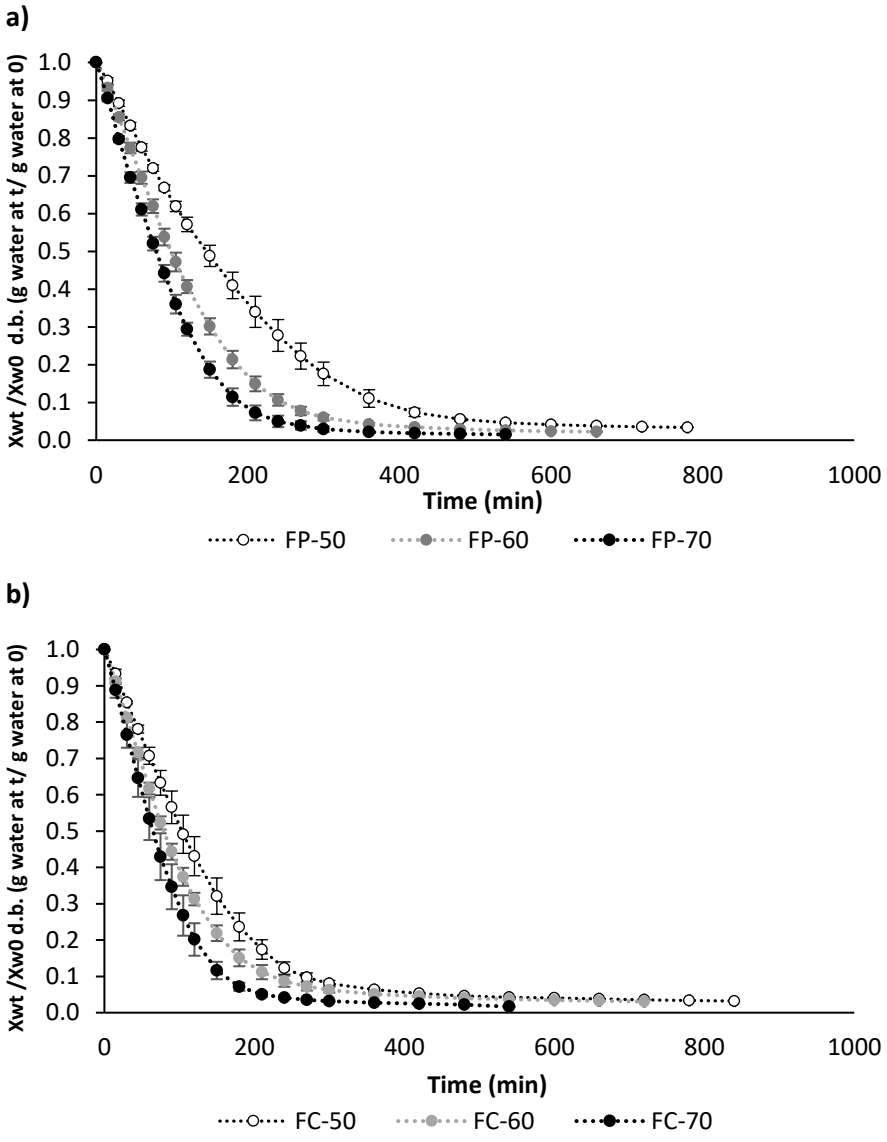


Figure 5.4. Air-drying curves (X^w_t/X^w_0 (g water at t/g water at 0)) at 50, 60 and 70 °C of: (**a**) fermented Pardina (FP), and (**b**) fermented Castellana (FC).

In the case of the Pardina lentil, drying with air at 50 °C demands approximately 9 h until the product reaches a constant weight. This time is reduced to 7 and 5.5 h when drying at 60 °C and 70 °C, respectively. In the case of the Castellana variety, the same tendency occurs, needing 8 and 6 h to get a constant weight after drying at the lowest temperatures. Increasing the temperature to 70 ◦C decreased the time required to 4.5 h. Although when obtaining a functional food ingredient, it is necessary to consider the effect of air-drying temperature on bioactive compounds (phenols and antioxidants) content, processing time, and total energy consumption are key factors when setting up an industrial process. In this sense, drying at 70 °C would provide faster drying.

Air-drying curves were fitted following the Lewis model, and the constants and statistical coefficients of the model are shown in Table 5.5. In all cases, the R squared is superior to 0.97. Besides, the parameter K, related to the drying rate, shows a linear correlation with the air temperature. In the case of Pardina lentil, the slope was 0.012, the intersection -0.348, and the coefficient of determination was 0.9974. On the other hand, for Castellana lentil, the slope was 0.010, the intersection -0.240, and the coefficient of determination was 0.9601.

These parameters were used to estimate the drying time needed at each temperature to obtain fermented lentil flours with 7% final moisture content.

Table 5.5. Drying kinetic parameters k (min^{-1}) and R squared at different temperatures.

Temperature	**Fermented Pardina**		**Fermented Castellana**	
(°C)	**k**	**R^2**	**k**	**R^2**
50	0.2218	0.9788	0.2854	0.9746
60	0.3467	0.9771	0.3519	0.9768
70	0.4514	0.9698	0.4910	0.9732

On the other hand, drying affects the microbiological quality of the product regardless of the air-drying properties. In fact, dehydration of the product reduces the amount of water available for microbial growth leading to microbial inhibition or even death. However, the temperature and speed of air drying can affect the rate of destruction of microbes. The microbiological quality parameters (Table 5.6) demonstrate that the obtained flours fit the minimal safety requirements.

Table 5.6. Microbiological analysis in Pardina flour (PF) and Castellana flour (CF), and fermented Pardina flour (FPF) and fermented Castellana flour (FCF) dried by hot air (50, 60 and 70 °C) and lyophilisation (L).

	Aerobic mesophilic bacteria count (UFC/g)	**Yeast and mould count (UFC/g)**	***E. coli* count (UFC/g)**	***Salmonella* spp. detection (Presence /Absence)**	***Listeria monocytogenes* (Presence /Absence)**
Pardina lentil					
PF	$2.5x10^3$	$<10^2$	$<10^2$	Absence	Absence
FPF-50	$5x10^2$	$1x10^2$	$<10^2$	Absence	Absence
FPF-60	$<10^2$	$<10^2$	$<10^2$	Absence	Absence
FPF-70	$<10^2$	$<10^2$	$<10^2$	Absence	Absence
FPF-L	$3x10^2$	$<10^2$	$<10^2$	Absence	Absence
Castellana lentil					
CF	$6x10^2$	$<10^2$	$<10^2$	Absence	Absence
FCF-50	$4x10^2$	$<10^2$	$<10^2$	Absence	Absence
FCF-60	$1x10^2$	$<10^2$	$<10^2$	Absence	Absence
FCF-70	$<10^2$	$<10^2$	$<10^2$	Absence	Absence
FCF-L	$4x10^2$	$<10^2$	$<10^2$	Absence	Absence

3.3. Impact of processing on particle size, color, and phytic acid of fermented flours.

The drying conditions frequently affect the structure of dried foodstuff (Guiné, 2018), which is why the particle sizes of the resulting flours were affected by the drying process used during the previous dehydration step. Figure 5.5 shows the

particle size distribution of fermented flours obtained by different drying processes for Pardina and Castellana cultivars.

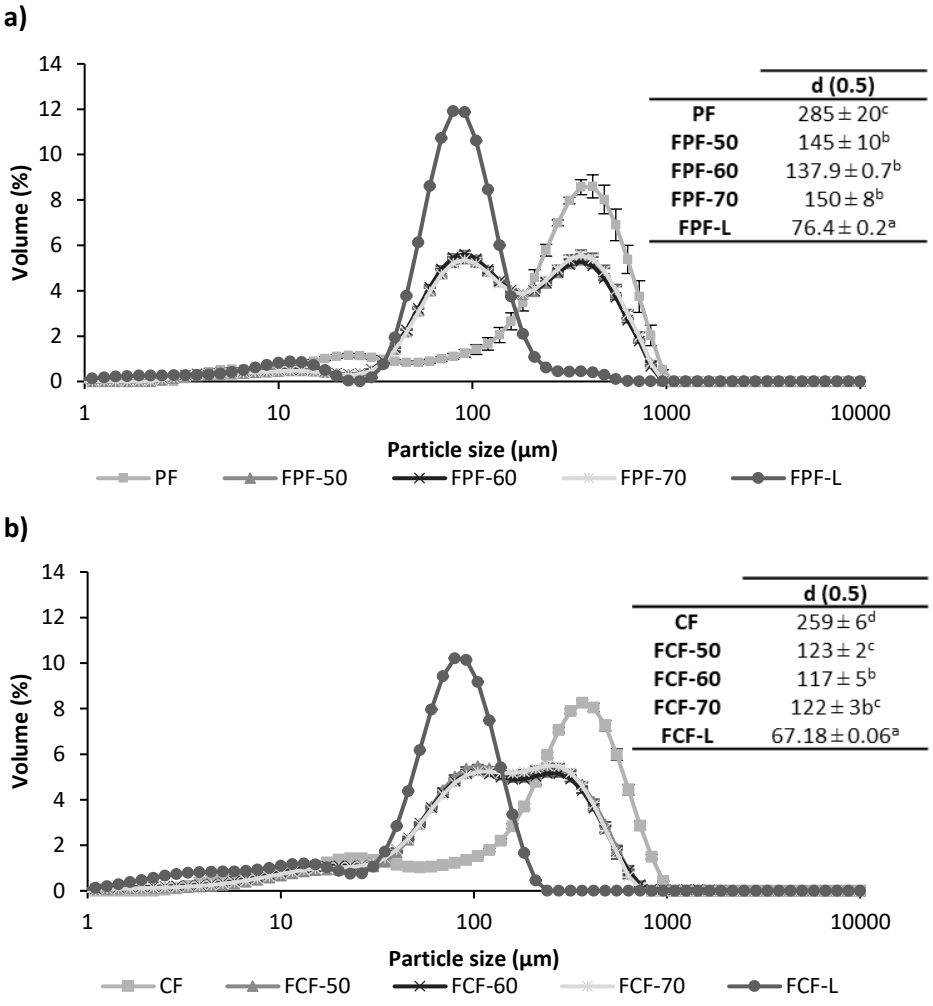


Figure 5.5. Particle size (volume (%)) in: (**a**) Pardina flour (PF) and fermented Pardina flour (FPF) obtained at different air-drying temperatures, and (**b**) Castellana flour (CF) and fermented Castellana flour (FCF) obtained at different air-drying temperatures. [a,b,c,d] Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level.

Those flours obtained from fermented and dried lentils have a smaller particle size than raw lentil flours. Choe et al. (2022) found the same results in thermally treated common bean flours, attributing this change to decreased seed hardness and less cohesiveness than the raw counterparts. In both cultivars, the same effect of fermentation and drying on the distribution and size of particles is observed. The drying method, air-drying or freeze-drying, significantly impacts the particle size, although the air-drying temperature does not affect this parameter. Raw lentil flour and fermented freeze-dried lentil flour present a monomodal distribution with an average particle size of 284 µm and 76.4 µm, respectively. Fermentation and hot-air drying shifted the particle size distributions towards smaller sizes, exhibiting a multimodal pattern. The reduction in particle size due to fermentation and drying was slightly more significant in the Castellana lentil flour. Nonetheless, Marchini et al. (2021) studied the influence of different particle sizes in red lentil flour for its use in bakery products. Although they found a slight influence on the water holding capacity decreasing as the particle size is smaller, the multivariable statistics demonstrated that the particle size of the lentil flours is not the major factor affecting the rheology. In this case, technologically speaking, the reduction in particle size due to fermentation and drying would not be a problem.

One of the parameters in the assessment of flour colour is the L* a* b* difference (ΔE). This parameter significantly increased with the combined process of SSF and drying (Table 5.7). It can be observed the substantial decrease in the L* value as well as the increase of the a* value compares to the unfermented flours in all cases. Changes in L* value and a* value could be related to Maillard reactions, caramelization, and/or pigment degradation (J.-Y. Yi et al., 2017) during the drying process. The drying method also significantly impacts the final colour of fermented

lentil flours, with the freeze-dried fermented samples having fewer colour changes compared with the unprocessed one.

Table 5.7. Colour of obtained flours from Pardina flour (PF) and Castellana flour (CF), fermented Pardina (FP) and fermented Castellana (FC), and fermented Pardina flour (FPF) and fermented Castellana flour (FCF) dried by hot air (50, 60 and 70 °C) and lyophilisation (L).

Pardina flours			Castellana flours		
	**L***	80.2 ± 0.3^{d}		**L***	82.86 ± 0.09^{d}
	a*	1.70 ± 0.06^{a}		**a***	0.905 ± 0.007^{a}
	b*	15.5 ± 0.2^{a}		**b***	18.34 ± 0.10^{e}
	C*	15.5 ± 0.2^{d}		**C***	18.36 ± 0.10^{e}
	h	83.72 ± 0.15^{d}		**h**	87.175 ± 0.008^{c}
PF	**ΔE**	-	**CF**	**ΔE**	-
	**L***	61.9 ± 0.2^{b}		**L***	58.7 ± 0.4^{a}
	a*	4.42 ± 0.03^{c}		**a***	7.18 ± 0.11^{d}
	b*	11.22 ± 0.09^{ab}		**b***	15.63 ± 0.09^{b}
	C*	12.06 ± 0.10^{ab}		**C***	17.20 ± 0.13^{b}
	h	68.49 ± 0.04^{b}		**h**	65.3 ± 0.2^{a}
FPF-50	**ΔE**	18.96 ± 0.41^{b}	**FCF-50**	**ΔE**	25.1 ± 0.4^{c}
	**L***	61.73 ± 0.06^{ab}		**L***	60.4 ± 0.2^{b}
	a*	4.30 ± 0.02^{b}		**a***	6.972 ± 0.012^{c}
	b*	10.91 ± 0.05^{a}		**b***	16.18 ± 0.08^{c}
	C*	11.73 ± 0.06^{a}		**C***	17.61 ± 0.07^{c}
	h	68.48 ± 0.03^{b}		**h**	66.68 ± 0.14^{b}
FPF-60	**ΔE**	19.20 ± 0.23^{b}	**FCF-60**	**ΔE**	23.4 ± 0.2^{b}
	**L***	61.3 ± 0.3^{a}		**L***	58.37 ± 0.14^{a}
	a*	4.43 ± 0.07^{c}		**a***	7.44 ± 0.02^{e}
	b*	11.44 ± 0.19^{b}		**b***	16.38 ± 0.09^{d}
	C*	12.3 ± 0.2^{b}		**C***	17.99 ± 0.07^{d}
	h	68.83 ± 0.02^{c}		**h**	65.57 ± 0.17^{a}
FPF-70	**ΔE**	19.51 ± 0.46^{b}	**FCF-70**	**ΔE**	25.4 ± 0.2^{c}

	**L***	62.5 ± 0.3^{c}		**L***	63.01 ± 0.04^{c}
	a*	5.01 ± 0.09^{d}		**a***	6.51 ± 0.02^{b}
	b*	12.6 ± 0.3^{c}		**b***	14.32 ± 0.03^{a}
	C*	13.6 ± 0.3^{c}		**C***	15.73 ± 0.03^{a}
	h	68.30 ± 0.09^{a}		**h**	65.55 ± 0.06^{a}
FPF-L	**ΔE**	18.23 ± 0.17^{a}	**FCF-L**	**ΔE**	21.02 ± 0.03^{a}

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e} Different lowercase letters indicate significant differences for each parameters L*, a*, b*, C*, h, and ΔE for Pardina and Castellana with a 95% ($p < 0.05$) significance level.

The effect of SSF and drying on the final colour of flours was higher in Castellana variety than in Pardina, especially in the a* coordinate, which increases significantly. Similar results were found for dried apples (Djekic et al., 2018), where the colour differences varied between 11.37 in the case of the freeze-dried apple and 21.11 in the case of air-drying at 60 °C. These differences in colour may be due to non-enzymatic Maillard reactions. Furthermore, the porous structure of dried products also differs, affecting mainly lightness of material due to the presence of air voids and pores (Nowak & Jakubczyk, 2020).

3.4. Impact of processing on antioxidant and anti-hypertensive properties of fermented flours.

The potential health benefits of lentils have been attributed to secondary metabolites such as phenolic compounds, which exhibit antioxidant properties. These compounds can reduce the activity of reactive oxygen species by different mechanisms (scavenging the free radicals generated, complexing pro-oxidant metals, and quenching singlet oxygen) (Aguilera et al., 2010; Madhujith & Shahidi, 2005; Ranilla et al., 2009). Table 5.8 shows the antioxidant activity evaluated by ABTS, DPPH, and FRAP, together with each antioxidant indexes, the APCI, and total phenol content.

Table 5.8. Antioxidant activity (mg trolox/g dry basis) by ABTS, DPPH, and FRAP method, total phenol content (mg GA/g dry basis), and phytic acid content (mg PA/g dry basis) in Pardina flour (PF) and Castellana flour (CF), fermented Pardina (FP) and fermented Castellana (FC), and fermented Pardina flour (FPF) and fermented Castellana flour (FCF) dried by hot air (50, 60 and 70 °C) and lyophilisation (L).

	Antioxidant activity							Total phenol content	Phytic acid
	ABTS	ABTS index	DPPH	DPPH index	FRAP	FRAP index	APCI*		
Pardina lentil									
PF	9.5 ± 0.4^{d}	100	2.07 ± 0.09^{c}	100	7.62 ± 0.17^{b}	100	100	3.8 ± 0.2^{c}	9.7 ± 0.7^{b}
FP	5.7 ± 0.5^{c}	60.7	0.64 ± 0.04^{b}	30.8	0.311 ± 0.019^{a}	4.09	31.9	2.10 ± 0.08^{b}	7.3 ± 0.6^{a}
FPF-50	3.81 ± 0.10^{b}	40.3	0.49 ± 0.03^{a}	23.6	0.30 ± 0.02^{a}	3.9	22.6	2.21 ± 0.09^{b}	7.4 ± 1.4^{a}
FPF-60	4.03 ± 0.19^{b}	42.7	0.486 ± 0.016^{a}	23.5	0.32 ± 0.02^{a}	4.2	23.5	2.39 ± 0.19^{b}	7.6 ± 1.2^{a}
FPF-70	3.91 ± 0.16^{b}	41.4	0.516 ± 0.010^{a}	25	0.351 ± 0.007^{a}	4.6	23.7	2.37 ± 0.12^{b}	7.7 ± 0.9^{a}
FPF-L	3.20 ± 0.04^{a}	33.9	0.502 ± 0.014^{a}	24.3	0.310 ± 0.16^{a}	4.07	20.7	1.59 ± 0.08^{a}	8.0 ± 1.4^{ab}
Castellana lentil									
CF	8.4 ± 0.4^{d}	100	1.634 ± 0.015^{bc}	72	8.3 ± 0.2^{e}	100	90.7	4.13 ± 0.10^{c}	7.3 ± 0.3^{d}
FC	2.50 ± 0.09^{a}	29.9	2.27 ± 0.13^{d}	100	1.10 ± 0.03^{a}	13.4	47.7	2.75 ± 0.11^{a}	0.9 ± 0.5^{c}
FCF-50	6.1 ± 0.3^{c}	73.2	1.61 ± 0.02^{b}	70.8	6.21 ± 0.19^{c}	75.2	73.1	6.9 ± 0.3^{d}	0.4 ± 0.4^{bc}
FCF-60	5.48 ± 0.07^{b}	65.4	1.568 ± 0.016^{b}	69.07	6.3 ± 0.2^{c}	76.2	70.2	7.13 ± 0.12^{e}	0.21 ± 0.07^{b}
FCF-70	6.2 ± 0.2^{c}	73.9	1.71 ± 0.02^{c}	75.1	7.0 ± 0.3^{d}	85.3	78.1	7.71 ± 0.15^{f}	0.02 ± 0.01^{a}
FCF-L	2.32 ± 0.16^{a}	27.7	1.093 ± 0.016^{a}	48.2	2.14 ± 0.05^{b}	25.9	33.9	3.42 ± 0.02^{b}	0.26 ± 0.04^{b}

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e,f} Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level. *APCI: Antioxidant potency composite index.

The results demonstrate significant differences between the phenolic compounds due to the processing (Table 5.8). It was found that processed Pardina samples were characterised by reduced phenolic content. At the same time, this parameter was higher for the all hot-air dried Castellana variety samples without a significant effect on temperature.

Comparing drying methods, freeze-drying implied a higher decrease in total phenol content than hot-air drying, regardless the variety. Similar results are found in literature when studying different legumes. In the case of bean sprouts, the total phenol content after being treated by hot-air drying was always significantly higher than that found when treated by freeze-drying either at low temperatures (20 °C) or high temperatures (80 °C) (R.-Y. Gan et al., 2017). Same tendency was also found for pinto beans (Anton et al., 2008). This reduction has been observed in other processes, such as boiling, and could be attributed to compound destruction, oxidation or chemical rearrangement involving binding with other compounds (S. Sharma et al., 2022). Aguilera et al. (2010) reported similar results under ordinary boiling for Pardina lentils. On the other side, heat treatments were expected to increase TPC compounds due to the partial destruction of cellular structure and then the release of bound compounds and/or the formation of Maillard reaction products with phenol and reducing agents above 40 °C (Zou et al., 2018). Hot-air drying better retained phenolic compounds or undergone a higher release of those than freeze-drying in lentils. Regarding the antioxidant activity (ABTS, DPPH, and FRAP assays), similar trends were found evidencing the relationship on these parameters and TPC (Table 5.8). Thus, fermentation and drying led to significant ($p < 0.05$) reduction in these activities with some exceptions. Only for Castellana lentils, an increase of FRAP and ABTS antioxidant activity was found in fermented flours due to hot-air drying.

DPPH antioxidant activity, however, was the only parameter experimented a rise because of fermentation for subsequently being negatively affected by drying. Que et al., 2008) studied the influence of hot air-drying and freeze-drying on the antioxidant activities of pumpkin flours. They showed significantly higher total antioxidant activity in hot air-dried pumpkin flour than in freeze-dried flour. The authors attributed this to the creation of Maillard products or their intermediates with potent antioxidant activity. In our case, the initial content of reducing sugars is much higher in fermented Castellana than in fermented Pardina samples (see Table 5.4). This high concentration of reducing sugars can generate a greater number of compounds with greater antioxidant capacity after undergoing the Maillard reaction. Finally, among the processed flours, the highest APCI was obtained for fermented Castellana and Pardina flours air-dried at 70 °C with an APCI of 78.1% and 23.7%, respectively.

Results of chromatogram profiles and concentrations of phenolic compounds identified, phenolic acids and flavonoids, are shown in Tables 5.9 and 5.10 for Pardina and Castellana samples, respectively. In all cases, two extractions were made to obtain phenolic compounds taking part in the free and bound fractions. However, in the case of Pardina samples, only unquantifiable traces were found for p-Coumaric acid, Epicatechin and Ferulic acid for the second extraction (bound fraction). In the case of Castellana lentil, gallic acid, 4-Hydroxybenzoic acid, and p-Coumaric acid as bonded phenols were detected. In this last case, both results were summed. Therefore, it can be said that most of the phenols are in their free form in the samples because of the native form or due to a release along processing, fermentation and/or drying. This fact and the reduction in most phenols revealed that a destruction of these compounds is the main mechanism that occurred instead of rearrangement with other molecules or chemical conversion into other phenolic derivates in

Pardina. The losses in Pardina samples are consequence of the great reduction of 4-O-caffeoylquinic and epicatechin, the most abundant phenolic acid and flavonoid in raw Pardina lentil, respectively, along fermentation and further drying. Only a significant increase of vanillic acid and generation of trans-Cinnamic acid were found due to fermentation and hot-air drying. For Castellana samples, a notable increase in free gallic acid was given during fermentation and drying, together with a drastic decrease in rutin and disappearing of epicatechin, among the most abundant compounds. As a result of these variations, a net increase of phenols was found in fermented hot-air dried samples, being more accused as long as the drying temperatures increases.

It has been reported the capability of some fungus genus, such as *Rhizopus* or *Aspergillus,* to synthesise microbial enzymes, tannases, able to release low-molecular weight phenolic acids from tanninic complex molecules (Aguilar-Zárate et al., 2014; Bajpai & Patil, 2008). This fact together with the heating-induced depolymerization of condensed tannins could be also responsible of the increase of some phenolic acids found in Pardina and Castellana fermented and dried samples.

On the other hand, legumes are rich in phytates, which has traditionally been considered a disadvantage since these compounds (present in legumes, whole grains, seeds, and nuts) hinder the absorption of specific vitamins and minerals (especially calcium, iron, and zinc). However, processing legumes are usually used to reduce antinutrients in legumes, and in this study, phytic acid content was analysed to assess the impact of SSF and drying temperature (Table 5.8). The initial content of phytic acid in the raw flours was within the range of the values published for these two cultivars (Thavarajah et al., 2009), and in both cases, they were much lower than the harmful range of 10-60 mg/g (Farinde et al., 2018) .

Table 5.9. Phenolic content (µg/g dry basis) in raw Pardina flour (PF), fermented Pardina (FC), dried at 50, 60, and 70 °C (FPF-50, FPF-60, FPF-70), and lyophilised (FPF-L).

	PF	FP	FPF-50	FPF-60	FPF-70	FPF-L
Phenolic acids						
Gallic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	10.6 ± 0.4^{BC}	n.d.	n.d.	n.d.	n.d.	n.d.
p-Coumaric acid	7.8 ± 0.2^{bABC}	9.47 ± 0.04^{dC}	8.5 ± 0.5^{cC}	10.2 ± 0.5^{dC}	9.5 ± 0.2^{dC}	5.2 ± 0.1^{aA}
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	264 ± 6^{dG}	143.5 ± 0.06^{cF}	121 ± 3^{aG}	123 ± 2^{aF}	127 ± 3^{bF}	152 ± 5^{cD}
4-Hydroxybenzoic acid	11.0 ± 0.2^{aC}	11.78 ± 0.03^{aD}	11.0 ± 0.3^{aD}	11.2 ± 0.3^{aC}	10.7 ± 0.3^{aC}	10.1 ± 0.1^{aB}
Vanillic acid	23.0 ± 0.3^{aE}	34.7 ± 0.9^{bE}	47 ± 3^{cF}	47 ± 2^{cE}	46 ± 4^{cE}	34 ± 2^{bC}
Ferulic acid	6.6 ± 0.2^{cA}	5.3 ± 0.1^{bA}	2.8 ± 0.01^{aA}	2.92 ± 0.03^{aA}	2.88 ± 0.06^{aA}	2.90 ± 0.03^{aA}
trans-Cinnamic acid	traces	6.6 ± 0.2^{bB}	20.78 ± 0.04^{cE}	29 ± 2^{dD}	26.8 ± 0.6^{dD}	4.98 ± 0.05^{aA}
Flavonoids						
Rutin	11.6 ± 0.8^{C}	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	71 ± 2^{F}	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	6.70 ± 0.02^{cAB}	traces	3.38 ± 0.05^{aAB}	3.30 ± 0.03^{aA}	3.2 ± 0.2^{aA}	3.76 ± 0.09^{bA}
Apigenin-7-glucoside	16.9 ± 0.1^{D}	traces	traces	traces	traces	Traces
Quercetin	5.7 ± 0.2^{cA}	11.2 ± 0.1^{dD}	5.89 ± 0.07^{aB}	6.05 ± 0.03^{aB}	5.95 ± 0.03^{aB}	5.76 ± 0.05^{bA}
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C,D,E,F,G} Different capital letters indicate significant differences ($p < 0.05$) between phenolic compounds. n.d.: not detected. Traces: not quantifiable.

Table 5.10. Phenolic content (µg/g dry basis) in raw Castellana flour (CF), fermented Castellana (FC), dried at 50, 60, and 70 °C (FCF-50, FCF-60, FCF-70), and lyophilised (FCF-L).

	CF	FC	FCF-50	FCF-60	FCF-70	FCF-L
Phenolic acids						
Gallic acid	45 ± 5^{aE}	67 ± 2^{bD}	143 ± 10^{dE}	129 ± 15^{dE}	181.6 ± 0.8^{eE}	102 ± 7^{cE}
Caffeic acid	13 ± 2^{bC}	8.2 ± 0.5^{aC}	8 ± 0.2^{aC}	7.6 ± 0.4^{aC}	8.1 ± 0.8^{aBC}	8.3 ± 0.5^{aC}
p-Coumaric acid	9 ± 0.1^{C}	traces	traces	traces	traces	Traces
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	4.5 ± 0.2^{aB}	9.8 ± 0.4^{bC}	12.1 ± 0.6^{cD}	12.2 ± 0.3^{cD}	10 ± 1^{bC}	12.5 ± 0.5^{cD}
Vanillic acid	2.7 ± 0.2^{A}	n.d.	n.d.	n.d.	n.d.	n.d.
Ferulic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trans-Cinnamic acid	n.d.	5.9 ± 0.7^{aB}	9.7 ± 0.2^{bCD}	11.5 ± 0.4^{cD}	14 ± 2^{dD}	5.2 ± 0.3^{aB}
Flavonoids						
Rutin	43.3 ± 0.2^{dF}	6.3 ± 0.7^{cB}	5.5 ± 0.1^{bB}	4.8 ± 0.2^{abB}	4.7 ± 0.4^{aB}	4.3 ± 0.3^{aB}
Epicatechin	19 ± 4^{D}	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	4.1 ± 0.1^{bB}	4.2 ± 0.2^{bA}	3.0 ± 0.2^{aA}	2.8 ± 0.1^{aA}	3.0 ± 0.5^{aA}	n.d.
Quercitrin	traces	traces	traces	traces	traces	3.2 ± 0.8^{A}
Apigenin-7-glucoside	3.4 ± 0.1^{aA}	traces	4.7 ± 0.1^{bB}	7.2 ± 0.3^{cC}	7.5 ± 0.9^{cB}	4.7 ± 0.3^{bB}
Quercetin	3.9 ± 0.1^{B}	n.d.	n.d.	n.d.	n.d.	n.d.
Naringenin	n.d.	n.d.	6 ± 1^{bB}	6.5 ± 0.2^{bC}	7.5 ± 0.9^{bB}	3.0 ± 0.1^{aA}
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

^{a,b,c,d,e} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C,D,E,F} Different capital letters indicate significant differences ($p < 0.05$) between phenolic compounds. n.d.: not detected. Traces: not quantifiable.

The reduction in phytic acid can be attributed to the hydrolysis by the endogenous phytase enzyme, which can be activated during processing. However, the reduction of these compounds because of processing (soaking, cooking, fermentation, drying, etc.) depends on the type of legume and the processing conditions (S. Sharma et al., 2022). In this study, significant differences between cultivars were observed in phytic acid reduction because of SSF. In contrast, the phytic acid content is almost negligible in fermented Castellana lentils; no significant differences were observed between raw and fermented Pardina lentils. This result reveals that probably endogenous phytase enzyme in Pardina lentils is quickly inactivated by the autoclaving of the substrate before the inoculation and incubation for fermentation. The impact of drying in phytic acid content on the fermented flour was also analysed (Table 5.8). No significant differences can be attributed to the drying process, temperature, or drying type. The early inactivation of endogenous phytases in the Pardina during the processing before drying could explain these results, while Castellana's endogenous phytase seems more resistant to processing inactivation.

However, it should be noted that a moderate-small amount of phytates in the diet is even beneficial since these compounds, in preclinical studies, have been shown to inhibit the proliferation of colon cancer cells (Barahuie et al., 2017). Phytic acid, specifically hexaphosphate (inositol), is receiving special attention because, in cell and animal studies, it has been shown to have an anticancer action (Schröterová et al., 2010; Shamsuddin, 2002) both in colon and prostate cancer (Gu et al., 2009) and leukemia cells, among others (Vucenik & Shamsuddin, 2006). Much remains to be investigated in this regard, but this shows that, in cooked or germinated legumes, there is no problem with a certain amount of phytates since it will hardly influence the absorption of nutrients and, on top of that, seems to bring us certain benefits.

Regarding the capacity of the samples to inhibit the angiotensin I-converting-enzyme (ACE), fermentation plus drying increased the potential cardiovascular benefits of the samples compared to their counterparts' raw lentils flours (Figure 5.6).

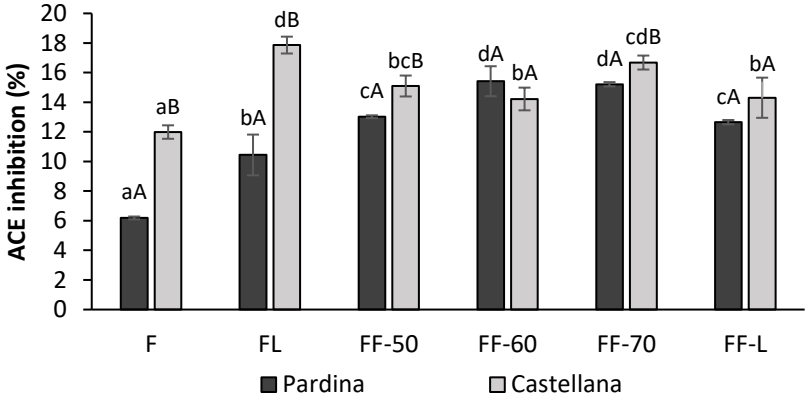


Figure 5.6. ACE inhibitory activity (%) values in Pardina and Castellana flour (F), fermented lentil (FL) and fermented flour (FF) dried by hot air (50, 60, and 70 °C) and lyophilisation (L). Results represent the mean of three repetitions with their standard deviation. [a,b,c,d] Different lowercase letters indicate significant differences between dried flours and [A,B] different capital letters indicate significant differences between lentil varieties with a significance level of 95% ($p < 0.05$).

The impact of processing was more notable on Pardina than the Castellana variety; even though Castellana fermented and dried flours exhibited a slightly, but statistically significant, higher ACE-inhibitory activity than Pardina samples. According to the literature, the ACE-inhibitory activity would correspond to protein fractions and mainly to low molecular weight peptides. Some plant proteins have been reported to be a source of various bioactive ACE-inhibitory peptides with anti-hypertensive activity. This is the case of some protein hydrolysates or isolates from soybean, bean, pea, sesame, rice and zein (Hong et al., 2008). Moreover, increased

dietary intake of plant protein was reported to exert a more beneficial effect on blood pressure compared to protein from animals (Hong et al., 2008). It is also noticeable how only the fermentation with *Pleurotus* significantly increases the ACE-inhibitory capacity in both varieties of lentils in about 5 percentage points. *Pleurotus ostreatus* is well known for its potential for ACE inhibition. Abdullah et al. (2012) reported that all *Pleurotus* fungi studied performed ACE activity mainly due to its protein content. The effect of the type of drying on ACE capacity also performs an influence. In our case, the lyophilisation performs lesser activity that the lentils dried at 70 °C. Piskov et al. (2020a) studied various drying methods on the ACE inhibition activity of *Pleurotus ostreatus* and concluded that *P. ostreatus* dried using freeze-drying performed lower ACE inhibitory capacity than the same mushroom dried with hot air in concordance with our data.

On the other hand, Mohamad Ansor et al. (2013) reported the capability of some mycelia such as of G*. lucidum* in lowering blood pressure levels. Apparently, four proteins (cystathionine beta synthase-like protein, DEAD/DEAH box helicase-like protein, paxillin-like protein, and alpha/beta hydrolase-like protein) derived from edible mushrooms would be responsible of the ACE inhibition. Therefore, the partial hydrolysis of native proteins along fermentation together with the mycelium could be responsible of an improvement in the benefits for cardiovascular health of the samples. Several studies have established a relationship between the chemical structure of peptides and their ability to inhibit ACE (Lin et al., 2023). Peptides with hydrophobic or aromatic terminal amino acids are more likely to interact with the active site of ACE and present highest ACE inhibitory activity. The presence of C-terminal aromatic amino acid residues and N-terminal hydrophobic amino acid residues can also enhance the peptide's activity in inhibiting ACE (Lin et al., 2023).

4. Conclusions

In the present study, the implications of solid-state fermentation (SSF) together with stabilization (by hot-air or freeze-drying) on some functional and technological properties, which play a relevant role in ingredients' quality, were evaluated in fermented lentil flours. The obtained results demonstrated the Castellana variety was the most suitable substrate for fungal solid-state fermentation with edible fungus *Pleurotus ostreatus*, with a significant reduction in antinutrient phytic acid (from 7.3 to 0.9 mg/g dry basis) as well as the insoluble fibre (15 to 11 g/100 g dry basis). Besides, the SSF increased the ACE inhibitory capacity in both varieties of lentils in about 5 percentage points. With respect to dehydration, hot-air drying, and specifically at 70 °C, entailed in an increase in total phenolic content, inhibition of ACE and lower phytic acid content in fermented Castellana lentil, compared to freeze-drying. Drying significantly decreased the particle size from around 270 μm to less than 150 μm reaching only 76.4 μm after lyophilisation. Fermented freeze-dried lentil flour presents a monomodal distribution in both varieties of lentil, while air-drying flours shifted the particle size distributions towards smaller sizes, exhibiting a multimodal pattern. Colour changes have also been notable after fermentation and drying with (ΔE>20), being more pronounced when lyophilising. Regarding the phenolic profile, it has been observed how fermentation changes the profile, decreasing some compounds and increasing others, such as p-coumaric acid, vanillic acid, and quercetin for the Pardina variety and gallic acid, trans-cinnamic acid and naringenin for the Castellana variety. Regarding the antioxidant capacity, the SSF negatively affected the antioxidant capacity of lentils, however, air-drying processing at 70 °C significantly increased the values obtained by ABTS and FRAP in the case of Castellana lentil flour. Therefore, fermented Castellana flours obtained by solid-state fermentation with *P. ostreatus* and air-dried at 70 °C might be considered a promised

rich protein ingredient with improved functionality and new optical properties. *In vitro* digestion evaluation, however, would be interested to go deep into the healthy benefits of these flours.

ARTÍCULO 3

ARTÍCULO 3: Fermented quinoa flour: Implications of fungal solid-state bioprocessing and drying on nutritional and antioxidant properties.

ABSTRACT

Quinoa (Chenopodium quinoa Willd) is a pseudocereal rich in protein and excellent source of vitamins, antioxidants, and minerals. Developing new quinoa-based products would be a strategy to extend its consumption. The aim of this study is to evaluate the impact of solid-state fermentation with *Pleurotus ostreatus* and drying (hot-air at 50, 60 and 70 ºC and lyophilisation) on the nutritional and functional properties of fermented white and black quinoa flours. Changes in proximal composition revealed that white variety was more suitable for solid-state fermentation (SSF), according to the higher biomass production, higher increase of protein (2.11%) and insoluble fibre (7.82%) content. SSF was highly effective in phytic acid reduction (90%) regardless the variety. However, after fermentation, quinoa seeds presented reduced total phenolic content (TPC) and antioxidant capacity, especially on black variety. Nonetheless, hot-air drying improved the antioxidant properties (increased TPC and antioxidant capacity) and promoted changes in the phenolic profile. These changes were characterized by a release of gallic acid together with a notable reduction of vanillic, caffeic and ferulic acids, quercetin-3-glucoside and/or quercetin. A notable increase of the angiotensin converting enzyme-I inhibitory activity was found on the fermented flours mainly due to SSF.

Keywords: fermentation, *Pleurotus ostreatus*, colour, particle size, antioxidants.

1. Introduction

The increase in the world population, together with the negative impact of climate change on the productivity of arable land, could cause less accessibility to food in the next future (Ray et al., 2019). This fact promotes the search for cultivable species that supply the population with nutrient-rich foods, especially those in vegetal proteins. Fortunately, underexploited but nutritionally essential crops, such as pseudocereals, are highly resistant to hostile environments (drought, salinity, and extreme temperatures) and present high yields with limited resources (Rodríguez et al., 2020). Therefore, new pseudocereal-based ingredients could positively contribute to food sovereignty.

Among pseudocereals, quinoa (*Chenopodium quinoa Wild* Var. Real), an Andean plant from Peru and Bolivia, is a genus of the Amaranthaceae family with more than 250 species. Few varieties are used for human nutrition, including white, red, and black. The main difference in seed colorations is likely related to betalains with antioxidant activity properties, more than with phenolic compounds (Ballester-Sánchez et al., 2019), even if higher phenolic content has been reported for red and black varieties compared to white one. Regardless of the quinoa seed cultivars, they are rich in proteins of high nutritional value. Specifically, it is made up mainly of soluble proteins such as 2S albumins and 11S-type globulins, which are more accessible to proteases than glutenins and gliadins; the latter is present mainly in cereals. It has a high carbohydrate content (51%–61% on a dry basis) that allows it to be used like cereals for flours production (Mastebroek et al., 2000; Repo-Carrasco-Valencia et al., 2010b). Its fibre content varies from 7 to 26.5% (Joshi et al., 2019), and it is composed mainly of insoluble polysaccharides (78% of the total dietary fibre content) that include homogalacturonans and rhamnogalacturonan-I, as well as

xyloglucans, highly branched (30%) and cellulose (Lamothe et al., 2015), providing it of prebiotic effect. Likewise, it contains much higher lipid content (4%–7.5%) than other cereals where unsaturated fatty acids are predominant (71%–84.5% of total lipids) (Pachari Vera et al., 2019). Additionally, quinoa is an excellent source of vitamins B and E, antioxidants (α and γ-tocopherols), and minerals such as calcium, iron, potassium, magnesium, copper, and manganese. Beyond its excellent nutritional profile, the absence of gluten and its low glycaemic index makes it a good alternative for developing highly palatable products for celiac and/or diabetic people.

Despite its nutritional goodness, the presence of anti-nutritional factors can hinder the health benefits of quinoa. Among its main antinutritional factors, saponins, phytic acid, tannins, nitrates, oxalates, and trypsin inhibitors can be cited. The saponins confer a bitter taste. Likewise, the presence of phytates (myo-inositol-6-phosphate) and tannins reduce the bioavailability of certain minerals and, therefore, their bioabsorption. Phytic acid binds to positively charged cations such as iron, zinc, calcium, and proteins, forming stable phytate complexes at intestinal pH (6-7) (Schlemmer et al., 2009). Therefore, its degradation is essential to improve the bioaccesibility of these minerals. Certain heat treatments, such as roasting or drying, can be applied to reduce the antinutrient content. Likewise, certain bioprocesses such as germination or microbial fermentation also seem to have appositively impact reduction of these compounds. Specifically, fermentation could improve the nutritional and organoleptic quality of the substrate, as well as increase the bioaccessibility of bioactive compounds (P. Thakur et al., 2021) and decrease the presence of anti-nutrients (Castro-Alba et al., 2019; Kumitch et al., 2019). Solid-state fermentation (SSF) presents advantages over submerged fermentation, including higher productivity, lower energy and water requirements, easy aeration, low need

for sterility, easier downstream processing, and being environmentally friendly (Dey et al., 2016). This is why SSF is well aligned with some of the sustainable development goals of 2030.

Within the wide range of microorganisms susceptible to fermentation, the fungoid kingdom presents certain advantages over other microorganisms such as its high protein content (20%–30% in dry matter) and its protein quality. In addition, they act as a source of dietary fibre and have a high content of B vitamins and low-fat content. In a previous study, solid-state fermentation (SSF) with *Pleurotus ostreatus* proved to be an efficient way to enhance the nutritional profile of lentils (Sánchez-García et al., 2023). The bioconversion of vegetal materials into stable fermented ingredients such as flour involves further stabilization operations by hot-air convective drying or freeze-drying, altering their physicochemical properties. Therefore, the aim of this work is to evaluate the impact of fungal solid-fermentation with *Pleurotus ostreatus* and the air-drying temperature (50, 60, and 70 C) on the final properties of fermented white quinoa and black quinoa flours in some relevant properties such as antioxidant properties, ACE-inhibitory capacity, phytic acid, colour, and particle size.

2. Materials and methods

2.1. Materials

Quinoa of white and black varieties were purchased at local stores in Valencia (Spain) from Hacendado® and Nut&me brands, respectively. *Pleurotus ostreatus* strain was obtained from the Spanish Type Culture Collection (CECT20311). Sulphuric acid, sodium hydroxide, sodium chloride, thioglycolic acid, phytic acid sodium salt hydrated from rice, 2,2'-bipyridine, 4-dimethylamino benzaldehyde, acetylacetone,

ascorbic acid, ethyl acetate, formic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), folin ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), N-Hippuric-His-Leu hydrate, Angiotensin Converting Enzyme (ACE) from rabbit lung (≥2.0 units/mg protein) (A6778-25UN), glucose, mycopeptone, chloramphenicol and tryptone bile X-glucuronide agar were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). For HPLC analysis vanillic acid, quercetin 3-glucoside, quercetin, quercitrin, 4-hydroxybezoic acid, rutin, epicatechin, trans-cinnamic acid, ferulic acid, naringenin, caffeic acid, 4-O-caffeoylquinic, p-coumaric acid, apigenin-7-glucoside, kaempferol, and sinapic acid were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) as analytical standard (HPLC grade).

Acetic acid glacial, concentrated hydrochloric acid, ethanol absolute, diethyl ether, sodium carbonate, ammonium iron (III) sulphate dodecahydrate and EDTA Calcium Disodium Salt were obtained from Panreac AppliChem (Barcelona, Spain). Acetonitrile (HPLC grade), methanol (HPLC grade), iron (III) chloride hexahydrate, sodium acetate trihydrate, and potassium persulphate were obtained from Honeywell Fluka (Morris Plains, NJ, USA). Plate-count agar, Malt extract, agar and Sabouraud dextrose agar were obtained from Scharlau (Barcelona, Spain).

2.2. Fungal solid-state fermentation

For the solid-state fermentation process, in the first place a starter culture was prepared with quinoa seeds and then the fermentation process was started by inoculating 1/8 portion of the starter in 35 g of quinoa seeds following the methodology used in Sánchez-García et al., 2023.

2.3. Drying and milling of fermented grains/seeds

Fermented quinoa seeds were dried by two methods: hot air drying at three different temperatures (50, 60, and 70 °C) using a convective dryer (Pol-Eko-Aparatura, CLW 750 TOP+) and freeze-drying using a freeze dryer (Telstar, Lyoquest-55) at -45 °C and 0.8 mBar for 48 h. Milling process for unfermented (raw) and dry fermented samples was carried out using a food processor (Thermomix®, TM6-1), applying 10000 rpm at 15 s intervals for 1 min.

2.4. Drying kinetics and modelling

Drying curves of the fermented samples at each drying temperature were obtained by measuring mass variation at a determined time interval, obtaining several measurements over time until a constant weight was reached. Lewis model was selected as one of the most common mathematical models used for modelling purposes of agricultural products (Chkir et al., 2015; Lewis, 1921), according to Equation 5.5:

$$\frac{X_t^w}{X_0^w} = e^{-k \cdot t} \qquad (5.5)$$

Where: X_t^w is the moisture at a determined time, X_0^w is the moisture at time 0, k is the model constant, and t is the time (min).

2.5. Analytical determinations

Proximal composition of the seeds

Moisture, protein, lipid, and ash were analysed according to standardized methodologies of the Association of Official Analytical Chemist (AOAC, 2000). Briefly,

the moisture content was determined gravimetrically, considering the mass variation of the samples before and after the drying process until reaching their constant weight. Crude protein was measured from the total nitrogen content of the food samples using the Kjeldahl method (using a Kjeldahl factor of 6.25). Lipid content of the samples was determined gravimetrically using the Soxhlet method using petroleum ether (60:40). The ash content was measured gravimetrically by calcining the samples in a muffle at 550 °C for 10 hours (Select-Horn, JP-SELECTA). Finally, carbohydrates were analysed by difference. Total fibre and soluble and insoluble fibre content were determined according to the AOAC Method 991.43 and AACC Method 32-07.01. Results were expressed in g/100 g dry basis.

Fungus biomass

Fungal biomass determination was carried out according to the protocol described by Aidoo et al. (1981) and Tomaselli Scotti et al. (2001). A dry sample (100 mg) was mixed with 2.4 mL of 72% H_2SO_4 at 25 °C for 24 h. Samples were diluted with 55 mL of distilled water and then sterilized at 121 °C for 2h. To neutralize the hydrolysate to pH 7, 10 mol/L and 0.5 mol/L NaOH were added. A 1 mL aliquot of the neutralized hydrolysate was mixed with 1 mL of acetylacetone reagent and heated in a boiling water bath for 20 min. Samples were cooled to room temperature, and 6 mL of ethanol and 1 mL of Erhlick's reagent were added. Absorbance was measured at 530 nm after incubating the mixture at 65 °C for 10 min and cooling it to room temperature. Results were expressed as mg glucosamine/g dry basis.

Colour

A spectro-colorimeter (Minolta, CM-3600D) was used to measure the colour of the different flours, considering a standard illuminant D65 and a standard observer

of 10°. CIE-L*a*b* colour coordinates were measured, and tone (h) and chroma (C*) values were calculated by the device with the a* and b* coordinates. Colour differences (ΔE) were calculated according to Equation 5.6:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (5.6)$$

Particle size

A laser diffraction device (Mastersizer 2000, Malvern Instruments Limited) was used to measure the particle size by dry method. Results were reported as the volume weighted mean D[4,3], surface weighted mean D[3,2], and percentile particle size d(0.5).

Phytic acid content

The protocol described by (Haug & Lantzsch, 1983) and modified by Peng et al. (2010) was followed for phytate content determination. Briefly, the extract was prepared by mixing 50 mg of sample with 10 mL of 0.2 mol/L HCl and left overnight at 4 °C. An aliquot of 1 mL of ferric solution was added to 500 µL of the extract. It was incubated in a water bath for 30 minutes and then cooled to room temperature. Samples were centrifuged for 30 min at 3000 ×*g*, and 1 mL of the supernatant was taken and mixed with 1.5 mL of 2,2'-bipyridine solution. Absorbance was measured at 519 nm against distilled water. Results were expressed as mg phytic acid (PA)/g dry basis using a calibration curve.

Phenolic compounds by HPLC analysis

The methodology described by Caprioli et al. (2018) and Giusti et al. (2019) was used to extract phenolic compounds following first an acid hydrolysis and secondly

an alkaline hydrolysis. Both extracts were filtered with a 0.45 µm PTFE filter, and then analysed by HPLC separately.

Extracts were analysed using a HPLC 1200 Series Rapid Resolution coupled to a diode detector Serie (Agilent, Palo Alto, CA, USA) following the methodology described by Tanleque-Alberto et al. (2020) using a Brisa-LC 5 µm C18 column (250 x 4.6 mm) (Teknokroma, Spain). Mobile phase A was 1% formic acid, and mobile phase B was acetonitrile (ACN). The gradient program used to analyse each compound was the same as described by Tanleque-Alberto et al. (2020). All compounds were identified by comparison of chromatographic retention times and UV spectral characteristics (200−400 nm) of unknown analytes with authentic standards (Sánchez-García et al., 2023).

Total phenolic content (TPC)

Folin-Ciocalteu method described by Chang et al. (2006) was used to determine TPC. The same extract described for acid extraction in phenolic compounds was used. A 125 µL aliquot of the extract was mixed with 500 µL of bidistilled water and mixed with 125 µL of folin ciocalteu reagent for 6 min. Then, 1.25 mL of 7% Na_2CO_3 and 1 mL of bidistilled water were added and finally incubated at room temperature in darkness for 30 min. Absorbance was measured at 760 nm, and the results were expressed as mg gallic acid (GA)/g dry basis using a standard curve.

Antioxidant activity

Three different methods (ABTS, DPPH, and FRAP) were used to determine antioxidant activity according to the protocol described by Thaipong et al. (2006). The same acid extract described above was used. For all antioxidant determination

methods, results were expressed as mg trolox/g dry basis using a standard curve using trolox such as standard (0-200 mg/L).

Angiotensin converting enzyme inhibitory activity (ACE ia (%))

ACE ia (%) of the samples was determined according to the method proposed by Akillioğlu & Karakaya (2009) and Hernández-Olivas et al. (2022) following first a protein extraction and precipitation by adjusting the pH to 4.5 (isoelectric point) and then measuring the ACE inhibitory capacity analysing the precipitation of solid hippuric acid.

Microbiological analysis

Total aerobic mesophilic bacteria count, mold and yeast count and Escherichia coli count were performed plating 0.1 mL of each serial dilution onto Plate-count agar, Sabouraud dextrose agar with 50 mg/L of Chloramphenicol and Tryptone Bile X-Glucuronide agar (TBX chromogenic selective medium) and then incubated at 30 °C for 72h, 25 °C for 5-7 days, and 44 °C for 24h, respectively. Salmonella spp. and Listeria monocytogenes detection analyses were carried out according to ISO 11290-1:2017 and ISO 6579-1:2017.

2.6. Statistical analysis

Analytical determinations were performed in triplicate, and results were reported as mean ± standard deviation. One-Way ANOVA with 95% confidence interval ($p < 0.05$) was conducted to assess possible differences between different drying temperatures using the statistical program Statgraphics Centurion-XV. Principal component analysis (PCA) was also performed to describe the relationship between SSF and drying of white and black quinoa on health-promoting effects (ACE inhibitory

activity, antioxidant activity and TPC). Statgraphics Centurion XVII was used with a confidence level of 95% ($p < 0.05$).

3. Results and discussion

3.1. Changes in the proximal composition of white and black quinoa seeds due to solid-state fermentation with *P. ostreatus*.

Although fungi have been found to be the main starters used in quinoa seed fermentation, up to the authors' knowledge, *P. ostreatus* used has not been previously reported (Starzyńska-Janiszewska et al., 2019; L. N. Xu et al., 2019). Table 5.11 shows the proximate analysis of raw and fermented white and black quinoa seeds, including moisture, protein, lipids, ashes, carbohydrates, and fibre (total, soluble and insoluble fractions). Solid-state fermentation (SSF) significantly ($p < 0.05$) modified the nutritional value of quinoa seeds. Nevertheless, the magnitude of these changes was dependent on the quinoa variety. SSF was applied to enrich the substrate with unicellular protein, among other purposes. Nevertheless, the net protein content change was positive in white quinoa but negative in black. This fact, together with the reduction in lipid content in both varieties would be related to the nutritional requirements of *P. ostreatus* along SSF. Quinoa seeds supply the fungus with carbon, nitrogen, and sulphur, among other nutrients resulting in most cases of a reduction of some macronutrients. Oyster mushrooms have a major role in the biotransformation of lignocellulosic materials into simple and soluble biomolecules through the secretion of lignocellulolytic enzymes like cellulase, amylase, protease, laccase, lipase, catalase, xylanase, lignin peroxidase and manganese peroxidase (Munir et al., 2015). This enzymatic machinery allows the fungus to obtain metabolizable nutrients from seed macronutrients. In white quinoa, nevertheless, protein content increased along SSF according to the greater fungal biomass

production, thus providing unicellular protein, compared to black seeds. Therefore, unicellular protein compensates for the protein consumed the microorganism to obtain nitrogen and sulphur sources. Fungal biomass expressed as mg N-acetil-D-glucosamine per g^{-1} of dry basis was 64.2 ± 1.9 and 39 ± 2 in white and black seeds, respectively. Glucosamine is the monomer of chitin, the main structural polysaccharide of the cell wall of fungus (Tomaselli Scotti et al., 2001). A higher increase of insoluble fibre was found in white seeds compared to black ones because of the greater biomass production in white seeds. This fact also impacted on total carbohydrates content, which includes fibre. Dietary insoluble fibre consumption has been associated with a prebiotic effect together with cholesterol reduction. Studies on dietary insoluble fibre intake showed that consumption of soybean hulls modulates the intestinal microbiota composition promoting the population of *Lactobacillus* and *Bifidobacterium* (L. Yang et al., 2020), also, consumption of bamboo shoot improves the abundance of *Bifidobacterium* and *Lactobacillus* and increases the content of short-chain fatty acids (W. Wu et al., 2020).

Table 5.11. Proximate composition (g/100 g dry basis) of white and black quinoa before and after solid-state fermentation.

Parameter	White quinoa (Raw)	Fermented white quinoa	Black Quinoa (Raw)	Fermented black quinoa
Moisture	8.60 ± 0.13^{a}	127.1 ± 0.5^{b}	10.18 ± 0.06^{a}	96.1 ± 1.0^{b}
Protein	13.87 ± 0.14^{a}	15.98 ± 0.11^{b}	14.28 ± 0.18^{b}	12.0 ± 0.3^{a}
Lipids	6.94 ± 0.07^{b}	2.21 ± 0.07^{a}	6.1 ± 0.3^{b}	3.54 ± 0.19^{a}
Ashes	2.45 ± 0.02^{a}	2.576 ± 0.011^{b}	2.999 ± 0.011^{b}	2.464 ± 0.006^{a}
Total carbohydrates*	76.7 ± 0.2^{a}	79.2 ± 0.5^{b}	76.6 ± 0.5^{a}	81.97 ± 1.14^{b}
Total fibre	7.37 ± 0.10^{a}	13.71 ± 0.10^{b}	12.29 ± 0.10^{a}	14.3 ± 0.2^{b}
Soluble fibre	2.53 ± 0.10^{b}	1.16 ± 0.10^{a}	1.45 ± 0.10^{a}	2.25 ± 0.10^{b}
Insoluble fibre	4.84 ± 0.10^{a}	12.66 ± 0.10^{b}	11.2 ± 0.2^{a}	12.26 ± 0.10^{b}

Results represent the mean of three repetitions with their standard deviation. ^{a,b} Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level as a function of fermentation. * Total carbohydrates calculated by difference.

3.2. Impact of hot-air temperature on drying kinetics, microbiological safety, particle size, and colour of fermented quinoa seeds.

Drying behaviour of fermented white and black quinoa seeds dried at 50, 60, and 70 °C is shown in Figure 5.7. All drying curves followed a similar trend for a constant rate period and falling rate period before reaching equilibrium moisture content (end of drying). As expected, results showed a considerable reduction in drying time as the drying temperature increased. The influence of temperature on the drying kinetics is also confirmed by the k values (Table 5.12) obtained from Lewis's equation. The good adjustment obtained (R^2) confirms that this model can be confirmed as a useful tool for calculating the drying time once the final humidity of the product is established at each temperature. In addition, the results of the microbial analysis (Table 5.13) confirmed that the obtained flours fitted the standard of microbial quality and are safe to be used as new ingredients. However, the choice of the most suitable drying temperature might also be done considering relevant bioactive compounds for human health, sensorial properties, or technological ones, among others.

Table 5.12. Kinetic constant (k (min^{-1}) obtained by means of Lewis' equation applied to hot-air drying curves of fermented white quinoa and fermented black quinoa dried at different temperatures.

	Drying temperature (°C)	k (min^{-1})	R^2
Fermented white quinoa	50	0.0069 ± 0.0002^a	0.935 ± 0.007
	60	0.00958 ± 0.00014^b	0.960 ± 0.013
	70	0.0133 ± 0.0007^c	0.96 ± 0.03
Fermented black quinoa	50	0.00746 ± 0.00014^a	0.949 ± 0.007
	60	0.00101 ± 0.0002^b	0.956 ± 0.019
	70	0.0135 ± 0.0004^c	0.981 ± 0.006

Results represent the mean of three repetitions with their standard deviation. Lowercase letters evidence significant differences at 95% ($p < 0.05$) confidence level.

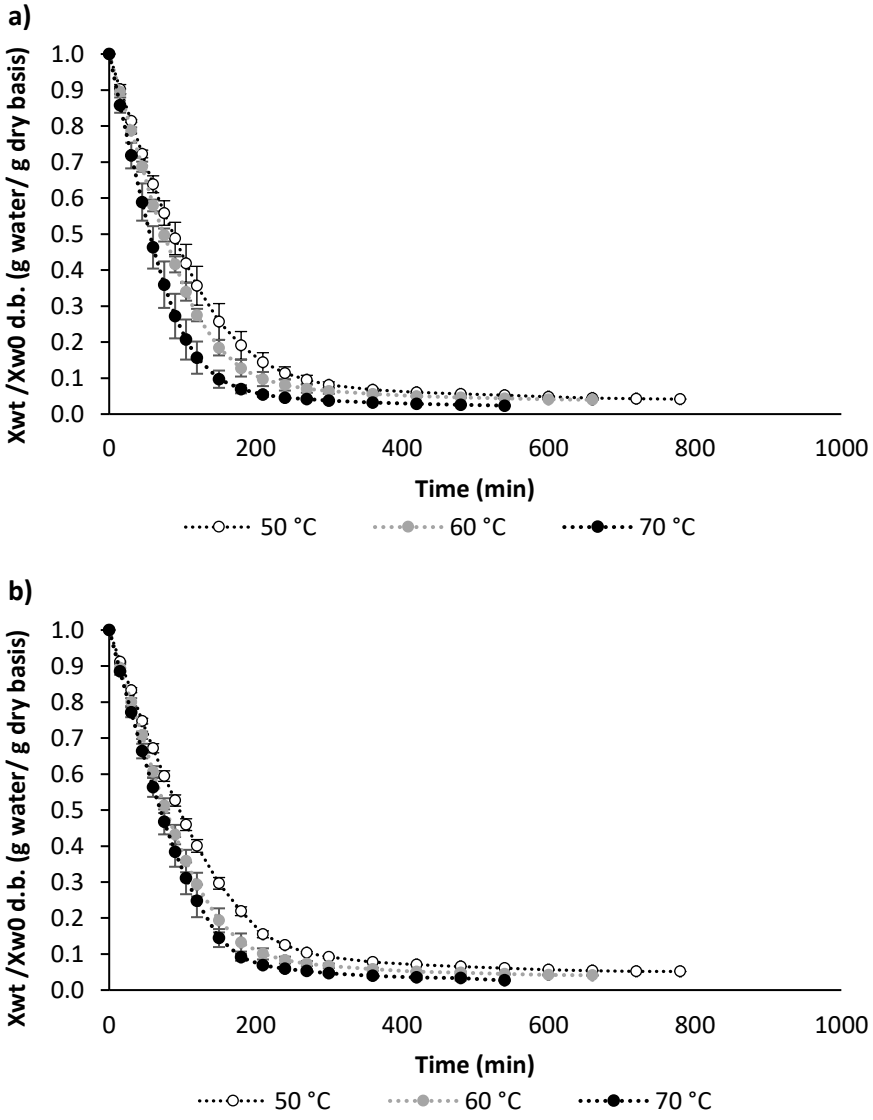
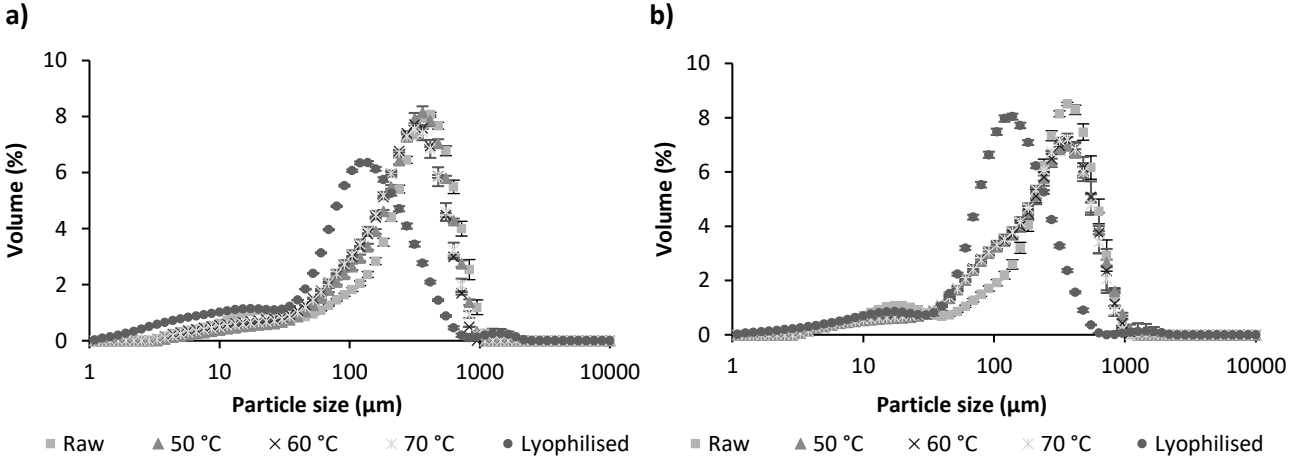


Figure 5.7. Hot air-drying curves (X^w_t/X^w_0 (g water/g dry basis)) of fermented white quinoa (**a**) and fermented black quinoa (**b**) at 50, 60 and 70 °C.

Table 5.13. Microbiological analysis in white and black quinoa flour (raw) and fermented white and black quinoa flour stabilized by hot-air drying (50, 60 and 70 °C) or lyophilisation.

	Aerobic mesophilic bacteria count (UFC/g)	**Yeast and mould count (UFC/g)**	***E. coli* count (UFC/g)**	***Salmonella* spp. detection (Presence/ Absence)**	***Listeria monocytogenes* (Presence/ Absence)**
White quinoa					
Raw	$5x10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-dried (50 °C)	$4x10^2$	$3x10^2$	$<10^2$	Absence	Absence
Fermented-dried (60 °C)	$2x10^2$	$1x10^2$	$<10^2$	Absence	Absence
Fermented-dried (70 °C)	$<10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-lyophilised	$1.4x10^3$	$<10^2$	$<10^2$	Absence	Absence
Black quinoa					
Raw	$9x10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-dried (50 °C)	$5x10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-dried (60 °C)	$2x10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-dried (70 °C)	$2x10^2$	$<10^2$	$<10^2$	Absence	Absence
Fermented-lyophilised	$3x10^2$	$<10^2$	$<10^2$	Absence	Absence

The particle size distribution is critical for quality control, packaging, handling, processing, product development, and research. Thus, particle size distribution was determined in flours, and the curve pattern and main parameters are shown in Figure 5.8.



	D [4,3]	D [3,2]	d (0.5)
Raw	303 ± 9^{d}	69.6 ± 1.3^{b}	283 ± 7^{d}
50 °C	276 ± 3^{c}	93 ± 2^{d}	253 ± 5^{c}
60 °C	239.9 ± 1.5^{b}	71.0 ± 0.6^{b}	217.8 ± 1.3^{b}
70 °C	248 ± 8^{b}	77 ± 3^{c}	221 ± 9^{b}
Lyophilised	149 ± 10^{a}	27.18 ± 0.17^{a}	107.8 ± 0.5^{a}

	D [4,3]	D [3,2]	d (0.5)
Raw	279 ± 12^{c}	67 ± 2^{b}	265 ± 9^{c}
50 °C	260 ± 4^{b}	71.9 ± 1.5^{c}	224 ± 5^{b}
60 °C	252 ± 17^{b}	71 ± 3^{c}	221 ± 11^{b}
70 °C	245 ± 11^{b}	70 ± 3^{c}	216 ± 10^{b}
Lyophilised	141 ± 17^{a}	40.4 ± 0.6^{a}	115.7 ± 1.5^{a}

Figure 5.8. Particle size (volume (%)) in white (**a**) and black (**b**) quinoa unfermented (raw) and fermented-dried by hot air (50, 60 and 70 °C) or lyophilised. ^{a,b,c,d} Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level.

According to the literature (Ciurzyńska & Lenart, 2011; Michalczyk et al., 2009), freeze-drying would be the method preserving the most characteristic of the raw materials, and for this reason, fermented quinoa seeds was also dehydrated by this method. All samples exhibited a monomodal distribution, although the maximum amplitude and situation depended on the drying method. Thus, the particle size distribution of fermented flours stabilized with freeze-drying became narrower and moved towards narrow and smaller particle sizes than those dried by hot-air drying. In fact, the mean particle size (d(0.5)) of hot-air-dried samples was two-fold that of lyophilised fermented flours. Besides, no effect of hot-air drying temperature was found on particle size distribution, which was similar to unfermented flour. This fact evidence that particle size distribution exclusively depends on the drying method and the intensity and time of milling, being the conditions of the latter mechanical operation common for all samples. The particle size of hot-air dried samples was characterized by a broader distribution consistent with the greater differences observed in the parameters D[3,2] and D[4,3], compared with lyophilised fermented flours. Therefore, they can be considered more heterogeneous. The D[3,2] represents the average size based on the specific surface per unit volume and allows characterizing of small particles and spherical shapes, while D[4,3] indicates the mean size distribution of particles by volume considering the unit mass of the particles and illustrates the largest particles and irregular shapes, such as aggregates. Both the values of D[4,3] and D[3,2] show that lyophilisation is the best method to obtain flour consisting of small spherical particles together with a reduced number of larger and irregular ones.

Optical properties were also determined because of the relevance of ingredients' colour in food formulations. Table 5.14 gathers CIE-L*a*b* values and illustrates the

colour changes experimented on quinoa seeds along with processing through images.

Table 5.14. CIE-L*a*b* parameters of white and black quinoa flour (raw) and fermented white and black quinoa flour stabilized by hot-air drying (50, 60 and 70 °C) or lyophilisation.

White quinoa flours			Black quinoa flours		
	**L***	86.51 ± 0.03^{e}		**L***	71.77 ± 0.10^{d}
	a*	0.83 ± 0.03^{a}		**a***	2.464 ± 0.011^{a}
	b*	13.99 ± 0.06^{a}		**b***	9.27 ± 0.04^{a}
	C*	14.01± 0.06^{a}		**C***	9.59 ± 0.04^{a}
	h	86.61 ± 0.13^{e}		**h**	75.11 ± 0.10^{c}
Raw	**ΔE**	-	**Raw**	**ΔE**	-
	**L***	67.7 ± 0.5^{a}		**L***	57.13 ± 0.13^{b}
	a*	8.4 ± 0.2^{d}		**a***	3.92 ± 0.04^{c}
	b*	24.81 ± 0.18^{c}		**b***	12.02 ± 0.16^{b}
	C*	26.2± 0.2^{c}		**C***	12.6 ± 0.2^{b}
	h	71.3 ± 0.4^{a}		**h**	71.93 ± 0.07^{a}
Fermented-dried (50 °C)	**ΔE**	23.0 ± 0.6^{c}	**Fermented-dried (50 °C)**	**ΔE**	14.97 ± 0.02^{b}
	**L***	69.7 ± 0.3^{c}		**L***	57.23 ± 0.07^{b}
	a*	8.03 ± 0.16^{c}		**a***	3.891 ± 0.009^{c}
	b*	25.28 ± 0.08^{e}		**b***	12.06 ± 0.07^{bc}
	C*	26.52± 0.13^{d}		**C***	12.67 ± 0.07^{bc}
	h	72.4 ± 0.3^{c}		**h**	72.12 ± 0.13^{b}
Fermented-dried (60 °C)	**ΔE**	21.5 ± 0.3^{b}	**Fermented-dried (60 °C)**	**ΔE**	14.88 ± 0.14^{b}
	**L***	68.87 ± 0.02^{b}		**L***	56.7 ± 0.2^{a}
	a*	8.207 ± 0.002^{cd}		**a***	4.01 ± 0.07^{d}
	b*	25.08 ± 0.09^{d}		**b***	12.3 ± 0.2^{c}
	C*	26.39± 0.09^{cd}		**C***	12.9 ± 0.3^{c}
	h	71.88 ± 0.06^{b}		**h**	71.97 ± 0.09^{ab}
Fermented-dried (70 °C)	**ΔE**	22.11 ± 0.07^{b}	**Fermented-dried (70 °C)**	**ΔE**	15.43 ± 0.17^{c}

	**L***	79.30 ± 0.13^{d}		**L***	63.40 ± 0.07^{c}
	a*	4.18 ± 0.02^{b}		**a***	3.04 ± 0.05^{b}
	b*	21.787 ± 0.008^{b}		**b***	13.49 ± 0.15^{d}
	C*	22.185± 0.007^{b}		**C***	13.83 ± 0.15^{d}
	h	79.14 ± 0.05^{d}		**h**	77.31 ± 0.10^{d}
Fermented-lyophilised	**ΔE**	11.14 ± 0.13^{a}	**Fermented-lyophilised**	**ΔE**	9.40 ± 0.02^{a}

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e} Different lowercase letters indicate significant differences for each parameters L*, a*, b*, C*, h, and ΔE for white and black quinoa with a 95% ($p < 0.05$) significance level.

SSF and stabilization implied significant colour changes perceivable by the human eye according to ΔE values higher than 5 (Witzel et al., 1973). However, the magnitude of those changes was significantly higher in white than black quinoa flours and in those samples dried by hot-air drying than lyophilisation. Statistically significant differences were found in CIE-L*a*b* parameters as a function of drying temperature. In particular, processing decreased brightness (L*) and increased a* and b* values. White quinoa flour moved from neutral yellow-grey colour (raw samples) with high luminosity to yellow-orange saturated chroma (higher C* values) with a lower level of tone (decreased h values). In the case of black quinoa, processing also implied an increase of C*, mainly due to b* positive variation characterized by more yellow-brown chromes. At the same time, tone and luminosity values went down, except fermented for lyophilised flours. Before drying, the optical properties of fermented white and black quinoa were also measured. For that, a paste of fermented quinoa was obtained and compared to unfermented quinoa paste with the same moisture (≈ 65%) and were measured with a 20 mm thick transparent plastic cell. In both cases, a notable increase of both a* and b* values were found in white quinoa compared to the unfermented paste (a* and b* values of white fermented paste: 8.97 ± 0.13 and 19.2 ± 0.2). In contrast, only a slight increase was found in b* parameter (7.82 ± 0.13) in black quinoa because of

fermentation. According to that, colour changes in white quinoa could mainly be attributed to SSF, and hot-air drying would just intensify those changes. Zhao et al. (2017) reported the conversion of phenolic compounds into dark pigments during SSF of wheat bran due to the enzymatic browning reactions induced by fungal polyphenol oxidases in presence of oxygen and water. Similarly, Gebru & Sbhatu (2020) reported a decrement of total phenolic compounds in white Teff during SSF with *P. ostreatus* (KACC 42738), partly due to the oxidation of phenols by these enzymes. Hot-air drying would continue with the autooxidation of the phenols due to hot air flow carrying on oxygen. Images in Table 5.14 demonstrated that the colour changes could be positive because of the attractive appearance of the obtained samples.

3.3. Assessment of processing on healthy properties of white and black quinoa seeds.

The effect of SSF and drying was determined on the antioxidant benefits, antihypertensive properties, and reduction in phytates of white and black quinoa seeds.

Concerning the antioxidants, Table 5.15 shows the total phenolic content (TPC) and antioxidant capacity estimated by ABTS, DPPH, and FRAP assays. White and black quinoa seeds differed between varieties, being higher in black than white quinoa, as expected (M. Liu et al., 2020). In addition, values were in the range of those found in literature in seeds grown in different geographies, such as Finland (Mattila et al., 2018) or China (Y. Han et al., 2019), superior to quinoa cultivated in Marrocco (Mhada et al., 2020), but lower than quinoa from Peru (Abderrahim et al., 2015).

Table 5.15. Antioxidant capacity (mg trolox/g dry basis) by ABTS, DPPH and FRAP assays, total phenol content (mg GA/g dry basis) and phytic acid content (mg PA/g dry basis) in white and black quinoa before (raw) and after solid-state fermentation, and after stabilization by hot-air drying (50, 60 and 70 °C) or lyophilisation.

	Antioxidant capacity			Total phenol content	Phytic acid
	ABTS	DPPH	FRAP		
White quinoa					
Raw	1.48 ± 0.08^{b}	1.070 ± 0.005^{c}	1.53 ± 0.05^{c}	1.89 ± 0.03^{b}	15.0 ± 1.4^{c}
Fermented	1.87 ± 0.08^{c}	1.334 ± 0.012^{d}	0.47 ± 0.03^{a}	1.88 ± 0.08^{b}	1.1 ± 0.7^{b}
Fermented- dried (50° C)	2.44 ± 0.11^{e}	0.790 ± 0.005^{a}	1.94 ± 0.18^{d}	3.67 ± 0.05^{d}	0.99 ± 0.04^{b}
Fermented-dried (60° C)	2.22 ± 0.08^{d}	0.799 ± 0.005^{a}	1.80 ± 0.04^{d}	3.47 ± 0.02^{c}	0.9 ± 0.3^{b}
Fermented-dried (70° C)	2.287 ± 0.006^{d}	0.80 ± 0.04^{a}	1.88 ± 0.09^{d}	3.70 ± 0.05^{d}	0.04 ± 0.01^{a}
Fermented-lyophilised	1.12 ± 0.04^{a}	1.00 ± 0.03^{b}	0.63 ± 0.03^{b}	1.44 ± 0.04^{a}	1.0 ± 0.4^{b}
Black quinoa					
Raw	2.48 ± 0.03^{c}	0.88 ± 0.03^{c}	2.74 ± 0.04^{e}	2.16 ± 0.04^{c}	17.5 ± 0.7^{b}
Fermented	1.69 ± 0.10^{b}	1.38 ± 0.02^{e}	0.52 ± 0.02^{a}	1.33 ± 0.03^{a}	2.5 ± 0.5^{a}
Fermented- dried (50° C)	1.71 ± 0.05^{b}	0.629 ± 0.01^{ab}	1.31 ± 0.05^{d}	2.25 ± 0.10^{c}	2.7 ± 0.6^{a}
Fermented-dried (60° C)	1.69 ± 0.08^{b}	0.579 ± 0.009^{a}	1.22 ± 0.06^{c}	2.26 ± 0.04^{c}	2.6 ± 0.3^{a}
Fermented-dried (70° C)	1.68 ± 0.06^{b}	0.666 ± 0.014^{b}	1.26 ± 0.04^{cd}	2.50 ± 0.04^{e}	2.0 ± 0.2^{a}
Fermented-lyophilised	1.33 ± 0.05^{a}	0.96 ± 0.06^{d}	0.82 ± 0.05^{b}	1.85 ± 0.04^{b}	2.4 ± 0.6^{a}

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d,e} Different lowercase letters indicate significant differences with a 95% ($p < 0.05$) significance level.

In general, values found in other studies suggest significant variability in quinoa TPC depending on several factors, such as genetic traits, growing conditions, and postharvest processing of quinoa seeds. Accordingly, black quinoa exerted higher antioxidant capacity than white ones. The fungal fermentation decreased the TPC of black quinoa seeds, while no impact was found in white seeds. However, DPPH-antioxidant capacity increased during SSF regardless of the variety, while FRAP-antioxidant capacity drastically decreased. ABTS assay reported a higher antioxidant capacity for fermented white seeds but a lower one for black ones compared to the unfermented raw material. The increase observed in DPPH-antioxidant capacity could be related to the bioconversion of some phenols into other molecules with increasing antioxidant capacity, such as free aglycones (Hur et al., 2014). FRAP reduction, however, could be due to a degradation/metabolization during SSF of some dihydroxybenzoic acids, such as 2.3-or 2.5-, with strong activity reducing ion Fe^{3+} to Fe^{2+} (Spiegel et al., 2020). Numerous studies report fermentation's effect on phenolic compounds and the antioxidant capacity of quinoa (Melini & Melini, 2022). During fermentation, bound phenolic compounds are bio-converted from their linked or conjugated forms to their free ones, because of the (a) breakdown of the bonds with the grain cell wall components; (b) activities of enzymes, such as β-glucosidase or reductases; and (c) metabolic activity of fermenting microorganisms (Adebo & Medina-Meza, 2020). On the other hand, upon fermentation, a decrease of free phenols can occur, because they might bind with other molecules present in the food matrix, be degraded by microbial enzymes and/or hydrolysed by specific microbial strains microorganisms (Adebo & Medina-Meza, 2020). Fermentation can thus increase or reduce the phenolic compound content and antioxidant capacity of grains (Gan et al., 2017), but the effect and the degree of influence depend on the starter employed and substrate (Hur et al., 2014). The phenolic profile has also been determined to go deeper in antioxidants changes (Tables 5.16 and 5.17).

Table 5.16. Phenolic content (μg/g dry basis) in white quinoa before (raw) and after solid-state fermentation, and after stabilization by hot-air drying (50, 60, and 70 °C) or lyophilisation.

	Raw	Fermented	Fermented-dried (50 °C)	Fermented-dried (60 °C)	Fermented-dried (70 °C)	Fermented-lyophilised
Phenolic acids						
Gallic acid	29 ± 1^{aC}	64 ± 4^{bD}	140 ± 20^{cD}	132 ± 9^{cD}	132 ± 9^{cC}	49 ± 4^{abE}
Caffeic acid	100 ± 4^{cE}	31 ± 4^{bC}	3.9 ± 0.6^{aA}	3.7 ± 0.1^{aA}	3.3 ± 0.4^{aA}	traces
p-Coumaric acid	4.1 ± 0.3^{A}	traces	traces	traces	traces	traces
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	traces	9 ± 1^{bB}	5.6 ± 0.8^{aAB}	16 ± 2^{cC}	6 ± 2^{aAB}	5.4 ± 0.4^{aB}
Vanillic acid	220 ± 6^{bF}	14 ± 4^{aB}	9.0 ± 0.7^{aC}	9.1 ± 0.1^{aB}	9.0 ± 0.1^{aB}	12.6 ± 0.1^{aD}
Ferulic acid	49 ± 1^{bD}	3.1 ± 0.2^{aA}	traces	traces	traces	traces
trans-Cinnamic acid	traces	traces	traces	traces	traces	traces
Flavonoids						
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	traces	traces	traces	traces	traces	traces
Quercetin 3-glucoside	18.8 ± 0.6^{B}	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	20.4 ± 0.5^{cB}	12 ± 3^{bB}	7.0 ± 0.6^{aB}	7.0 ± 0.3^{aB}	8 ± 1^{abB}	8.2 ± 0.8^{abC}
Apigenin-7-glucoside	n.d.	n.d.	11.0 ± 1.6^{cC}	8.6 ± 0.1^{bB}	9.2 ± 0.7^{bcB}	2.0 ± 0.1^{aA}
Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naringenin	n.d.	n.d.	traces	traces	traces	traces
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total phenolic content	443 ± 13^{d}	133 ± 15^{b}	180 ± 20^{c}	176 ± 11^{c}	166 ± 14^{c}	77 ± 5^{a}

^{a,b,c} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C,D,E,F} Different capital letters indicate significant differences ($p < 0.05$) between phenolic compounds. n.d.: not detected. Traces: not quantifiable.

Table 5.17. Phenolic content (μg/g dry basis) in black quinoa before (raw) and after solid-state fermentation, and after stabilization by hot-air drying (50, 60, and 70 °C) or lyophilisation.

	Raw	Fermented	Fermented-dried (50 °C)	Fermented-dried (60 °C)	Fermented-dried (70 °C)	Fermented-lyophilised
Phenolic acids						
Gallic acid	48 ± 4^{aC}	55 ± 3^{aE}	89 ± 8^{bD}	89 ± 11^{bE}	104 ± 11^{cE}	104 ± 3^{cF}
Caffeic acid	92 ± 9	traces	12 ± 2^{bB}	6.5 ± 0.8^{aB}	15 ± 1^{bC}	16 ± 2^{bC}
p-Coumaric acid	traces	n.d.	n.d.	n.d.	n.d.	n.d.
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	7.0 ± 0.2^{aA}	8.9 ± 0.5^{bcC}	9.1 ± 0.1^{cB}	6 ± 1^{aB}	7.2 ± 0.7^{abB}	7.2 ± 0.2^{aB}
Vanillic acid	300 ± 30^{bE}	30 ± 2^{aD}	26 ± 2^{aC}	24 ± 3^{aD}	31.2 ± 0.6^{aD}	33.7 ± 0.1^{aE}
Ferulic acid	54 ± 0.9^{cC}	5.0 ± 0.3^{bcA}	5.9 ± 0.1^{bA}	3.9 ± 0.3^{aA}	3.9 ± 0.4^{aA}	4.2 ± 0.3^{aA}
trans-Cinnamic acid	n.d.	n.d.	traces	traces	traces	traces
Flavonoids						
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	4.2 ± 0.1^{aA}	6.6 ± 0.3^{bB}	5.1 ± 0.1^{aA}	4.4 ± 0.4^{aA}	4.3 ± 0.2^{aA}	4.7 ± 0.1^{aA}
Quercetin 3-glucoside	17 ± 2^{B}	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	12 ± 2^{bB}	6.3 ± 0.9^{aAB}	20 ± 2^{cC}	10.1 ± 1.2^{bC}	18.7 ± 1.2^{cC}	23.2 ± 0.6^{cD}
Apigenin-7-glucoside	n.d.	n.d.	6 ± 3^{abAB}	3.5 ± 0.8^{aA}	7.1 ± 0.2^{bB}	3.9 ± 0.1^{aA}
Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naringenin	n.d.	n.d.	traces	traces	traces	traces
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total phenolic content	534 ± 50^{d}	113 ± 6^{a}	172 ± 16^{bc}	148 ± 20^{b}	190 ± 14^{c}	196 ± 5^{c}

^{a,b,c} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C,D,E,F} Different capital letters indicate significant differences ($p < 0.05$) between phenolic compounds. n.d.: not detected. Traces: not quantifiable.

The same compounds, mainly phenolic acids, were identified in both varieties, being black seeds richer in 4-Hydroxybezoic, vanillic, gallic, caffeic, and ferulic acids than white seeds. It is important to emphasize that two extractions were performed for each sample, the first extraction (acidic) to quantify the phenolic compounds of the free fraction and the second extraction (basic) for the bound fraction. In the second extraction, depending on the type of quinoa (white or black) and the type of treatment (raw, fermented or fermented-dried), traces of the compounds caffeic acid, p-Coumaric acid, 4-Hydroxybezoic acid and ferulic acid were found. However, in the samples with quantifiable amounts of phenolic compounds such as gallic acid, 4-Hydroxybezoic acid, vanillic acid, ferulic acid, and epicatechin, the free and bound fractions were summed. It should be noted that in black quinoa, gallic acid presented similar amounts in the bound and free fractions, while epicatechin could only be quantified in the bound fraction.

Fungal fermentation clearly modified the phenolic profile varying the contents of native phenols. However, the chromatographic analysis did not report the generation of new compounds, except hydroxybenzoic acid in white quinoa. Therefore, the mechanism-driven phenolic changes along SSF was the release of gallic acid, with a significative increase, from complex molecules such as vanillic, caffeic, and ferulic acids, quercetin-3-glucoside and/or quercetin. The synthesis pathway of gallic acid from caffeic acid, with 3,4,5-trihydroxycinnamic acid and protocatechuic acid as intermediate products, has been described (Metsämuuronen et al., 2019). However, it is unclear if the reduction of other phenols found in this study is related to the increase in gallic acid content. This fact was found in both varieties, corresponding to the 50% of TPC quantified by chromatography to gallic acid. This release/conversion from native phenols into gallic acid progressed along hot-air drying, being gallic acid until 80%–90% of the total phenolic content depending on the air temperature.

Miranda et al. (2010) evaluated the impact of hot-air drying temperature on quinoa seeds. They reported thermal degradation of TPC, especially at 60, 70, and 80 °C. However, this study found an increase after fermentation in samples stabilized by hot-air drying. Surprisingly, lyophilisation led to worse preservation of phenols. Lyophilisation implies a longer processing time, 24 h, than hot-air drying and previous freezing, which could be undergone drastic changes in TPC. Although some authors have reported that long drying times associated with low process temperature may promote a decrease in antioxidant capacity (Garau et al., 2007); in this study, it was not evidenced a clear effect of drying variables on antioxidant capacity. Thus, ABTS and FRAP-antioxidant activity increased along hot-air drying in fermented white quinoa, while DPPH decreased regardless of the temperature in both fermented varieties.

SSF was very efficient in decreasing phytates content (Table 5.15). A yield of reduction of approx. 90% were found in both varieties, so no additional reduction was found during the posterior drying regardless of the conditions applied. Approximately, the seeds and grains contain between 1%–2% phytic acid, although it can even be 3%–6% (Hídvégi & Lásztity, 2002). Phytic acid cannot be digested by monogastric animals and ends up being excreted. In humans, diets high in phytic acid can significantly reduce the absorption of essential micronutrients such as iron and calcium (Hurrell et al., 2003), zinc (Guttieri et al., 2006), and magnesium (Bohn et al., 2004). Additionally, it can bind to proteins or digestive enzymes (proteases and amylases), resulting in less protein solubility and proteolytic inhibition (López-Perea et al., 2019). The obtained white and quinoa fermented flours can be considered phytates-free, with higher potential digestibility and mineral bioaccessibility than the unfermented counterparts.

An increase in the prevalence of cardiovascular diseases, together with metabolic syndrome, among other diseases, has been reported in the last decades. Therefore, new ingredients production with improved health benefits concerns the food industry. *In vitro* antihypertensive activity by means of ACE-inhibition (%) was evaluated in the samples (Figure 5.9). As it can be seen, similar inhibitory capacity against the angiotensin-converting enzyme (ACE) was exerted by both varieties before and after fermentation, with a statistically significant increase during fermentation. A slight increase was also found without the statistical significance of either the drying method or temperature. Bioactive peptides have been reported to be responsible for ACE inhibitory capacity, and many of them have been identified in diverse plant protein sources such as wheat, soybeans, lentils, beans, rice, and maize among others (Daskaya-Dikmen et al., 2017; Guang & Phillips, 2009). Scientific studies reported that the bioactive peptide generation throughout fermentation processes, being the type of starter culture and fermentation conditions critical (Daskaya-Dikmen et al., 2017; Handa et al., 2020). Foods such as douchi (a Chinese fermented soybean product) fermented with *Aspergillus Egyptiacus* (J. H. Zhang et al., 2006), tempeh made from soybeans and fermented with *Rhizopus oryzae* (Handa et al., 2020), or chickpeas fermented with *Cordyceps militaris* SN-18 (Xiao et al., 2015) showed an increase ACE inhibitory activity due to the breakdown of proteins into new, smaller peptides (2 to 12 short amino acid sequences). The ACE inhibitory activity of these peptides is mainly governed by the presence of C-terminal amino acids, hydrophobic amino acids, and aromatic amino acids in their structure, thus competitive binding to the active site of ACE causes the inhibitory activity (Yuan et al., 2022). Therefore, SSF as a processing technology may have influenced the content and composition of the peptides, showing increased ACE inhibition in white and black quinoa seeds with an antihypertensive nature. Besides, some mycelia have been demonstrated *in vitro* antihypertensive action. Manoharan et al. (2017)

reported the capacity to inhibit ACE of tripeptides of Gly-Val-Arg isolated from *Pleurotus pulmonarius*. Similarly, Wu et al. (2019) demonstrated the *in vitro* antihypertensive action of the bioactive peptides: Gln-Leu-Val-Pro, Gln-Asp-Val-Leu and Gln-Leu-Asp-Leu isolated from the mycelium of *Ganoderma lucidum*. Mohamad Ansor et al. (2013) discovered four proteins derived from mushrooms (*Ganoderma lucidum*) that could affect lowering blood pressure due to inhibition of ACE activity; these proteins are cystathionine beta synthase-like protein, DEAD/DEAH box helicase-like protein, paxillin-like protein, and alpha/beta hydrolase-like protein. Finally, an additional increase in ACE inhibitory capacity was found in dried samples compared to undried ones. Therefore, neither the drying technology nor the drying temperature was influenced.

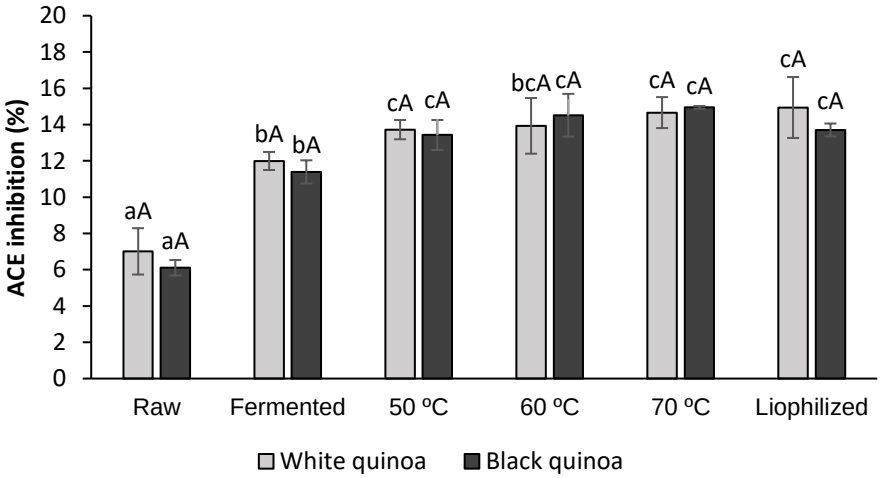


Figure 5.9. ACE inhibitory activity (%) values in white and black quinoa unfermented (raw), fermented and fermented-dried by hot air (50, 60 and 70 °C) or lyophilised. Data shown are mean values from triplicates and the standard deviation. [a,b,c] Different lowercase letters indicate significant differences between dried flours and [A] different capital letters indicate significant differences between quinoa varieties, with a significance level of 95% ($p < 0.05$).

3.4. Principal component analysis (PCA) applied to the obtained data.

Figure 5.10 shows the biplot from PCA and applied to the data obtained to explain the relationship between SSF and drying of white and black quinoa on health-promoting effects. As can be seen, the sum of the two main components (PC1 and PC2) explained 89.31% and 97.81% of the total variance of white and black quinoas, respectively. In the case of white quinoa, Figure 5.10 (A), PC1 distinguishes between the samples dried by hot-air (positive axis), from the rest (negative axis), showing higher ACE inhibitory activity, ABTS and FRAP antioxidant activity and total phenolic content (TPC). Likewise, PC2 clearly separates the raw white quinoa (negative axis) from the all fermented samples (positive axis), although the sample dried by hot-air at 50 °C is slightly also in the negative axis. With respect to the changes experimented by fermented black quinoa (Figure 5.10 (B), PC1 (54.87%) highlights the closed relationship and higher values of ACE inhibitory activity and DPPH found for fermented and dried by hot air and lyophilisation vs the raw black quinoa clearly separates in the positive axis with higher values in ABTS and FRAP. On the other hand, PC2 (42.94%) separates on the positive axis the samples fermented and dried by hot-air denoting higher values of ACE inhibitory activity and TPC.

Hence, it can be observed how there is a clear separation between the fermented and non-fermented samples in both quinoas, however, the effect that fermentation has on the different measured parameters clearly depends on the variety of quinoa studied.

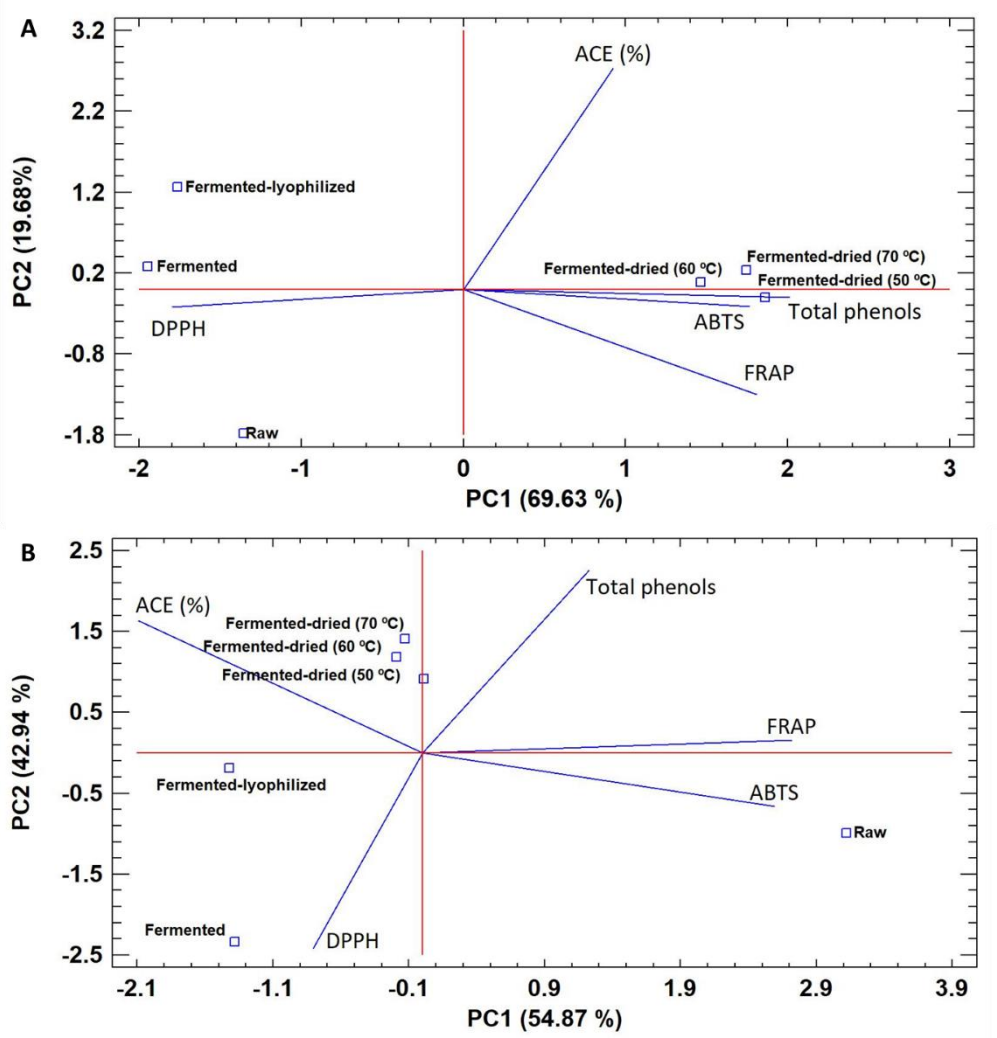


Figure 5.10. Biplot obtained by means of a principal component analysis (PCA) of white quinoa (**A**) and black quinoa (**B**) on health-promoting effects properties (total phenol content, DPPH, ABTS, FRAP antioxidant activities, and ACE inhibition), and their association with the fermentation/drying type.

4. Conclusions

To conclude, solid-state fermentation together with hot air drying led to nutritional and functional changes in quinoa seeds, being white variety the most suitable substrate for *Pleurotus ostreatus* according to the obtained results. SSF significantly increased protein, and total and insoluble fibre contents in white seeds and was very efficient in phytic acid reduction. Antioxidant activity and total phenolic content showed a reduction after 14 days of fermentation, notable in black seeds Nevertheless, antioxidant properties experimented an improvement because of stabilization step, being higher in samples dehydrated by hot-air drying, and especially at 70 ºC, than those by freeze-drying. The phenolic compound profile was modified by SSF and hot air drying by a release of gallic acid, making in white quinoa. The results also demonstrated the interest of SSF for obtaining quinoa flours with improved antihypertensive capacity. On the other hand, particle size of the fermented flours was not influenced by the hot air-drying temperature and showed a monomodal distribution similar to the unfermented flours. SSF plus drying underwent human eye appreciable changes in quinoa, resulting in a decrease in lightness (L*) and an increase in a* and b* values with yellow-orange colours in white quinoa and yellow-brown colours in black quinoa.

Finally, SSF with *P. ostreatus* can also involve changes in quinoa flours digestibility and bioaccessibility. Thus, it could be interesting to perform *in vitro* digestion studies of fermented seeds to elucidate the potential benefits of fermented quinoa on human health. To meet people's increasing demand, the quinoa-fungi products can be an alternative to healthier diets.

ARTÍCULO 4

ARTÍCULO 4: Volatile profile of quinoa and lentil flour under fungal fermentation and drying.

ABSTRACT

Solid-state fermentation reportedly improves the nutritional and sensory properties of legumes and pseudocereals. This study examined changes in the volatile profile using HS-SPME-GC-MS of two varieties of lentil and quinoa flour fermented with *Pleurotus ostreatus* and dried using hot-air drying and lyophilisation. Fermentation significantly increased the volatile profile. Pardina lentil flour showed a 570% increase in its volatile profile, and 10 compounds were created. In white quinoa, the total area rose from 96 to 4500, and 30 compounds were created. Compounds such as 1-octen-3-ol, benzaldehyde, 3-octanone and hexanal were generated during fermentation, providing a sweet, grassy, cocoa flavour. Hot-air drying led to decrease of over 40% in total peak area. Dried fermented flour retained higher levels of compounds that provide a sweet, cocoa aroma. Air-drying temperature had no significant influence on the volatile profile. This a allows the inclusion of these flours in a wide variety of food products.

Keywords: Volatile compounds, quinoa, lentil, flour, fermentation, *Pleurotus ostreatus*.

1. Introduction

Global concern for environmental sustainability and food security, coupled with a focus on healthy eating and low costs, is driving the search for new plant-based high-protein foods. Legumes and pseudocereals play a prominent role in ensuring a balanced human diet worldwide. They offer large quantities of macro- and micro-nutrients. They also have several environmental benefits. For instance, legume cultivation reduces greenhouse gas emissions, helps fix atmospheric nitrogen in the soil and decreases the carbon footprint (Stagnari et al., 2017).

Legumes are a rich source of protein. Their protein content ranges from 21% to 31% on a dry basis, depending on the species and crop. These proteins are high in essential amino acids such as lysine and leucine. Studies have emphasised the importance of consuming legumes not only because they offer a source of protein but also because they are high in dietary fibre, minerals, and polyphenols. Lentils (*Lens culinaris spp*.) are a type of legume cultivated in over 70 countries. They are valued around the world for their nutritional richness in proteins, dietary fibres, complex carbohydrates, and essential micronutrients such as iron, zinc and vitamin B complex (Liberal et al., 2023). In addition, lentil seeds have a higher antioxidant capacity than other legumes due to the presence of certain phenolic compounds (Grela et al., 2017).

Another group of crops that offer large quantities of macro- and micro-nutrients is pseudocereals. They are resilient crops capable of withstanding salinity and extreme temperatures, and they can be easily grown with limited resources (Rodríguez et al., 2020). Quinoa seeds *(Chenopodium Willd*) offer an alternative gluten-free protein source with nutritional value similar to that of cow's milk (Repo-Carrasco et al., 2006). The amino acid profile of quinoa includes all essential amino

acids, with high quantities of lysine. Quinoa is also rich in various vitamins and minerals, with a higher protein content than traditional grains (Repo-Carrasco et al., 2006).

Despite all their nutritional benefits, legumes and pseudocereals also contain antinutrients such as protease inhibitors, phytates, tannins and saponins. These antinutrients can affect the nutritional quality of these foods. Certain processing methods such as heat treatments, germination and fermentation appear to reduce these antinutrients and increase the bioaccessibility of bioactive compounds (P. Thakur et al., 2021). One such processing method is solid-state fermentation (SSF). It involves the microbial fermentation of a substrate without water, resulting in greater productivity than other forms of fermentation. Many microorganisms are susceptible to fermentation. The fungal kingdom offers certain advantages over other microorganisms. These advantages include a high protein content (20% to 30% in dry matter) and protein quality. Fungi also serve as a source of dietary fibre and have a high vitamin B content and low fat content.

During the fermentation process, fungi play a crucial role in the formation of different volatile compounds, primarily through the hydrolysis of large molecules such as lipids and proteins into fatty acids and amino acids. This process provides precursors for the formation of a variety of volatile organic compounds (VOCs), including acids, aldehydes, ketones, alcohols, esters and hydrocarbons (Zhong et al., 2022). Both fermentation and drying methods tend to affect the volatile profile of legumes, cereals, and other foods, leading to variations and the generation of new compounds. These variations can have a positive effect on the creation of new ingredients, with an improved volatile profile. Studies have shown that legumes fermented by *Lactobacillus* have more flavour, potentially reducing their beany

flavour while forming aldehydes, alcohols, acids, and sulphur compounds through further biotransformation (C. Yi et al., 2021). However, processes such as hot-air drying and freeze drying can affect VOCs, which are necessary to provide stable fermented ingredients such as flour. For instance, hot-air drying has been observed to decrease aldehydes, alcohols, and esters in volatile compounds of coffee beans (Zhang et al., 2022). Different drying methods can affect the final volatile profile in different ways (Rajkumar et al., 2017).

Despite the existence of studies of the effect of bacteria- and fungi-based fermentation on the volatile profile of fermented substrates, no studies have examined the influence of *Pleurotus ostreatus* fermentation on the volatile profile of quinoa and lentils. The hypothesis tested in the present study is that the volatile profile changes after fermentation and drying of quinoa and lentil flour. This process thereby modifies the final aroma, which is one of the main sensory considerations when choosing new food products. Thus, the aim of this study is to analyse the impact of SSF with *Pleurotus ostreatus* and drying (air-drying at 50 °C, 60 °C and 70 °C and lyophilisation) on the volatile profile of two varieties of lentil and quinoa flour. The findings will be useful for developing new products based on high-protein flour.

2. Materials and methods

2.1. Materials

Hacendado® brand lentils (*Lens culinaris*) of the Pardina and Castellana varieties were purchased from local shops in Valencia, Spain. Hacendado® and Nut&me brand quinoa (*Chenopodium quinoa Wild*) of white and black varieties, respectively, was also purchased from local shops in Valencia, Spain. The *Pleurotus ostreatus* strain was obtained from the Spanish Type Culture Collection (CECT20311). Sodium

chloride (NaCl) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Glucose and mycopeptone were also obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Malt extract and agar-agar were obtained from Scharlau (Barcelona, Spain).

2.2. Fungal solid-state fermentation

Starter culture preparation

A starter culture was prepared by hydrating 10 g of lentil and quinoa flour to 65% moisture. Sterilisation was performed using an autoclave (Vertical Stand Autoclave 4002136, JP Selecta™, Barcelona, Spain; for 20 min at 121°C). Then, 1 mL of *Pleurotus ostreatus* culture was added and kept for 14 days at 28 °C in a digital incubator (digital oven 2001249, JP Selecta™, Barcelona, Spain). *Pleurotus ostreatus* mycelium was previously grown in a culture broth prepared with 2% glucose, 2% malt extract and 0.1% mycopeptone. It was incubated for 14 days at 28 °C in a digital incubator (JP Selecta™ digital oven 4002136, Barcelona, Spain).

Fermentation process

The SSF process was conducted following the method described by Sánchez-García et al. (2023). In total, 35 g of lentils and quinoa at 65% moisture were placed in each glass jar and sterilised in an autoclave (Vertical Stand Autoclave 4002136, JP Selecta™, Barcelona, Spain) for 20 min at 121°C. To achieve a moisture content of 65%, a preliminary material balance calculation was performed. The samples were then mixed with water and left for 20 minutes. Next, the moisture content was checked and autoclaved. *Pleurotus ostreatus* was then inoculated from the starter culture by adding a 1/8 portion. The jars were incubated at 28 °C for 14 days in a digital oven (2001249, JP Selecta™, Barcelona, Spain).

2.3. Drying and milling of fermented grains and seeds

To prepare the different flours, 500 g of fermented quinoa and lentils were hot-air dried at three different temperatures (50, 60 and 70 °C) in a convective dryer (Pol-Eko-Aparatura, CLW 750 TOP+, Wodzisław Śląski, Poland) following the method described by Sánchez-García et al. (2023). As a control, freeze drying was performed using a freeze dryer (Lyoquest-55, Telstar, Terrassa, Spain), as described bySánchez-García et al. (2023). After drying, the samples were milled into flour using a food processor at 15-second intervals for 1 min at 10000 rpm (Thermomix® TM6-1, Vorwerk, Wuppertal, Germany). Multiple glass jars were inoculated to produce an adequate amount of fermented substrate to conduct the experiments. The fermented substrates were combined to create a uniform sample for the subsequent drying process and analysis.

2.4. HS-SPME-GC-MS volatile compounds

The volatile compounds in unfermented, fermented and fermented-dried quinoa and lentil flours were determined using headspace-solid phase microextraction (HS-SPME) and were analysed by gas chromatography/mass spectrometry (GC/MS) following the method described by Escriche et al. (2022). Briefly, 2.5 g of sample and 7.5 mL of 20% w/v sodium chloride were added to a 20 mL glass vial with a screw cap and a PTFE-silicone septum. The mixture was homogenised using a vortex mixer. The sample was then heated on a stirring heating platform at 50 °C and 250 rpm for 30 min. Volatile compounds were trapped using a divinylbenzene/carboxene/polydimethylsiloxane fibre (DVB/CAR/PDMS 50/30 µm). Volatile compounds were analysed using a gas chromatograph (Intuvo 9000, Agilent Technologies, Palo Alto, CA, USA) coupled with a triple quadrupole detector (7000 Series GC/TQ, Agilent Technologies, Palo Alto, CA, USA) that was fitted with an

electron ionisation source at 70 eV. A capillary column (DB WAX, 30 m × 0.25 mm × 0.25 µm, Agilent J&W, Santa Clara, CA, USA) with helium as the carrier gas at a constant flow rate of 1 mL/min was used. The MassHunter Workstation software (Unknown analysis) was used for the data analysis and identification of volatile compounds. The mass spectra of each compound were analysed using the NIST spectral library (NIST 17, National Institute of Standards and Technology). The procedure used a coincidence factor of ≥ 80% and the linear retention indices (LRI). The results are presented as peak area/100000 for ease of reading. The total amount of volatile compounds was estimated by summing all areas of the chromatogram.

2.5. Statistical analysis

The analytical data were analysed using one-way analysis of variance (ANOVA). The least significant difference (LSD) Fisher test was performed to identify homogeneous groups among different drying temperatures. The R programming language (version 4.2.2) was used for analysis. The confidence level was set to 95% (*p-value* < 0.05). All experiments were conducted in triplicate. The data are reported as the mean ± standard deviation.

3. Results and discussion

3.1. Changes in volatile profile due to fungal solid-state fermentation.

The fermentation process *per se* strongly influences the flavour and aroma of the final product. Fungal SSF with *Pleurotus ostreatus* causes different changes depending on the characteristics of the initial substrates and the cultivar. Hence, there may be significant differences in composition, structure, seed coat to cotyledon ratio and seed size (Espinosa-Páez et al., 2017). The use of HS-SPME-GC-MS showed

the generation of sufficient amounts of volatile compounds during fermentation in lentils and quinoa (Tables 5.18 to 5.21). In general, none of the unfermented flour types had a varied volatile profile before fermentation. More volatile compounds were found for white quinoa (Table 5.18) than for black quinoa (Table 5.19). Unfermented black quinoa had nine volatile compounds, constituting 32 ± 9 of the total peak area. These volatile compounds consisted of three aldehydes, one alcohol, one ketone, two heterocyclic compounds, one phenolic compound and one other compound. Unfermented white quinoa had a significantly superior aroma, with a sum of 96 ± 2 in the total peak area. Yang et al. (2021) also observed and correlated these differences between different colour quinoas. In unfermented white quinoa, the concentration and variety of alcohols was higher than in black quinoa. The highest compound was 1-Hexanol (Song et al., 2021; Yang et al., 2021). In unfermented white quinoa (UFWQ), 2-pentylfuran, 1-octen-3-ol, and benzaldehyde were the individual substances that contributed the most to its aroma, in addition to hexanol. The substance 1-Hexanol is correlated with a grassy/green odour, whereas 2-pentylfuran and benzaldehyde were found to give out strong nutty aromas (Yang et al., 2021; Zhang et al., 2019).

On the other hand, unfermented lentils had a greater volatile profile than that of black quinoa and almost double that of white quinoa (Tables 5.20 and 5.21). There were no statistically significant differences between lentil varieties. Nonetheless, the volatile profiles of Pardina and Castellana lentils differed both in concentration and compounds. Unfermented Pardina lentils (UFPL) (Table 5.20) had nine compounds, mainly belonging to alcohol and aldehyde chemical groups. Castellana lentils (Table 5.21) also contained heterocyclic compounds such as furfural and furfuryl methylamphetamine. In both lentil varieties, 1-hexanol and hexanal were highly present, but in different ratios. In unfermented Pardina lentils (UFPL), the aldehyde

hexanal contributed most to the aroma. Its peak area was almost four times lower in unfermented Castellana lentils. The alcohol 1-hexanol was the most present in unfermented Castellana lentils. Although there are no studies of the volatile profile of these lentil varieties, both 1-hexanol and hexanal have been found in red and green lentil flour, with different results in each case (Paucean et al., 2018). Hexanal provided a green, grassy, leafy odour. Furfural, the heterocycle compound, also played an important role in the odour of the unfermented Castellana lentils (UFCL), providing a soil/roasted odour that was absent in unfermented Pardina lentils (UFPL) (Paraskevopoulou et al., 2012).

Regarding the impact of fungal fermentation, the volatile profiles changed notably during fermentation, with *Pleurotus ostreatus* significantly raising the flavour. Fungi are highly polymorphic in their ability to produce a unique profile of VOCs. Each species emits a profile that changes qualitatively and quantitatively. Profiles vary according to the genotype of strains and species. In addition, VOC profiles are influenced by the physical environment, as well as factors such as the age of the fungal colony, the availability of water, the type of substrate, temperature,

and the presence of interacting species. Each mushroom species has a characteristic mixture of volatile flavour components that may include additional aliphatic, terpenoid, aromatic and sulphur-containing compounds (Inamdar et al., 2020). During the process of fermentation and fungal growth, complex biochemical changes take place to create the characteristic aroma. Recent data have shown that fermentation can modify the metabolism of flavour-related compounds (Zhang et al., 2021).

The Pardina lentil was the least affected by fermentation, despite increasing the total peak area by 570% with respect to the unfermented Pardina lentil. On the

contrary, fermented white quinoa (FWQ) was the most affected by fermentation, raising the total area from 96 to 4500. Four common compounds were observed in the volatile profile of the fermented lentils and quinoa. These compounds were also the main contributors to the aroma of the fermented flour. They were 1-octen-3-ol, benzaldehyde, hexanal and 3-methoxybenzaldehyde. The latter two compounds were not found in Pardina lentils. It is well known that the dominant aroma associated with mushrooms is due to a mixture of aliphatic, oxygenated and 8-carbon compounds. They all function as host location cues, especially 1-octen-3-ol, which is related to the odour of mushrooms (Xu et al., 2019; Yang et al., 2019). Indeed, Aisala et al. (2019) found that compounds containing 8-carbons are responsible for mushroom-like odour, being 1-octen-3-ol and 1-octen-3-one the most important. Other compounds such as 1-octanol, 3-octanol, octenal, 2-octenal, 3-octanona and 3-octen-2-one are also typically found in mushrooms. Therefore, the fungal metabolism could be expected to be responsible for generating these compounds during fermentation in the four flour types included in this study.

Benzaldehyde has also been linked to *Pleurotus ostreatus*, giving off sweet and fruity notes (Beltran-Garcia et al., 1997). Besides the sweet aroma, this aldehyde is found in cocoa beans and is linked to the aroma of cocoa (Escobar et al., 2021; Mohamadi Alasti et al., 2019). Unsurprisingly, benzaldehyde is the second most found compound in white and black quinoa flour, since when one smells the flour, the sweet and cocoa aroma is evoked. Fermentation with *Pleurotus ostreatus* transformed the initially odourless unfermented lentil and quinoa samples into sweet-cocoa smelling flour, generating a pleasant and appetizing flavour that does not typically exist in legumes and pseudocereal flour.

Table 5.18. Volatile profile of unfermented white quinoa flour (UFWQ), fermented white quinoa (FWQ) dried at 50, 60, and 70 °C (FWQ-50, FWQ-60, FWQ-70), and lyophilised fermented white quinoa (FWQ-L).

NAME	RT	LRI	UFWQ	FWQ	FWQ-50	FWQ-60	FWQ-70	FWQ-L
FREE ACIDS								
2-Methylpentanoic acid anhydride	22.13	1655	-	24 ± 4	-	-	-	-
Alcohols								
2-Methyl-1-butanol	10.85	1207	-	31 ± 5^{a}	7.2 ± 0.4^{b}	11 ± 2^{b}	10.0 ± 1.2^{b}	38 ± 4^{a}
1-Hexanol	14.80	1280	77.11 ± 0.73^{b}	36.0 ± 0.8^{e}	54 ± 2^{c}	48.4 ± 1.4^{d}	47.8 ± 0.9^{d}	91 ± 2^{a}
3-Octanol	15.85	1299	-	28 ± 8^{b}	15 ± 2^{b}	-	-	140 ± 4^{a}
1-Octen-3-ol	17.25	1452	2.19 ± 0.01^{e}	196 ± 2^{b}	119 ± 5^{dc}	115 ± 3^{d}	132.9 ± 0.4^{c}	395 ± 14^{a}
1-Heptanol	17.41	1459	1.56 ± 0.01^{e}	42 ± 5^{a}	9 ± 0.5^{d}	7.3 ± 0.2^{d}	21 ± 11^{b}	11 ± 3^{c}
2-Methyl-6-hepten-1-ol	17.60	1466	1.75 ± 0.14	-	-	-	-	-
4-Ethylcyclohexanol	19.50	1543	-	33.55 ± 0.08	-	-	-	-
1-Octanol	19.90	1560	-	51.8 ± 2.5^{a}	9.2 ± 0.3^{c}	8.53 ± 0.16^{c}	8.6 ± 0.4^{c}	22 ± 3^{b}
Benzyl alcohol	26.80	1871	0.61 ± 0.07^{d}	3.23 ± 0.06^{c}	3.6 ± 0.3^{c}	5.0 ± 0.3^{b}	14.5 ± 0.4^{a}	-
Phenylethyl Alcohol	27.50	1905	0.5 ± 0.2^{d}	3.2 ± 0.4^{a}	1.4 ± 0.3^{c}	3.37 ± 0.04^{a}	3.5 ± 0.3^{a}	2.15 ± 0.01^{b}
ALDEHYDES								
Hexanal	7.22	1169	-	1400 ± 18^{a}	840 ± 60^{b}	832 ± 92^{b}	741 ± 2^{b}	570 ± 47^{c}
Octanal	12.70	1240	-	99.26 ± 1.12^{a}	74 ± 7^{b}	78.61 ± 1.08^{b}	81 ± 2^{b}	21 ± 3^{c}
2-Heptenal	13.70	1259	-	38 ± 6^{a}	-	-	-	27 ± 5^{a}
Nonanal	15.60	1294	-	123 ± 6^{a}	97 ± 9^{b}	115 ± 4^{a}	129 ± 6^{a}	26.4 ± 0.2^{c}
5-Ethylcyclopent-1-enecarboxaldehyde	16.15	1408	-	45.98 ± 0.08^{a}	27 ± 2^{c}	27.6 ± 0.5^{cb}	29.05 ± 0.01^{b}	11.7 ± 0.9^{d}
2-Octenal	16.50	1422	-	156 ± 9^{a}	36 ± 0.3^{c}	34 ± 11^{c}	35.7 ± 1.5^{c}	65 ± 9^{b}
Decanal	18.30	1494	-	17.55 ± 0.18^{a}	14 ± 3^{a}	16 ± 2^{a}	17.6 ± 1.2^{a}	4.6 ± 1.2^{b}
Benzaldehyde	18.80	1514	1.91 ± 0.39^{c}	1221 ± 113^{c}	309 ± 10^{b}	295 ± 7^{b}	308 ± 29^{b}	405 ± 17^{b}
5-methyl-2-furancarboxaldehyde	20.05	1566	-	13.63 ± 0.35^{a}	4.6 ± 0.3^{c}	9.50 ± 1.14^{b}	12 ± 2^{ab}	-
Benzeneacetaldehyde	21.60	1632	-	3 ± 2^{c}	15 ± 3^{b}	25.1 ± 1.8^{a}	25.2 ± 0.3^{a}	6.43 ± 0.07^{c}
2-Decenal	21.73	1638	-	15 ± 2^{a}	-	4.83 ± 1.14^{b}	5.9 ± 0.4^{b}	4.3 ± 0.3^{b}
2-Butyl-2-octenal	22.30	1663	-	12.76 ± 2.8^{a}	6.8 ± 0.4^{b}	6.59 ± 0.04^{b}	6 ± 2^{bc}	1.8 ± 0.4^{c}

2-hydroxy-benzaldehyde	22.40	1667	-	2.9 ± 0.3	-	-	-	-
3-Methoxybenzaldehyde	29.65	>2000	-	345 ± 24^{a}	190 ± 26^{b}	235 ± 2^{b}	231 ± 5^{b}	318 ± 23^{a}
3-Chloro-4-methoxybenzaldehyde	32.78	>2000	-	4.5 ± 0.44^{b}	2.59 ± 0.13^{c}	3.0 ± 0.2^{c}	3.3 ± 0.4^{c}	8.9 ± 0.4^{a}
KETONES								
3-Octen-2-one	16.02	1403	-	65.11 ± 1.19^{a}	26 ± 4^{b}	28.8 ± 1.1^{b}	29.2 ± 0.6^{b}	11.3 ± 1.8^{c}
Methyl-1-cyclopenten-1-yl)-ethanone	20.60	1589	0.28 ± 0.07^{d}	6.32 ± 0.18^{c}	14 ± 2^{b}	18.4 ± 0.3^{a}	20.1 ± 0.7^{a}	1.1 ± 0.1^{d}
2-Undecanone	20.75	1595	-	11 ± 3	-	-	-	-
Acetophenone	21.80	1641	0.36 ± 0.01^{c}	2.39 ± 0.01^{b}	2.93 ± 0.04^{a}	2.7 ± 0.3^{a}	2.5 ± 0.4^{a}	1.2 ± 0.2^{a}
5-(Hydroxymethyl)dihydrofuran-2(3H)-one	23.00	1693	-	7.9 ± 0.2	-	-	-	-
HETEROCYCLIC COMPOUNDS								
2-Pentylfuran	11.25	1214	6.69 ± 0.31^{e}	123 ± 14^{c}	153 ± 7^{b}	230 ± 13^{a}	244 ± 6^{a}	97 ± 12^{d}
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	17.01	1261	1.54 ± 0.01^{b}	22.34 ± 0.09^{a}	-	-	-	-
2-Propylfuran	18.13	1487	-	4.36 ± 0.05	-	-	-	-
Pyrrole	18.70	1510	1.58 ± 0.18	-	-	-	-	-
2-n-Butyl furan	20.46	1583	-	10.4 ± 0.7^{a}	-	-	-	7.1 ± 1.3^{a}
3-Phenylfuran	26.22	1844	-	42 ± 14^{a}	1.8 ± 0.2^{b}	2.9 ± 0.2^{b}	3.6 ± 0.3^{b}	21 ± 10^{b}
2(3H)-Furanone, dihydro-5-pentyl-	29.78	>2000	-	2.3 ± 0.5^{c}	12 ± 2^{ab}	14.78 ± 0.10^{a}	10.28 ± 0.07^{b}	9.5 ± 0.5^{b}
PHENOLIC COMPOUNDS								
Phenol	29.34	1998	0.37 ± 0.02^{d}	2.1 ± 0.2^{c}	3.74 ± 0.01^{b}	4.6 ± 0.2^{a}	4.3 ± 0.5^{ab}	-
ESTERS								
Ethyl hexanoate	11.46	1218	-	200.06 ± 0.04^{a}	116 ± 2^{c}	140.4 ± 1.7^{bc}	122 ± 10^{c}	175 ± 30^{ab}
Ethyl octanoate	16.75	1432	-	58 ± 8^{a}	36 ± 2^{b}	57 ± 8^{a}	53 ± 2^{a}	35 ± 12^{b}
Ethyl benzoate	22.22	1660	-	-	2.7 ± 0.3^{b}	1.87 ± 0.10^{a}	2.09 ± 0.09^{b}	6.4 ± 0.5^{a}
Methyl 4-methoxybenzoate	30.96	>2000	-	7.6 ± 0.9^{a}	2.5 ± 0.6^{c}	4.80 ± 0.13^{b}	5.4 ± 0.2^{b}	5.5 ± 0.7^{b}
ETHER								
2-Propylphenol, methyl ether	26.07	1836	-	3.42 ± 0.16^{b}	1.9 ± 0.5^{c}	3.12 ± 0.01^{b}	4.2 ± 0.4^{a}	1.03 ± 0.04^{d}
TOTAL			96 ± 2^{c}	4500 ± 250^{a}	2200 ± 145^{b}	2389 ± 155^{b}	2360 ± 88^{b}	2550 ± 200^{b}

The data represent the peak area/100000. ^{a,b,c,d,e} Lowercase letters indicate significant differences at the 95% ($p < 0.05$) significance level between flours in the same row. RT: retention time. LRI: linear retention indices.

Table 5.19. Volatile profile of unfermented black quinoa flour (UFBQ), fermented black quinoa (FBQ) dried at 50, 60, and 70 °C (FBQ-50, FBQ-60, FBQ-70), and lyophilised fermented black quinoa (FBQ-L).

NAME	RT	LRI	UFBQ	FBQ	FBQ-50	FBQ-60	FBQ-70	FBQ-L
ALCOHOLS								
1-Pentanol	12.21	1232	-	32 ± 3^{a}	18.8 ± 1.8^{bc}	13 ± 4^{c}	-	25 ± 3^{ab}
1-Hexanol	14.80	1280	3.6 ± 0.1^{e}	12.6 ± 1.2^{d}	14.1 ± 0.5^{c}	11.88 ± 0.04^{d}	15.843 ± 0.009^{b}	29 ± 0.3^{a}
3-Octanol	15.85	1299	-	69 ± 17^{b}	25 ± 4^{c}	0.9 ± 0.2^{c}	-	165 ± 3^{a}
1-Octen-3-ol	17.25	1452	-	323 ± 25^{a}	50.5 ± 0.3^{a}	61.6 ± 1.5^{c}	62.6 ± 1.6^{c}	177 ± 3^{b}
1-Heptanol	17.41	1459	-	8.08 ± 0.02	-	-	-	-
1-Octanol	19.90	1560	-	14.1 ± 0.9^{a}	3.98 ± 0.03^{c}	1.9 ± 0.3^{d}	1.5 ± 0.8^{d}	7.8 ± 0.8^{b}
ALDEHYDES								
Hexanal	7.22	1169	-	724 ± 38^{a}	310 ± 3^{c}	279 ± 8^{c}	311 ± 31^{c}	490 ± 27^{b}
Octanal	12.70	1240	-	24.8 ± 1.5^{c}	40.1 ± 1.5^{b}	24.7 ± 1.0^{c}	40 ± 13^{b}	66 ± 3^{a}
2-Heptenal	13.70	1259	-	18.2 ± 0.8	-	-	-	-
Nonanal	15.60	1294	-	23 ± 4^{c}	30 ± 2^{b}	29.8 ± 0.6^{b}	38 ± 3^{a}	29 ± 3^{b}
5-Ethylcyclopent-1-enecarboxaldehyde	16.15	1408	-	17.1 ± 0.4^{ab}	13.2 ± 0.3^{d}	14.5 ± 1.0^{cd}	16 ± 0.06^{bc}	19 ± 2^{a}
2-Octenal, (E)-	16.50	1422	-	71 ± 3	-	-	-	-
Benzaldehyde	18.80	1514	3.9 ± 0.6^{c}	437 ± 22^{a}	130 ± 3^{b}	194 ± 23^{b}	187 ± 6.9^{b}	406 ± 77^{a}
5-methyl-2-furancarboxaldehyde	20.05	1566	-	16.1 ± 0.8^{a}	12 ± 3^{b}	11.0 ± 0.2^{b}	10.7 ± 0.3^{b}	17.2 ± 1.3^{a}
Benzeneacetaldehyde	21.60	1632	5 ± 5^{d}	13.7 ± 1.2^{c}	28 ± 2^{a}	25.7 ± 0.2^{ab}	29.7 ± 1.2^{a}	20.4 ± 0.6^{b}
2,3-dihydro-1H-indene-4-carbaldehyde	27.80	1921	-	-	0.658 ± 0.002^{a}	0.41 ± 0.02^{b}	0.34 ± 0.02^{c}	-
3-Methoxybenzaldehyde	29.65	>2000	2.3 ± 0.7^{d}	112 ± 6^{b}	90 ± 4^{c}	98 ± 4^{bc}	86 ± 4^{c}	202 ± 16^{a}
3-Chloro-4-methoxybenzaldehyde	32.78	>2000	-	2.7 ± 0.2^{b}	1.7 ± 0.2^{c}	1.94 ± 0.06^{c}	2.1 ± 0.3^{c}	3.4 ± 0.3^{a}
KETONES								
3-Octanone	11.85	1225	-	238 ± 63^{a}	-	-	-	122 ± 4^{a}
1-Hepten-3-one	13.25	1251	-	13 ± 3	-	-	-	-
	14.00	1264	-	10.4 ± 0.3	-	-	-	-
3-Octen-2-one	16.02	1403	-	34 ± 3^{c}	38.6 ± 1.2^{b}	31.9 ± 0.3^{c}	31 ± 0.3^{c}	44 ± 3^{a}
Acetophenone	21.80	1641	0.42 ± 0.1^{d}	1.4 ± 0.2^{c}	3.5 ± 0.4^{a}	2.8 ± 0.5^{b}	2.31 ± 0.04^{b}	2.37 ± 0.09^{b}

HETEROCYCLIC COMPOUNDS								
2-Pentylfuran	11.25	1214	-	75 ± 4^{c}	248 ± 17^{a}	223 ± 4^{a}	165 ± 32^{b}	93 ± 4^{c}
Furfural	17.39	1458	3.4 ± 0.2^{d}	7.5 ± 0.4^{ab}	8.81 ± 0.02^{a}	6.6 ± 0.9^{abc}	4.9 ± 1.7^{bcd}	4.2 ± 1.2^{cd}
Naphthalene	23.70	1725	2.9 ± 0.9	-	-	-	-	-
PHENOLIC COMPOUNDS								
Phenol	29.34	1998	0.83 ± 0.02^{b}	1.14 ± 0.08^{b}	3.4 ± 0.4^{a}	3.2 ± 0.2^{a}	3.1 ± 0.4^{a}	1.2 ± 0.1^{b}
ESTERS								
Ethyl octanoate	16.75	1432	-	-	7.8 ± 0.4^{a}	2.3 ± 0.3^{c}	5.0 ± 0.2^{b}	-
Methyl 4-methoxybenzoate	30.96	>2000	-	1.06 ± 0.06^{a}	0.99 ± 0.09^{a}	0.9 ± 0.2^{a}	0.86 ± 0.15^{a}	1.5 ± 0.2^{a}
OTHERS								
4-Isothiocyanate-1-butene	17.15	1448	7.6 ± 1.1	-	-	-	-	-
.beta.-Bisabolene	23.55	1719	-	-	2.9 ± 0.2^{a}	2.2 ± 0.2^{b}	2.3 ± 0.1^{b}	-
TOTAL			32 ± 9^{c}	2310 ± 200^{a}	1080 ± 45^{b}	1040 ± 50^{b}	1020 ± 97^{b}	1970 ± 150^{a}

The data represent the peak area/100000. ^{a,b,c,d,e} Lowercase letters indicate significant differences at the 95% ($p < 0.05$) significance level between flours in the same row. RT: retention time. LRI: linear retention indices.

Table 5.20. Volatile profile of unfermented Pardina lentil flour (UFPL), fermented Pardina lentil (FPL) dried at 50, 60, and 70 °C (FPL-50, FPL-60, FPL-70), and lyophilised fermented Pardina lentil (FPL-L).

NAME	RT	LRI	UFPL	FPL	FPL-50	FPL-60	FPL-70	FPL-L
ALCOHOLS								
1-Hexanol	14.80	1207	29 ± 2^{a}	-	1.9 ± 0.6^{c}	-	-	6 ± 2^{b}
3-Octanol	15.85	1299	-	98 ± 14^{a}	31 ± 3^{c}	35.6 ± 1.8^{bc}	49 ± 12^{c}	59 ± 4^{b}
1-Octen-3-ol	17.25	1452	-	353 ± 27^{a}	83 ± 2^{c}	102 ± 5^{c}	108 ± 21^{c}	207 ± 8^{b}
1-Heptanol	17.41	1459	2.5 ± 0.4	-	-	-	-	-
2-Ethyl-1-hexanol	18.25	1492	1.4 ± 0.1	-	-	-	-	-
Benzyl alcohol	26.80	1871	1.51 ± 0.02^{a}	2.14 ± 0.17^{a}	1.01 ± 0.03^{a}	-	-	2.1 ± 0.4^{a}
Phenylethyl Alcohol	27.50	1905	0.9 ± 0.1^{a}	0.47 ± 0.06^{b}	-	-	-	0.5 ± 0.1^{ab}
ALDEHYDES								
Hexanal	7.22	1169	132 ± 11^{a}	10 ± 5^{b}	17 ± 3^{b}	14 ± 2^{b}	15 ± 2^{b}	-
3,5,5-trimethyl-1-hexanal	18.10	1485	-	27 ± 1^{a}	-	-	-	22 ± 3^{a}
Benzaldehyde	18.80	1514	6.9 ± 0.6^{d}	405 ± 6^{b}	583 ± 8^{a}	129 ± 6^{c}	444 ± 47^{b}	473 ± 50^{b}
Benzeneacetaldehyde	21.60	1632	1.5 ± 0.3^{c}	39 ± 3^{a}	24.7 ± 1.5^{b}	25 ± 3^{b}	36 ± 4^{b}	29 ± 2^{b}
2,3-dihydro-1H-indene-4-carbaldehyde	27.80	1921	-	0.62 ± 0.04	-	-	-	-
3-Methoxybenzaldehyde	29.65	>2000	-	2.7 ± 0.2^{b}	1.7 ± 0.1^{b}	1.1 ± 0.1^{b}	1.8 ± 0.2^{b}	8.0 ± 1.5^{a}
3-Chloro-4-methoxybenzaldehyde	32.78	>2000	-	3.5 ± 0.3^{a}	4.4 ± 0.3^{a}	3.13 ± 0.06^{a}	4.6 ± 0.3^{a}	3.1 ± 1.4^{a}
KETONES								
3-Octanone	11.85	1225	-	172 ± 28^{a}	20 ± 7^{b}	34.1 ± 0.9^{b}	16 ± 6^{b}	43 ± 5^{b}
1-Hepten-3-one	13.25	1251	-	18 ± 1^{a}	-	-	-	11.3 ± 0.2^{b}
Acetophenone	21.80	1641	-	4.1 ± 0.8^{b}	2.5 ± 0.2^{b}	3.3 ± 0.2^{b}	3.28 ± 0.02^{b}	3 ± 1^{a}
HETEROCYCLIC COMPOUNDS								
3-Ethyl-2,5-dimethylpyrazine	17.06	1445	-	-	3.20 ± 0.06^{c}	8.7 ± 0.7^{b}	5.5 ± 0.8^{a}	-
3-Phenylfuran	26.22	1844	-	0.99 ± 0.03^{b}	0.85 ± 0.08^{b}	1.29 ± 0.06^{a}	1.54 ± 0.07^{a}	0.79 ± 0.09^{b}
PHENOLIC COMPOUNDS								
Phenol	29.34	1998	0.44 ± 0.06^{d}	0.85 ± 0.01^{ab}	0.73 ± 0.02^{bc}	0.6 ± 0.1^{cd}	1.05 ± 0.15^{a}	0.684 ± 0.001^{ab}
TOTAL			199 ± 18^{d}	1137 ± 87^{a}	780 ± 28^{b}	362 ± 18^{c}	685 ± 93^{b}	880 ± 36^{b}

The data represent the peak area/100000. ^{a,b,c,d} Lowercase letters indicate significant differences at the 95% ($p < 0.05$) significance level between flours in the same row. RT: retention time. LRI: linear retention indices.

Table 5.21. Volatile profile of unfermented Castellana lentil flour (UFCL), fermented Castellana lentil (FCL) dried at 50, 60, and 70 °C (FCL-50, FCL-60, FCL-70), and lyophilised fermented Castellana lentil (FCL-L).

NAME	RT	LRI	UFCL	FCL	FCL-50	FCL-60	FCL-70	FCL-L
ALCOHOLS								
1-Hexanol	14.80	1280	70.3 ± 0.4^{a}	28 ± 2^{b}	8.6 ± 0.5^{d}	5.6 ± 0.4^{e}	5.4 ± 0.5^{e}	17.7 ± 0.2^{c}
3-Octanol	15.85	1299	-	99 ± 15^{a}	-	-	-	14 ± 5^{b}
1-Octen-3-ol	17.25	1452	3.1 ± 0.3^{d}	302 ± 27^{b}	30 ± 2^{c}	45.4 ± 0.6^{c}	37.5 ± 1.5^{c}	460.6 ± 1.4^{a}
2-Ethyl-1-hexanol	18.25	1492	1.6 ± 0.2	-	-	-	-	-
1-Octanol	19.90	1560	-	6.8 ± 0.2^{a}	1.8 ± 0.2^{c}	-	-	3.9 ± 0.2^{b}
Cyclooctyl alcohol	21.25	1617	-	7.3 ± 0.6^{a}	-	-	-	4.24 ± 0.08^{b}
1-Nonanol	22.25	1661	1.2 ± 0.1	-	-	-	-	-
Benzyl alcohol	26.80	1871	4.7 ± 0.7^{a}	0.57 ± 0.02^{d}	0.9 ± 0.2^{cd}	1.5 ± 0.1^{c}	3.8 ± 0.2^{b}	1.6 ± 0.2^{c}
ALDEHYDES								
Hexanal	7.22	1169	38 ± 3^{b}	131 ± 11^{a}	48 ± 3^{b}	16 ± 3^{c}	-	45.9 ± 0.2^{b}
Nonanal	15.60	1294	-	10.7 ± 0.8^{a}	5.1 ± 0.2^{b}	-	-	8.9 ± 0.8^{a}
5-Ethylcyclopent-1-enecarboxaldehyde	16.15	1408	-	11.5 ± 0.6^{a}	4.1 ± 0.2^{b}	-	-	-
2-Octenal	16.50	1422	-	16 ± 2^{a}	17.8 ± 0.8^{a}	-	-	-
Decanal	18.30	1494	-	6.9 ± 1.4	-	-	-	-
Benzaldehyde	18.80	1514	4.5 ± 0.2^{e}	250 ± 19^{b}	91 ± 0.7^{d}	118 ± 2^{d}	149.1 ± 0.7^{c}	530 ± 22^{a}
2-Nonenal, (E)-	19.20	1531	-	3.8 ± 0.3	-	-	-	-
5-methyl-2-furancarboxaldehyde	20.05	1566	4.5 ± 1.6^{ab}	5.4 ± 0.2^{a}	4.1 ± 0.1^{abc}	3.4 ± 0.2^{bc}	3.81 ± 0.02^{abc}	2.4 ± 0.8^{c}
Benzeneacetaldehyde	21.60	1632	-	27 ± 2^{b}	45.7 ± 1.6^{a}	49.5 ± 0.4^{a}	46.8 ± 1.9^{a}	26.9 ± 0.4^{b}
3-Ethylbenzaldehyde	23.00	1694	-	1.39 ± 0.06^{a}	0.78 ± 0.07^{b}	-	-	0.95 ± 0.01^{b}
2-Phenylpropenal	25.10	1790	-	2.02 ± 0.09	-	-	-	-
Benzeneacetaldehyde, .alpha.-ethylidene-	27.80	1921	-	0.61 ± 0.01^{c}	1.9 ± 0.2^{a}	1.24 ± 0.01^{b}	1.2 ± 0.2^{b}	0.963 ± 0.002^{b}
3-Methoxybenzaldehyde	29.65	>2000	-	698 ± 20^{b}	145 ± 10^{d}	260 ± 11^{c}	260 ± 6^{c}	838 ± 19^{a}
3-Chloro-4-methoxybenzaldehyde	32.78	>2000	-	28.3 ± 0.4^{b}	5.7 ± 0.5^{e}	15.9 ± 0.9^{d}	17.6 ± 0.8^{c}	31.7 ± 0.03^{a}
KETONES								
1-Hepten-3-one	13.25	1251	-	14 ± 3^{a}	-	-	-	13 ± 2^{a}

Acetophenone	21.80	1641	-	1.7 ± 0.5^{b}	5.5 ± 0.5^{a}	5.9 ± 0.3^{a}	5.6 ± 0.3^{a}	1.5 ± 0.2^{b}
HETEROCYCLIC COMPOUNDS								
Furfurylmethylamphetamine	11.00	1210	2.1 ± 0.2^{c}	9 ± 2^{b}	-	-	-	24.8 ± 0.5^{a}
2-Pentylfuran	11.25	1214	-	-	87.3 ± 0.02^{a}	20 ± 5^{b}	14 ± 4^{b}	-
2,6-Dimethylpyrazine	14.02	1265	-	-	16.4 ± 1.9^{a}	8.9 ± 0.4^{b}	13.6 ± 0.6^{a}	-
3-Ethyl-2,5-dimethylpyrazine	17.06	1445	-	-	5.3 ± 0.4^{a}	3.8 ± 0.2^{b}	4.988 ± 0.001^{a}	-
Furfural	17.39	1458	27 ± 9^{a}	21 ± 4^{ab}	13 ± 3^{bc}	11.5 ± 1.7^{bc}	11.6 ± 0.02^{bc}	10 ± 2^{c}
2-Ethyl-5-methylpyrazine	18.10	1486	-	16 ± 0.4^{a}	4.4 ± 0.7^{c}	6.1 ± 0.2^{b}	6.9 ± 0.3^{b}	-
2-n-Butyl furan	20.46	1583	-	1.11 ± 0.05	-	-	-	-
3-Phenylfuran	26.22	1844	-	-	2.14 ± 0.09^{ab}	1.8 ± 0.2^{b}	2.6 ± 0.3^{a}	0.63 ± 0.08^{c}
PHENOLIC COMPOUNDS								
Phenol	29.34	1998	1.2 ± 0.2^{cd}	1.00 ± 0.01^{d}	2.9 ± 0.1^{c}	2.22 ± 0.14^{b}	1.4 ± 0.1^{c}	2.8 ± 0.3^{a}
ESTERS								
Ethyl 4-(ethyloxy)-2-oxobut-3-enoate	13.85	1262	-	2.5 ± 0.3^{a}	2.1 ± 0.2^{a}	-	-	-
Methyl benzoate	21.17	1614	-	1.03 ± 0.07	-	-	-	-
Methyl 4-methoxybenzoate	30.96	>2000	-	2.38 ± 0.01^{a}	-	-	-	2.3 ± 0.2^{a}
OTHERS								
Methane-d, trichloro-	5.58	1154	-	28 ± 5^{a}	-	-	-	-
TOTAL			160 ± 15^{d}	1730 ± 120^{b}	554 ± 28^{c}	585 ± 27^{c}	592 ± 18^{c}	2052 ± 56^{a}

The data represent the peak area/100000. ^{a,b,c,d,e} Lowercase letters indicate significant differences at the 95% ($p < 0.05$) significance level between flours in the same row. RT: retention time. LRI: linear retention indices.

In addition to these major compounds, a cascade of reactions was triggered during fermentation. These reactions increased the complexity of the volatile profile in both lentil and quinoa flour. In fermented Castellana lentils (FCL), six alcohols, 14 aldehydes, two ketones, four heterocyclic compounds, one phenolic compound and three esters were formed, enriching their aroma. Nonanal provided citrus notes, benzeneacetaldehyde provided grassy and flowery aromas, and 2-Ethyl-5-methylpyrazine provided a nutty, roasted, and chocolaty flavour (Aisala et al., 2019; FAO, 2023; Qian et al., 2019). The compound 1-hexanol was prevalent in unfermented Castellana lentils. This compound decreased significantly with benzyl alcohol during fermentation.

Fermented Pardina lentils presented only four alcohols, seven aldehydes, three ketones, one heterocyclic compound and one phenolic compound after fermentation. Ketones mainly provided fresh, herbaceous, woody, and fruity flavours (Xu et al., 2019). Other volatile components such as 1-hepten-3-one provided an earthy, green odour (Ebert et al., 2022).

Finally, in quinoa seeds the number of compounds increased significantly after fermentation. The white quinoa profile increased from 13 to 41 compounds, whereas the black quinoa profile rose from 9 to 26. In both cases, aldehydes were the most affected chemical group. The aroma of fermented black quinoa (FBQ) was also characterised by other compounds such as 2-heptanal, nonanal and furfural. Despite being found in lower concentrations, these compounds also provided the FBQ with a fruity, sweet, cooked-bean aroma (Hao et al., 2023; Sharan et al., 2022). In fermented white quinoa, other compounds such as 1-heptanol and 2-methyl-1-butanol also formed. The concentration of the alcohol 1-heptanol multiplied 40 times, contributing to a sweeter final aroma after fermentation (S. Wu et al., 2021).

Compounds such as decanal increased this sweet aroma. After fermentation, furan compounds such as 2-Pentylfuran, ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate, 2-propylfuran, 2-nButyl-furan, 3-penthylfuran, and 2(3H)-Furanone, dihydro-5-pentyl- increased/formed. The generation of furan compounds in the volatile profile resulted from sterilisation prior to fermentation rather than the fungal metabolism itself (Maga & Katz, 2009). Finally, esters were also generated after fermentation in fermented white quinoa. They were responsible for fruity odours (Ouellette & Rawn, 2014). In short, the aroma of white quinoa was the most affected by SSF with *Pleurotus ostreatus,* generating a new, more complex, sweeter aroma than that of unfermented white quinoa.

3.2. Impact of dehydration on the volatile profile of fermented quinoa and lentil flour.

After fermentation, the samples were submitted to hot-air drying at three temperatures (50, 60 and 70 °C), as well as lyophilisation. The impact of drying on the volatile profile seemed to depend on the substrate. The results appear in Tables 5.18 to 5.21.

Dried fermented white quinoa flour (Table 5.18) decreased in total volatile volume from 4500 to less than 2500 total peak area. This decrease means that the total volatile compounds fell by 50%. There were no significant differences between the results for different hot-air drying temperatures and drying methods. However, the profile differed slightly between white quinoa flour. Some compounds, such as 4-Ethylcyclohexanol, 2-hydroxy-benzaldehyde and 2-undecanone, disappeared completely after drying. Meanwhile, the presence of others decreased or increased significantly. The amount of hexanal and 2-pentylfuran decreased by more than 50% after lyophilisation. In contrast, the amount of 3-Octanol and 1-Octen-3-ol increased

significantly. This combination resulted in lyophilised fermented white quinoa (FWQ-L) with a less nutty aroma but a stronger mushroom odour than hot-air dried fermented white quinoa. Similar results are reported in the literature for the comparison between hot-air drying and freeze drying. Rajkumar et al. (2017) reported that some compounds were formed after freeze drying and/or hot-air drying of cabbage, whereas other compounds did not withstand drying and disappeared. Four main routes are involved in the formation of volatile flavour compounds during drying. These routes include Maillard reactions, long-chain compound degradation and lipid oxidation and degradation (Deng et al., 2015; Yang et al., 2016). The loss of volatile flavour compounds is more common with increased temperature at the same moisture content due to thermal degradation, volatilisation, and other chemical reactions (Ge et al., 2020).

In the case of fermented black quinoa flour (Table 5.19), the sum of volatile compounds in the hot-air dried samples decreased by approximately 65% with respect to fermented black quinoa flour. There were no significant differences between fermented black quinoa dried at 50, 60 and 70 °C. However, FBQ-L did not change significantly (1960 ± 150) from fermented black quinoa (2310 ± 200). Almost all compounds that increased (hexanol, 3-Octanol and 1-Octen-3-ol) and decreased (hexanal) in FWQ-L also decreased in lyophilised fermented black quinoa. However, other compounds, such as 3-methoxybenzaldehyde increased significantly in FBQ-L. Thus, the concentration of volatiles was equal to that found in fermented black quinoa. The results imply that hot-air drying significantly reduced all volatile compounds, except for 2-pentylfuran, which increased. In addition, another three compounds (ethyl octanoate, beta-bisabolene and 2,3-dihydro-1H-indene-4-carbaldehyde) were generated with drying, regardless of temperature. As noted earlier, the formation of furan compounds such as 2-pentylfuran increased with

temperature. Ge et al. (2020) studied the volatile flavour in peppers dried at different temperatures, finding that some furan compounds increased with temperature under Strecker degradation. They also found that the compound ethyl octanoate increased significantly ($p < 0.05$) due to drying.

There were also changes in fermented lentil flour following drying. The total sum of volatiles in Pardina lentil flour (Table 5.20) decreased the least after drying, falling by only around 30%. However, an interesting effect occurred at 60 °C in fermented Pardina lentils, where the total sum of the areas decreased by 70%. This finding is mainly due to a decrease in the aldehyde benzaldehyde area from 405 to 129. Meanwhile, an increase in this compound was found in FPL dried at 50 °C, 70°C, and lyophilised. According to previous studies, this compound commonly increases after drying due to the degradation of benzoic acid into benzaldehyde (Pei et al., 2016). However, this compound decreased or maintained its presence in all other hot-air dried flour. This volatile compound was the most prevalent in Pardina lentil flour, giving it a nutty smell. Therefore, FPL-60 flavour would be milder in this hue than its equivalents.

The total peak area of fermented Castellana lentils dried at the three different temperatures (FCL-50, FCL-60, and FCL-70) (Table 5.21) decreased by 70% with respect to fermented Castellana lentils. Even though fermentation enriched the volatile profile in Castellana lentils to a greater extent than in Pardina lentils, hot-air drying led to a loss of aroma because of the drastic reduction of relevant compounds such as 1-octen-3-ol, hexanal, benzaldehyde and 3-methoxybenzaldehyde. However, lyophilised fermented Castellana lentil (FCL-L) significantly improved in terms of the concentration of these compounds. This greater concentration intensified the flavour of fermented Castellana lentil flour by 118%. Alcohols and aldehydes were the most

affected chemical groups, gradually diminishing as air temperature increased. Similar results have been reported for hot-air dried peppers (Ge et al., 2020). Given these changes, lyophilised fermented Castellana lentils had a green, grassy, leafy odour with nut and mushroom aromas. In the case of fermented Castellana lentils dried at 50, 60 and 70 °C, the aroma had sweet, burnt, and baked notes due to the pyrazines and 3-pentylfuran generated by Strecker degradation and Maillard reactions in hot-air drying (Fischer et al., 2017).

In sum, lyophilisation was much better in terms of maintenance of volatile compounds in the samples of black quinoa and Castellana lentils. In black quinoa, there was no loss of volatiles. In Castellana lentils, the total peak area increased by 14%. However, for white quinoa, there were no differences between types of drying or temperatures of hot-air drying. All reduced total peak area by 50%. In the case of Pardina lentils, drying at 60 °C provided lower results. In contrast, hot-air drying at 50 °C and 70 °C and freeze drying did not lead to significant differences. These processes affected the sample in the same way. Thus, even though hot-air drying led to a loss of the aroma generated during fermentation, the sweet, fruity, cocoa smell with hints of mushrooms and cooked substrates that was observed in the undried fermented samples was preserved, given the high concentrations of benzaldehyde, hexanal, nonanal, furfural and 1-octen-3-ol.

3.3. Principal component analysis of the volatile profile of different flours.

Principal component analysis (PCA) was performed to visualise the contribution of the volatile compounds to the flavour profile of each flour. This analysis highlighted differences in the generation of volatiles during SSF and the drying of samples. Figure 5.11 to 5.14 show the biplot for each substrate (Castellana lentils, Pardina lentils, white quinoa, and black quinoa). The sum of the first two principal

components (PC1 and PC2) explained 74.49%, 75.02%, 80.74% and 83.53% of the total variance of Pardina lentils, Castellana lentils, white quinoa, and black quinoa, respectively. A consistent pattern in sample clustering was observed for all substrates. PC1 distinguished unfermented samples (negative axis) from fermented and lyophilised samples (positive axis), except for white quinoa. Lyophilised fermented white quinoa was closer to UWQ. PC2 distinguished hot-air dried samples (positive axis) from unfermented and fermented samples (negative axis). In the case of lyophilised flour, PC2 grouped these samples together with hot-air dried samples for Castellana lentils and black quinoa. However, it distinguished between the samples for Pardina lentils and white quinoa. Therefore, freeze drying had different effects on the volatile profile depending on the substrate. The values of the variables defining the PC1 and PC2 equations were standardised by subtracting the means and dividing by the standard deviations of the volatile compounds. More strongly positive or negative values meant a greater contribution to explaining variability in the data. The volatile compounds that contributed the most to discriminating Pardina lentil samples (Figure 5.11) in PC1 were the alcohols 2-ethyl-1-hexanol, 1-hexanol and 1-Heptanol and the aldehyde hexanal. In PC1, the concentrations of the heterocyclic compound 3-ethyl-2,5-dimethylpyrazine, phenylethyl alcohol and the aldehydes 3-chloro-4-methoxybenzaldehyde, 3,5,5-trimethyl-1-hexanal and 1-Hepten-3-one distinguished hot-air dried samples from the other samples. For Castellana lentils (Figure 5.12), differences in concentrations of some alcohols (3-octanol, 1-octanol and cyclooctyl alcohol), the ester methyl 4-methoxybenzoate and some aldehydes (Hexanal, 4-Ethylbenzaldehyde and 2-Phenylpropenal) distinguished unfermented flour from fermented and lyophilised flour (PC1). Hot-air dried fermented Castellana flour was characterised by higher concentrations of acetophenone, benzeneacetaldehyde, furans and pyrazines. The aroma of unfermented flour was

characterised by the presence of higher concentrations of some alcohols (1-hexanol, 2-ethyl-1-hexanol, 1-nonanol and benzyl alcohol) and furfural (PC2). Briefly, white quinoa flour had the most complex profile (Figure 5.13). Fermentation gave a new flavour to the flour, as reflected by the location of unfermented white quinoa with respect to fermented white quinoa (PC1) and the volatile compounds near the samples. White quinoa hot-air dried samples at three temperatures (50, 60 and 70 °C) (FWQ-50, FWQ-60, FWQ-70) differed from UWQ and FWQ (PC2). In contrast, the fermented lyophilised samples had similarities with unfermented flour. A similar trend was observed for black quinoa (Figure 5.14). Unfermented black quinoa was characterised by naphthalene and 4-Isothiocyanate-1butene. In contrast, high concentrations of 3-octanone, Hexanal, 1-octen-3-ol and benzaldehyde defined FBQ. Finally, PC2 distinguished hot-air dried samples based on 2,3-dihydro-1H-indene-4-carbaldehyde, furfural and ethyl octanoate from the other samples.

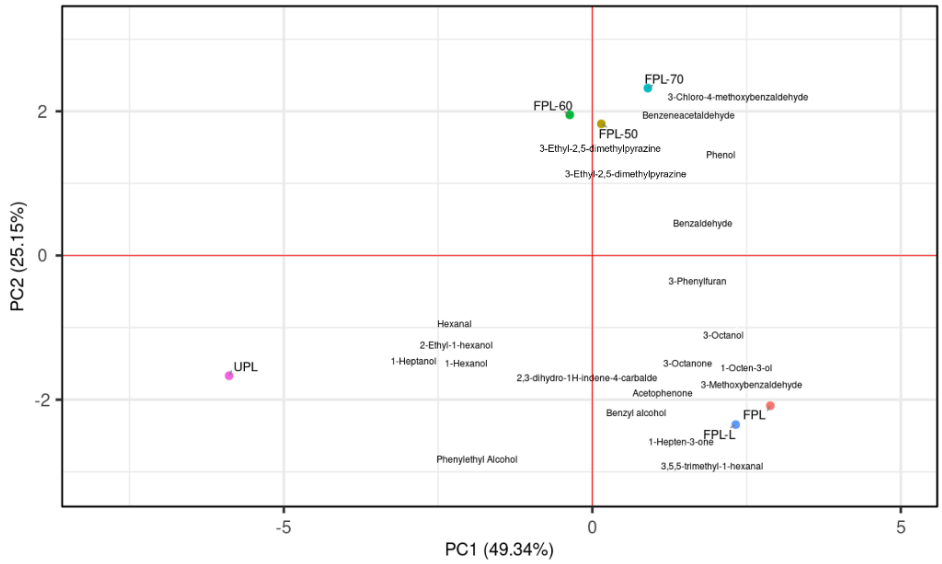


Figure 5.11. Biplot based on principal component analysis (PCA) of the different volatile compounds found in Pardina lentil before and after fermentation and drying.

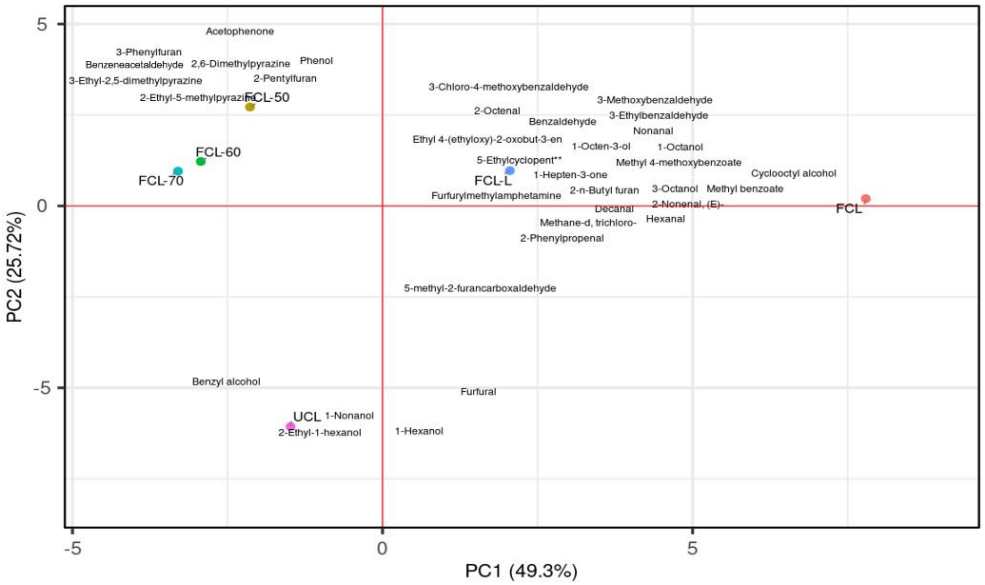


Figure 5.12. Biplot based on principal component analysis (PCA) of the different volatile compounds found in Castellana lentil before and after fermentation and drying.

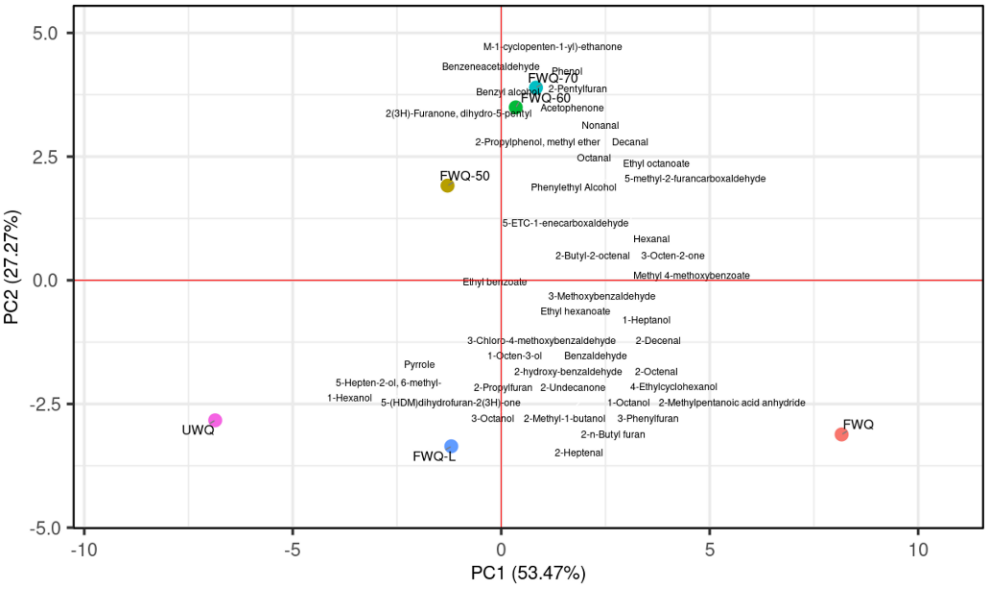


Figure 5.13. Biplot based on principal component analysis (PCA) of the different volatile compounds found in white quinoa before and after fermentation and drying.

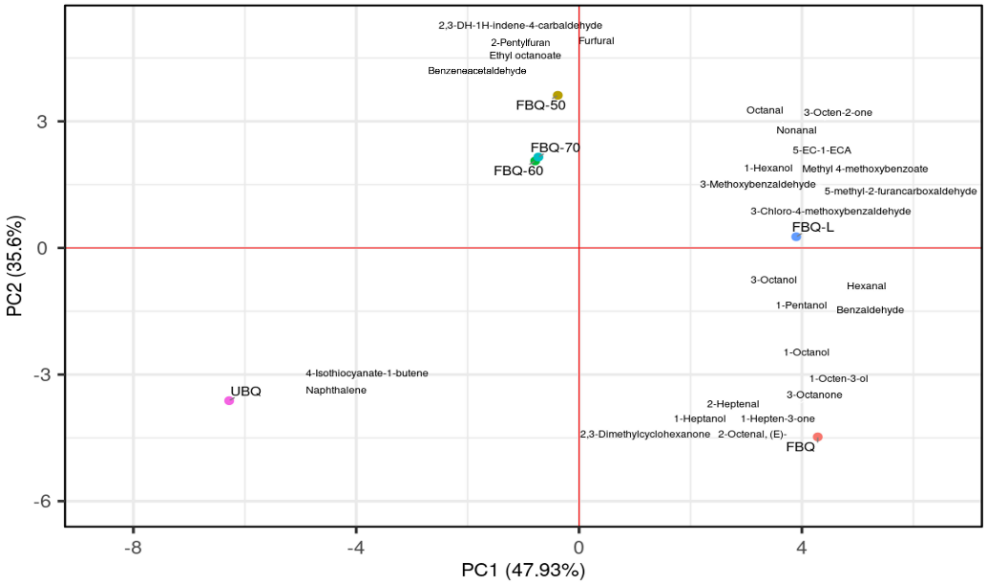


Figure 5.14. Biplot based on principal component analysis (PCA) of the different volatile compounds found in black quinoa before and after fermentation and drying.

These findings suggest that freeze drying is the optimal method for preserving desirable odours in the types of flour under study. However, despite its effectiveness for certain ingredients such as black quinoa and Pardina lentils, freeze drying is rarely used in the industry due to cost. Instead, hot-air drying is more common. This method provides comparable results to freeze drying for white quinoa and Castellana lentils.

Therefore, it is crucial to evaluate the impact of hot-air drying on sensory attributes such as odour when developing new products. Freeze drying should be used as a control. All temperature variations tested in this study had similar effects on the samples. Considering only the influence on volatile compounds, higher temperatures would be preferred because they enable faster drying and reduce energy consumption. However, further studies on other physicochemical

characteristics are necessary before recommending specific temperature ranges. The main limitation of this study is that these results cannot be extrapolated to other substrates. A possible escalation would require fine-tuning and readjustments. Larger-scale trials should be carried out to check possible changes in the volatile profile.

4. Conclusions

Solid-state fermentation (SSF) with *Pleurotus ostreatus* enhances the volatile profile of lentils and quinoa. Unfermented white and black quinoa had the least aroma, with a total peak area of 96 and 32, respectively. In contrast, unfermented lentils had a stronger, green, grassy, leafy odour, with a total peak area of more than 160. After fungal fermentation, the volatile profiles gained in complexity and intensity. Pardina lentils were the least affected by fermentation, with a 570% increase in total peak area and the generation of 10 compounds. White quinoa aroma was the most affected by fermentation. Total area rose from 96 to 4500, and 30 compounds were created. Even though the volatile profile varied among samples, 8-carbon volatile compounds were found in all fermented samples due to fungal fermentation. Benzaldehyde, hexanal and 3-methoxybenzaldehyde were formed after fermentation, providing sweet, green, cocoa aromas to the fermented lentils and quinoa. Hot-air drying significantly reduced the total aromatic compounds by up to 40% in total peak area for fermented black quinoa flour. No significant differences were found between different drying temperatures.

Dried fermented flour retained higher levels of the key compounds that provide a sweet, cocoa aroma. Lyophilisation preserved the volatile compounds generated in fermentation to a greater extent than hot-air drying in black quinoa and Castellana lentils (more than 150% in the total peak area). In conclusion, fermented lentil and

quinoa flours offer a richer and more intense flavour than unfermented lentil and quinoa flours. This finding presents a new opportunity for the inclusion of these flours in a wide variety of food products.

CONCLUSIONES DEL CAPÍTULO I

- La aplicación de la FES con *P. ostreatus* modifica el perfil nutricional de los sustratos fermentados en términos de aumento de proteínas con incrementos en semillas y harina de quinoa blanca del 26% y 7%, respectivamente, y en harina de lenteja Pardina del 21%.
- La FES provoca una marcada reducción en el contenido de ácido fítico, especialmente notable en las harinas de lenteja Pardina y quinoa blanca, con descensos que alcanzan hasta un 90%.
- El contenido fenólico total (CFT) y la actividad antioxidante disminuyen en quinoa blanca y lenteja Pardina, tanto en grano/semilla como en harina, a medida que avanza el tiempo de fermentación, con pérdidas del 20% y 50%, respectivamente.
- El hongo *P. ostreatus* creció mejor en los granos/semillas en comparación con las harinas, con aumentos de 30 a 52 mg glucosamina/g base seca en granos/semillas y de 32 a 45 mg glucosamina/g base seca en harinas.
- Dentro del conjunto de variedades de granos de lenteja y semillas de quinoa fermentadas, la lenteja Castellana y la quinoa blanca presentaron un mayor incremento en el contenido de proteína en comparación con las variedades de lenteja Pardina y quinoa negra. Se registraron incrementos del 2% y del 15%, respectivamente, con respecto a sus análogos sin fermentar.
- La FES mostró un mejor efecto en la reducción del contenido de ácido fítico en las muestras fermentadas en grano de lenteja Castellana, y en semillas de quinoa blanca y negra, logrando reducciones superiores al 90%.
- El secado por aire caliente a 50, 60 y 70 °C de los granos/semillas fermentadas de lenteja Castellana y Pardina, así como de quinoa blanca y negra, no tuvo efecto adicional sobre la reducción del contenido de ácido fítico.

- En el grupo de variedades de granos de lenteja y semillas de quinoa fermentadas, la lenteja Castellana y la quinoa blanca presentaron las menores reducciones en el CFT, con pérdidas del 30% y 1%, respectivamente, en comparación con la lenteja Pardina y quinoa negra que mostraron reducciones del 45% y 38%.
- Las variedades fermentadas de grano de lenteja Castellana y semillas de quinoa blanca presentaron las menores reducciones en la actividad antioxidante, con valores de índice compuesto de potencia antioxidante (APCI) de 48% y 70%, respectivamente, en contraste con los granos de lenteja Pardina y las semillas de quinoa negra que mostraron un APCI de 32% y 62%, respectivamente.
- El secado por aire caliente a 50, 60 y 70 °C de los granos fermentados de lenteja Castellana y semillas de quinoa blanca mejoró el CFT y la actividad antioxidante, especialmente a 70 °C. En ambos sustratos, se presentaron incrementos superiores al 90% en el CFT y un aumento del APCI, alcanzando valores entre 80% y 90%.
- La FES y el secado por aire caliente (50, 60 y 70 °C) promovieron cambios en el perfil fenólico de los granos de lenteja (Castellana y Pardina) y semillas de quinoa (blanca y negra) fermentados, disminuyendo algunos compuestos e incrementando otros, como el ácido gálico, que llegó a aumentar significativamente de 4 a 5 veces su contenido inicial (sin fermentar) en las variedades de lenteja Castellana y quinoa blanca.
- La FES y el secado por aire caliente (50, 60 y 70 °C) incrementa la capacidad para inhibir la enzima conversora de angiotensina (ECA) en las muestras fermentadas de lenteja Pardina en grano, y semillas de quinoa blanca y negra, con incrementos entre el 70% y el 135%. Solo los granos fermentados de lenteja Castellana presentaron un incremento entre el 20% y el 50%.
- El efecto de la FES y del secado por aire caliente (50, 60 y 70 °C) sobre el color final de los granos/semillas fermentadas fue mayor en la variedad de lenteja

Castellana y quinoa blanca con una diferencia de color (ΔE) ente 22 y 25, presentando coloraciones naranjas y marrones intensas, en contraste con las variedades de lenteja Pardina y quinoa negra que registraron un ΔE entre 15 y 19.

- El secado por aire caliente (50, 60 y 70 °C) de los sustratos fermentados produce tamaños de partícula más pequeños que sus análogos sin fermentar después de la molienda, con una disminución significativa del 50% en granos fermentados de lenteja Castellana y Pardina, y del 20% en semillas de quinoa blanca y negra.
- La FES mejora el perfil volátil de las variedades de granos de lenteja Castellana y Pardina, y semillas de quinoa blanca y negra. La variedad de quinoa blanca fue la más afectada con un aumento en el área total del pico de 96 a 4500, y se crearon 30 compuestos volátiles.
- Durante la FES se generaron compuestos volátiles como el 1-octen-3-ol, belzaldehído, hexanal y 3-metoxibenzaldehído, proporcionando aromas dulces, a hierba/verde y a cacao en las dos variedades de granos de lentejas y semillas de quinoa fermentadas.
- La FES generó compuestos volátiles de 8 carbonos como el 1-octen-3ol característicos del olor a champiñón.
- El secado por aire caliente (50, 60 y 70 °C) redujo significativamente los compuestos volátiles entre un 40% y un 70% el área total del pico en las dos variedades fermentadas de granos de lentejas y semillas de quinoa.
- Las muestras fermentadas secadas por aire caliente mantuvieron niveles elevados (entre 400 y 2500 el área total del pico) de compuestos que confieren un aroma dulce, afrutado y con matices a cacao, acompañado de notas de setas y sustratos cocidos, debido a las concentraciones de benzaldehído, hexanal, nonanal, furfural y 1-octen-3-ol presentes en las muestras fermentadas secas.
- La liofilización, utilizada como método de secado de referencia, disminuyó aún más el CFT, la actividad antioxidante y el perfil fenólico de las muestras

fermentadas en comparación con el secado por aire caliente, mientras que preservó en mayor medida los compuestos volátiles. Además, presentó un tamaño de partícula dos veces menor que las muestras secadas por aire caliente, así como menores diferencias de color ΔE >10.

- Los granos de lenteja Castellana y las semillas de quinoa blanca demostraron ser los sustratos más idóneos para la fermentación fúngica, junto con el secado por aire caliente a 70 °C. Por lo tanto, sus harinas pueden considerarse como ingredientes ideales para ser incluidos en el desarrollo de nuevos productos ricos en proteínas, con funcionalidad mejorada, nuevas propiedades sensoriales y libres de gluten. Estos productos podrían ser aptos para grupos de población específicos con altos requerimientos proteicos, como ancianos, deportistas, veganos o individuos con trastornos gastrointestinales.

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CAPÍTULO II

CAPÍTULO II: DIGESTIBILIDAD DE NUTRIENTES Y BIOACCESIBILIDAD DE COMPUESTOS BIOACTIVOS Y ANTINUTRIENTES DE LAS NUEVAS HARINAS DE LENTEJA Y QUINOA FERMENTADAS.

ARTÍCULO 5

Sánchez-García, J., Muñoz-Pina, S., García-Hernández, J., Tárrega, A., Heredia, A., & Andrés, A. (2023). In Vitro Digestion Assessment (Standard vs. Older Adult Model) on Antioxidant Properties and Mineral Bioaccessibility of Fermented Dried Lentils and Quinoa. *Molecules*, 28(21), 7298.

ARTÍCULO 6

Sánchez-García, J., Muñoz-Pina, S., García-Hernández, J., Tarrega, A., Heredia, A., & Andrés, A. (2023). Protein digestibility and ACE-Inhibitory activity of fermented flours under older adult and standard gastrointestinal simulation. **Enviado**, revista *Food Research International*. Número de manuscrito: FOODRES-D-23-09281.

RESUMEN DEL CAPÍTULO II

El **CAPÍTULO II** presenta los resultados obtenidos del estudio de la digestibilidad de macronutrientes y la bioaccesibilidad de algunos compuestos bioactivos y antinutrientes (ácido fítico) de las nuevas harinas de lenteja y quinoa fermentadas. En este estudio se simuló la digestión gastrointestinal (GI) de las dos harinas de lenteja (Pardina y Castellana) y quinoa (blanca y negra) sin fermentar, fermentadas y fermentadas-secadas a 70 °C y liofilizadas. Se seleccionó la temperatura de secado a 70 °C en lugar de la de 50 y 60 °C, dado que las muestras secadas a 70 °C presentaron mejores propiedades antioxidantes según el estudio presentado en el capítulo 1. En el presente capítulo se planteó evaluar la digestibilidad de las proteínas, las propiedades antioxidantes de los digeridos, la bioaccesibilidad de algunos minerales, y la inhibición de la enzima convertidora de angiotensina (ECA), todo ello mediante simulación *in vitro* de las condiciones gastrointestinales del adulto mayor.

En este estudio, el modelo de digestión GI del adulto sano (estándar) se utilizó como referencia para poder evaluar el impacto de las alteraciones típicas del modelo del adulto mayor. Las condiciones del modelo estándar se especifican a continuación, y seguido, entre paréntesis y negrita, se indican las alteraciones del modelo del adulto mayor: (i) Fase oral: amilasa 75 U/mL (**112.5 U/mL**), pH 7, 2 min; (ii) Fase gástrica: pepsina 2000 U/mL (**1200 U/mL**), pH 3 (**pH 3.7**), 2 h; (iii) Fase intestinal: pancreatina 100 U/mL (**80 U/mL**), sales biliares 10 mM (**7 mM**), pH 7, 2 h.

En los digeridos obtenidos, se evaluaron las propiedades antioxidantes en la fracción bioaccesible, observándose que la digestión GI promovió el incremento del contenido de los **compuestos fenólicos**, tanto en las muestras de lenteja como en las de quinoa, independientemente del tipo de procesamiento y del modelo de

digestión. Este incremento fue mayor en las muestras fermentadas-secadas a 70 °C de lenteja Pardina y Castellana, alcanzando valores de 7.5 y de 10.5 mg ácido gálico/g base seca, respectivamente, digeridas según el modelo de digestión estándar. Por otro lado, el **perfil fenólico** de los digeridos obtenidos de las muestras fermentadas y secadas a 70 °C, se caracterizó por un aumento de los ácidos vanílico y cafeico en lenteja Castellana de 6.2 a 20 y de 3.4 a 10.8 µg/g base seca, respectivamente. En el caso de la lenteja Pardina se observó un aumento del ácido vanílico de 7.6 a 20.7 µg/g base seca, mientras que, en la quinoa blanca y negra, se observó un aumento significativo del ácido gálico de 20 a 139 y de 30 a 42 µg/g base seca, respectivamente. En cuanto a la **actividad antioxidante**, los digeridos de las harinas fermentadas-secadas a 70 °C presentaron valores más altos en comparación con los digeridos de las muestras tras la fermentación o las muestras fermentadas y liofilizadas. Concretamente, en las harinas fermentadas-secadas a 70 °C se obtuvieron índices de actividad antioxidante (APCI) del 67% y 99% en lenteja Pardina y Castellana, respectivamente, y del 88% y 64% en quinoa blanca y negra, respectivamente.

Por otro lado, se observó una disminución del **contenido en minerales** (Mg, Ca y Fe) en la fracción bioaccesible en todas las muestras. No obstante, el proceso de fermentación y secado mejoró la bioaccesibilidad mineral en las dos variedades de lenteja y quinoa, resultando en incrementos del 3% al 30% para el Mg, del 18% al 124% para el Ca y del 63% al 329% para el Fe. Estos incrementos en la bioaccesibilidad de los minerales son consistentes con una menor concentración de **ácido fítico** observada después de la digestión GI. Esto, beneficiado por una reducción de este antinutriente durante la FES con el hongo *P. ostreatus* (Capítulo 1).

En relación a la digestibilidad proteica, se observó que la FES y el secado a 70 °C incrementó la **hidrólisis de las proteínas** hasta en un 20% con respecto a las harinas sin fermentar. La proteólisis fue mayor en las dos variedades de quinoa que en las de lenteja, presentando valores de 80 y 50 g proteína soluble en TCA/100 g de proteína, respectivamente. Por otro lado, la FES incrementó el porcentaje de **péptidos** más pequeño (12.5 kDa), alcanzando hasta un 35%, debido a la actividad proteolítica del hongo *P. ostreatus*. Las muestras fermentadas de lenteja Castellana y quinoa blanca presentaron las proporciones más elevadas de péptidos de 12.5 kDa. Después del secado a 70 °C los péptidos de 12.5 kDa representaron entre el 10% y el 20% de la fracción peptídica total. Luego, durante el proceso de digestión, se observó un aumento de la proporción de péptidos de menor tamaño (<0,19, 0,45 y 1,4 kDa) en todos los sustratos y sus respectivos tratamientos (sin fermentar, fermentado y fermentado-seco). Péptidos de 1.4 y 0.45 kDa representaron entre el 11% y el 28%, mientras que los péptidos <0.19 kD representaron el 60%. Además, la FES incrementó hasta 2 veces el contenido de la mayoría de los **aminoácidos** en lenteja Castellana y quinoa blanca. En lenteja Pardina y quinoa negra únicamente se observó un incremento en valina (Val), leucina (Leu), isoleucina (Ile) y triptófano (Trp). Estos resultados en las dos variedades de lenteja y quinoa favorecen el aumento de aminoácidos de cadena ramificada (Val, Leu e Ile) necesarios para la síntesis de proteínas musculares, relevante en el adulto mayor. Sin embargo, el secado por aire caliente a 70 °C afectó negativamente al contenido de aminoácidos en todos los sustratos fermentados, observándose reducciones de hasta un 40%. Esta disminución en el contenido de aminoácidos podría atribuirse a la formación de enlaces entre aminoácidos y azúcares reductores durante las reacciones de Maillard provocadas durante el secado con aire caliente. A pesar de esta disminución, los contenidos no descienden por debajo del contenido inicial de sus homólogos sin

fermentar. Por otro lado, se observó un aumento del contenido de aminoácidos hidrofóbicos (HAA) y aminoácidos cargados negativamente (NCAA) en los digeridos de las muestras fermentadas y fermentadas-secas a 70 °C. Estos incrementos fueron mayores en las dos variedades de lenteja, con contenidos >70 mg/g base seca en HAA y >20 mg/g base seca en NCAA.

Con respecto a la **actividad inhibitoria de la ECA**, se observó una reducción de hasta 35 puntos porcentuales la en la fracción bioaccesible de los digeridos de las muestras fermentadas en comparación con las muestras sin fermentar, probablemente debido a una mayor hidrólisis de la proteína tanto en la FES como durante la posterior digestión GI. Concretamente, las muestras fermentadas de lenteja pardina y quinoa negra fueron las que presentaron el mayor porcentaje de inhibición de la ECA (60%). Por el contrario, el secado a 70 °C de las muestras fermentadas de lenteja pardina, y quinoa blanca y negra se tradujo en un aumento de la actividad inhibidora de la ECA hasta valores comparables con sus homólogas sin fermentar; este resultado podría estar relacionado con la actividad inhibidora de algunos de los productos de las reacciones de Maillard, como por ejemplo las melanoidinas.

Por último, las **alteraciones gastrointestinales típicas en la digestión del adulto mayor**, parece no comprometer la bioaccesibilidad de los minerales estudiados en las muestras fermentadas y secadas a 70 °C. Sin embargo, si afectaron al perfil fenólico, reduciendo significativamente la diversidad y abundancia de la mayoría de los compuestos, y también a la actividad antioxidante hasta en un 15% en comparación con el modelo estándar. Asimismo, en las alteraciones GI simuladas para el adulto mayor se encontraron valores más bajos de digestibilidad de las proteínas y, por tanto, de péptidos más pequeños, del perfil de aminoácidos y una

actividad inhibidora de la ECA reducida de las harinas fermentadas. Se observaron disminuciones de hasta el 10% en la proteólisis, hasta el 20% en los grupos de aminoácidos (HAA y NCAA), y el 30% en la actividad inhibidora de la ECA, en comparación con el modelo de digestión estándar.

ARTÍCULO 5

ARTÍCULO 5: *In vitro* digestion assessment (standard vs. older adult model) on antioxidant properties and mineral bioaccessibility of fermented-dried lentils and quinoa.

ABSTRACT

The growing number of older adults necessitates tailored food options that accommodate the specific diseases and nutritional deficiencies linked with ageing. This study aims to investigate the influence of age-related digestive conditions *in vitro* on the phenolic profile, antioxidant activity, and bioaccessibility of minerals (Ca, Fe, and Mg) in two types of unfermented, fermented, and fermented dried quinoa and lentils. Solid-state fermentation, combined with drying at 70 °C, significantly boosted the total phenolic content in Castellana and Pardina lentils from 5.05 and 6.6 to 10.5 and 7.5 mg gallic acid/g dry basis, respectively, in the bioaccessible fraction following the standard digestion model, compared to the unfermented samples. The phenolic profile post-digestion revealed elevated levels of vanillic and caffeic acids in Castellana lentils, and vanillic acid in Pardina lentils, while caffeic acids in Castellana lentils were not detected in the bioaccessible fraction. The highest antioxidant potency composite index was observed in digested fermented dried Castellana lentils, with white quinoa samples exhibiting potency above 80%. Mineral bioaccessibility was greater in fermented and fermented dried samples compared to unfermented ones. Finally, the digestive changes that occur with ageing did not significantly affect mineral bioaccessibility but compromised the phenolic profile and antioxidant activity.

Keywords: *Pleurotus ostreatus*; Phenolic profile; Antioxidant activity; Total phenol content; Phytic acid.

1. Introduction

The ageing population is predominantly due to decreased fertility rates and increased life expectancy. It is projected that by 2050, the number of individuals over the age of 60 will reach approximately 2 billion, representing 22% of the global population, with the majority residing in developing nations (WHO, 2022). Therefore, the forthcoming expansion of older adults' population will cause substantial increased demands for food products that are specifically formulated to meet their preferences and nutritional requirements. Ageing frequently causes digestive disorders related to changes in the oral cavity, including tooth loss and wearing dentures, gingivitis, and reduced saliva production. Furthermore, reduced sense of taste and smell can decrease food palatability and increase inappetence, leading to changes in the type and quantity of food consumed (Brownie, 2006). Gastric emptying slows down, and the gastric lipase and pepsin enzyme secretions are reduced, leading to an alkalisation of the gastric environment. Furthermore, peristalsis in the small intestine decreases, resulting in reduced secretion of pancreatic enzymes and bile salts (Makran et al., 2022; Rémond et al., 2015; Shang et al., 2022). These gastrointestinal tract alterations may contribute to age-related malnutrition, causing deficiencies in micronutrients, particularly minerals, which can lead to functional decline, fragility, and difficulty in maintaining independent living (Vural et al., 2020). Furthermore, phytochemicals such as polyphenols may be considerable in chronic diseases, including cardiovascular disease, type II diabetes, cancer, osteoporosis, and neurodegenerative diseases (Shang et al., 2022). Therefore, it is crucial to include antioxidants and minerals in one's diet to maintain brain function, support bone and teeth health, aid cellular and thyroid metabolism, and strengthen the immune system of older individuals (Bourre, 2006; Lobine & Mahomoodally, 2022; Quintaes & Diez-Garcia, 2015; Thangthaeng et al., 2016).

In this scenario, assessing the impact of common gastrointestinal conditions in older adults on the digestibility of novel ingredients with enhanced antioxidant properties and improved digestibility is important. This assessment is crucial for creating new highly nutritious foods adapted to older adults. Therefore, grains and seeds, such as lentils or quinoa, could be considered good candidates as raw materials for developing protein-rich functional ingredients. The antioxidant activity of these plant materials is associated with a high content of phenolic compounds. Lentils have a higher reported total phenolic content (7.53 mg GAE/g sample) than other legumes, including peas, chickpeas, soybeans, red kidney, and black beans (B. J. Xu & Chang, 2007). Quinoa has a total phenolic content (TPC) of 5.18 mg GAE/g sample (Tang et al., 2015), possessing antioxidant properties that are more effective than those of other cereals and pseudocereals such as brown rice, millet, whole wheat, barley, oats, rye, Job's tears, corn, and amaranth (Hirose et al., 2010). Furthermore, some of the phenolic compounds present in lentils are flavonoids, including kaempferol glycosides, catechin/epicatechin glycosides, and procyanidins (B. Zhang et al., 2015). Phenolic acids, namely vanillic acid, ferulic acid, and their derivatives, and flavonoid compounds like quercetin, kaempferol, and their glycosides, have been found in quinoa (Safarov, 2020; Tang et al., 2015). Nevertheless, consumption of these compounds may not offer full health advantages because of factors such as antinutrients and limited digestibility. Solid-state fermentation (SSF), however, enhances the antioxidant properties and nutritional quality of diverse legumes and cereals. Thus, it is possible for the TPC of fermented plant materials to increase because of the release of phenolic compounds. These compounds are produced due to the structural breakdown of the cell wall after fungal colonisation, the action of ligninolytic and hydrolytic enzymes, or the synthesis of soluble phenolic compounds conducted by the fermentative micro-organism

(Bhanja Dey et al., 2016). It is important to note that this increase is not subjective but based on scientific evidence and observations. The variability in antioxidants altered by SSF relies on the binomial substrate-microorganism and process variables, precluding the generalisation of findings across studies. Furthermore, studies have shown that the TPC of fermented black bean, kidney bean, and oat samples increased up to twice as much compared to unfermented digested samples after gastrointestinal digestion, mimicking the healthy adult digestion model (Espinosa-Páez et al., 2017).

The bioavailability of minerals in plant materials is relatively low due to certain molecules, such as phytates or phenols, forming complexes (Ojo, 2020). However, SSF has been discovered to decrease phytates by endogenous phytase action, which is activated during fermentation. This leads to mineral release and increased bioavailability (Nkhata et al., 2018).

This study aims to analyse the effect of common age-related digestive conditions on the phenolic profile and antioxidant activity of the bioaccessible fraction together with the bioaccessibility of minerals (Ca, Fe, and Mg) of unfermented, fermented, and fermented dried (hot air drying or lyophilisation) quinoa (white and black) and lentils (Castellana and Pardina). Furthermore, all samples were subjected to *in vitro* digestion under healthy standard GI conditions for comparison.

2. Materials and methods

2.1. Materials

Lentils (*Lens culinaris*) of the Pardina and Castellana varieties from Hacendado® and quinoa (*Chenopodium quinoa Wild*) of white and black varieties from the

Hacendado® and Nut&me brands, respectively, were obtained from local stores in Valencia (Spain). The *Pleurotus ostreatus* strain was acquired from the Spanish Type Culture Collection (CECT20311).

Pepsin from porcine gastric mucosa (≥3200 U/mg), pancreatin from porcine pancreas (8 x USP), bovine bile (dried, unfractionated), p-toluene-sulfonyl-L-arginine methyl ester (TAME, T4626), analytical grade salts (potassium chloride, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, magnesium chloride hexahydrate, ammonium carbonate, and calcium chloride), potato starch, sodium phosphate, maltose standard, 3,5-dinitrosalicylic acid (DNS), potassium sodium tartrate tetrahydrate, sodium hydroxide, thioglycolic acid, phytic acid sodium salt hydrated from rice, 2,2'-bipyridine, formic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Folin–Ciocalteu reagent, 2,4,6-tripyridyl-s-triazine (TPTZ), gallic acid, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), glucose, and mycopeptone were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

For HPLC analysis, vanillic acid, quercetin 3-glucoside, quercetin, quercitrin, 4-hydroxybenzoic acid, rutin, epicatechin, trans-cinnamic acid, ferulic acid, naringenin, caffeic acid, 4-O-caffeoylquinic, p-coumaric acid, apigenin-7-glucoside, kaempferol, and sinapic acid were obtained from Sigma-Aldrich as an analytical standard (HPLC grade). Acetic acid glacial, concentrated hydrochloric acid, ethanol absolute, sodium carbonate, and ammonium iron (III) sulphate dodecahydrate were obtained from Panreac AppliChem (Barcelona, Spain). Acetonitrile (HPLC grade), methanol (HPLC grade), iron (III) chloride hexahydrate, sodium acetate trihydrate, and potassium persulphate were obtained from Honeywell Fluka (Morris Plains, NJ, USA). The malt extract and agar were obtained from Scharlau (Barcelona, Spain).

2.2. Fungal solid-state fermentation (SSF) and flour production

To perform SSF, a starter culture was first prepared by growing *P. ostreatus* mycelium in a Petri dish containing lentil or quinoa grains/seeds. The fermentation was then conducted by inoculating a portion of the starter culture into a glass jar containing 35 g of lentil or quinoa grains/seeds following the methodology used previously (Sánchez-García et al., 2023b). Fermented lentils and quinoa grains/seeds were dried using hot air drying and freeze drying methods, the latter used as the reference drying method because it was expected to have the best preservation of the sample properties according to literature. Hot air drying was conducted using a convective dryer (Pol-Eko-Aparatura, CLW 750 TOP+, Kokoszycka, Poland) at 70 °C with an air rate of 10.5 ± 0.2 m/s and an air humidity of 8.7 ± 1.2%. The samples were dried for 3.5 to 4 h to a target product moisture of 7% (wet basis). Freeze drying was performed using a freeze dryer (Telstar, Lyoquest-55, Terrassa, Spain) at - 45 °C and 0.8 mBar for 48 h. Unfermented and fermented dried samples were then milled using a food processor (Vorwerk, Thermomix® TM6-1, Wuppertal, Germany), applying 10000 rpm at 15-s intervals for 1 min.

2.3. Simulated *in vitro* gastrointestinal digestion under standard and older adult conditions

Unfermented, fermented, and fermented dried samples were digested under two static *in vitro* digestion models: the older adult model (Menard et al., 2023) and the healthy adult model (standard; as a control) (Brodkorb et al., 2019; Minekus et al., 2014) (Table 5.22). Enzymatic activities were determined before each experiment according to the supplementary information in the protocol published by Brodkorb et al. (2019). Simulated salivary (SSF), gastric (SGF), and intestinal (SIF) fluid were

prepared daily for the standard and older adult digestion model considering the concentrations of enzymes, bile salts, and pH of each digestive phase.

To perform the oral stage, 5 g of sample was mixed with 5 mL of SSF containing the enzyme concentration according to the digestion model (Table 5.22), with adjusted pH, mixed at 25 rpm using an Intelli-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia), and incubated in a thermostatic chamber (J.P. Selecta SA, Barcelona) at 37 °C for 2 min. For the gastric stage, 10 mL of SGF was added to the food bolus according to the conditions simulated in each model (Table 5.22), the pH, mixed at 55 rpm, and incubated at 37 °C for 2 h.

Table 5.22. Gastrointestinal conditions established for an *in vitro* digestion model for a healthy adult (standard) (Brodkorb et al., 2019; Minekus et al., 2014) and an older adult (Menard et al., 2023).

Digestive stage	Digestion models	
	Healthy adult (standard)	**Older adult**
Oral stage	Amylase (75 U/mL) pH 7 2 min	**Amylase (112.5 U/mL)** pH 7 2 min
Gastric stage	Pepsin (2000 U/mL) pH 3 2 h	**Pepsin (1200 U/mL)** **pH 3.7** 2h
Intestinal stage	Pancreatin (100 U/mL) Bile salts (10 mM) pH 7 2 h	**Pancreatin (80 U/mL)** **Bile salts (7 mM)** pH 7 2 h

The alterations made to the model for older adults compared to the standard model are highlighted in bold text.

For the intestinal stage, 20 mL of SIF was added to the gastric chyme according to the concentration of enzyme and bile salts (Table 5.22), adjusted the pH, mixed at 55 rpm, and incubated at 37 °C for 2 h. After gastrointestinal digestion, enzyme activity

was inhibited by adjusting the pH to 5 and keeping the samples in an ice bath. Finally, the samples were centrifuged at 8000 ×*g* for 10 min and aliquots of the bioaccessible fraction were taken for analytical determinations.

2.4. Analytical determinations

Total phenolic content (TPC)

TPC of the samples before and after undergoing *in vitro* digestion was determined using the Folin–Ciocalteu methodology as outlined by Chang et al. (2006). For the samples that were not digested, phenolic compounds were extracted by blending 2.5 g of the sample with 7.5 mL of the extraction solvent (a mixture of double distilled water and ethanol at 70:30) and adjusting the pH to 2 with 2 M HCl. The mixture was then treated to ultrasonic bath (J.P. Selecta, 3000840) at 30 °C for 2 h. The pH was adjusted to 2 with 2 M HCl. The samples were centrifuged at 8000 ×*g* for 15 min. and the extraction process was repeated twice, with subsequent mixing of both extracted samples. The bioaccessible fraction determined the digested samples. An aliquot of 125 µL of the extract/digest was taken and mixed with 500 µL of bidistilled water, followed by 125 µL of the Folin–Ciocalteu reagent. This was left to react for 6 min. Then 1.25 mL of 7% sodium carbonate and 1 mL of bidistilled water were added. The sample was incubated for 30 minutes at room temperature in darkness. Afterward, the absorbance was measured at 760 nm, and the results were presented in mg gallic acid/g dry basis using a standard curve.

Antioxidant activity

The antioxidant activity of the samples before and after *in vitro* digestion was determined by three methods: ABTS, DPPH, and FRAP, following the methodology

described by Thaipong et al. (2006). The same extracts used in the TPC section were used for undigested and digested samples.

For the ABTS test, the working solution (7.4 mM ABTS and 2.6 mM potassium persulphate in a 1:1 ratio) was allowed to react for 12 h at room temperature in darkness. The working solution (1 mL) was diluted with methanol to obtain an absorbance close to 1.1 at 734 nm. Extract/digest (150 µL) was reacted with 2.85 mL of ABTS working solution for 2 h in darkness and absorbance was measured at 734 nm.

For the DPPH test, a fresh working solution of 0.039 g/L DPPH was prepared in pure methanol to get an absorbance close to 1.1 at 515 nm. Extract/digest (75 µL) was reacted with 2.925 mL of DPPH working solution for 30 min in darkness and absorbance was measured at 515 nm.

For FRAP test, fresh working solution was prepared by mixing 300 mM acetate buffer (3.1 g sodium acetate trihydrate and 16 mL acetic acid glacial in 1 L water, pH 3.6), TPTZ solution (10 mM 2,4,6-tripyridyl-s-triazine dissolved in 40 mM HCl), and 20 mM iron (III) chloride hexahydrate solution in a 10:1:1 ratio, respectively, and incubated at 37 °C before use. Extract/digest (150 µL) was reacted with 2.85 mL of FRAP working solution for 30 min in darkness, and the absorbance was measured at 593 nm. The results were expressed as mg Trolox/g dry basis using a standard curve for the three antioxidant determination methods.

The antioxidant index was calculated for each sample for all antioxidant activity assays (ABTS, DPPH, and FRAP). An antioxidant index value of 100 was assigned to the highest sample score in each assay. Then, the antioxidant index was calculated

for the entire group of samples in each assay according to Equation 5.7 (S. Sharma et al., 2022):

$$Antioxidant\ index\ (\%) = \left(\frac{sample\ score}{highest\ sample\ score}\right) \times 100 \qquad (5.7)$$

The overall APCI was calculated by averaging each sample's antioxidant index (%) of each antioxidant activity assay.

Phenolic profile by HPLC analysis

The phenolic profile of the samples after *in vitro* digestion was determined by filtering the bioaccessible fraction of the digest with a 0.45 µm PTFE filter. The samples were analysed using an HPLC 1200 Series Rapid Resolution coupled to a diode detector Serie (Agilent, Palo Alto, CA, USA) according to the methodology described by Tanleque-Alberto et al. (2020). A Brisa-LC 5 µm C18 column (250 x 4.6 mm) (Teknokroma, Spain) was used. Mobile phase A was 1% formic acid, and mobile phase B was acetonitrile (ACN). The following gradient program was used: 0 min, 90% A; 25 min, 40% A; 26 min, 20% A; held for 30 min; 35 min, 90% A; held for 40 min. Flow rate, injection volume, and working temperature of the column was 0.5 mL/min, 10 µL, and 30 °C, respectively. Unknown compounds were identified by comparing chromatographic retention times with reference standards according to the following wavelengths for each compound: 250 nm for vanillic acid; 260 nm for 4-hydroxybenzoic acid, rutin, quercetin 3-glucoside, and quercitrin; 280 nm for gallic acid, epicatechin, quercetin and trans-cinnamic acid; 290 nm for naringenin; 320 nm for 4-O-caffeoylquinic, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid, and apigenin-7-glucoside; and 380 nm for kaempferol. The results were expressed as µg/g dry basis using a standard curve.

Phytic acid content

The phytic acid content was measured before and after *in vitro* digestion following the protocol described by Haug & Lantzsch (1983) and modified by Peng et al. (2010). For undigested samples, the extract was prepared by mixing 50 mg of sample with 10 mL of 0.2 M HCl and left overnight at 4 °C. For digested samples, the determination was performed on the bioaccessible fraction. An aliquot of 500 µL of the extract/digest was taken, and 1 mL of ferric solution (0.2 g of ammonium iron (III) sulphate dodecahydrate dissolved in 100 mL of 2 M hydrochloric acid and made up to 1 L with distilled water) was added. It was incubated in a boiling water bath for 30 min and then cooled to room temperature. The sample was centrifuged for 30 min at 3000 ×*g*, and 1 mL of the supernatant was taken and mixed with 1.5 mL of 2,2'-bipyridine solution (10 g of 2,2'-bipyridine and 10 mL of thioglycolic acid dissolved in distilled water and made up to 1 L). The results were expressed as mg phytic acid/g dry basis using a standard curve made with a stock solution of 1.3 mg/mL phytic acid concentration and diluted with 0.2 M hydrochloric acid between 0.1 and 1 mL (3.16-31.6 µg/mL phytate phosphorus).

Mineral quantification

Quantification of minerals (Fe, Ca, and Mg) before and after gastrointestinal digestion was performed by inductively coupled plasma mass spectrometry (ICP-MS). The mineral extract was prepared according to the methodology published by Barrera et al. (2009). A 5 g sample was weighed for undigested food, and a 3.5 mL aliquot was taken from the bioaccessible fraction of digested food. Samples were incinerated at 600 °C for 10 h. Ashes were dissolved with 1 mL of 69% nitric acid and re-incinerated until completely white ashes were obtained. The white ashes were suspended in 1.5 mL of 69% nitric acid and 4 mL of bi-distilled water.

Samples were analysed using an ICP-MS equipped with an autosampler (iCAP Q, Thermo, USA) according to the methodology proposed by Chen et al. (2020). Working equipment conditions were: Radio frequency power (1550 W), cool gas flow (14 L/min), auxiliary gas flow (0.8 L/min), nebuliser gas flow (1.08 L/min), peristaltic pump speed (40 rpm), sampling depth (5 mm), spray chamber temperature (2.7 °C), dwell time (20 ms). Results were expressed as μg/g dry basis.

2.5. Statistical analysis

The experiments were conducted at least in triplicate and results reported as mean ± standard deviation. A one-way ANOVA with a 95% confidence interval ($p < 0.05$) was performed to determine the statistical significance of the variables studied (SSF, drying, and common GI conditions of older adults) on the antioxidant activity, phenolic, phytates, and mineral contents in the bioaccessible fraction in lentil and quinoa samples employing Statgraphics Centurion-XV as statistical software.

3. Results and discussion

3.1. Impact of GI conditions on the release of phenols and antioxidant activity of unfermented fermented, and fermented dried lentils and quinoa

TPC and antioxidant activity changes during digestion were analysed and shown in Figure 5.15. In undigested samples, SSF and hot air drying at 70 °C resulted in an increase in TPC content in quinoa and lentils, compared to unfermented flours. The reasons behind this increase are elaborated in detail by Sánchez-García et al. (2023b, 2023a). During digestion, the gastrointestinal process induced a rise of free phenols in the bioaccessible fraction, regardless of the simulated conditions (standard or older adult) and the processing conducted to obtain flour from the substrates.

However, this increase was more significant in lentils, as compared to quinoa, and in the fermented samples (FPL, FCL), compared to unfermented ones (UFPL, UFCL). Furthermore, samples fermented and dried at 70 °C (FPL-70, FCL-70) showed the highest TPC in the bioaccessible fraction. The optimal conditions for extracting phenols were pH, enzymatic activity, temperature, and stirring during the digestion process.

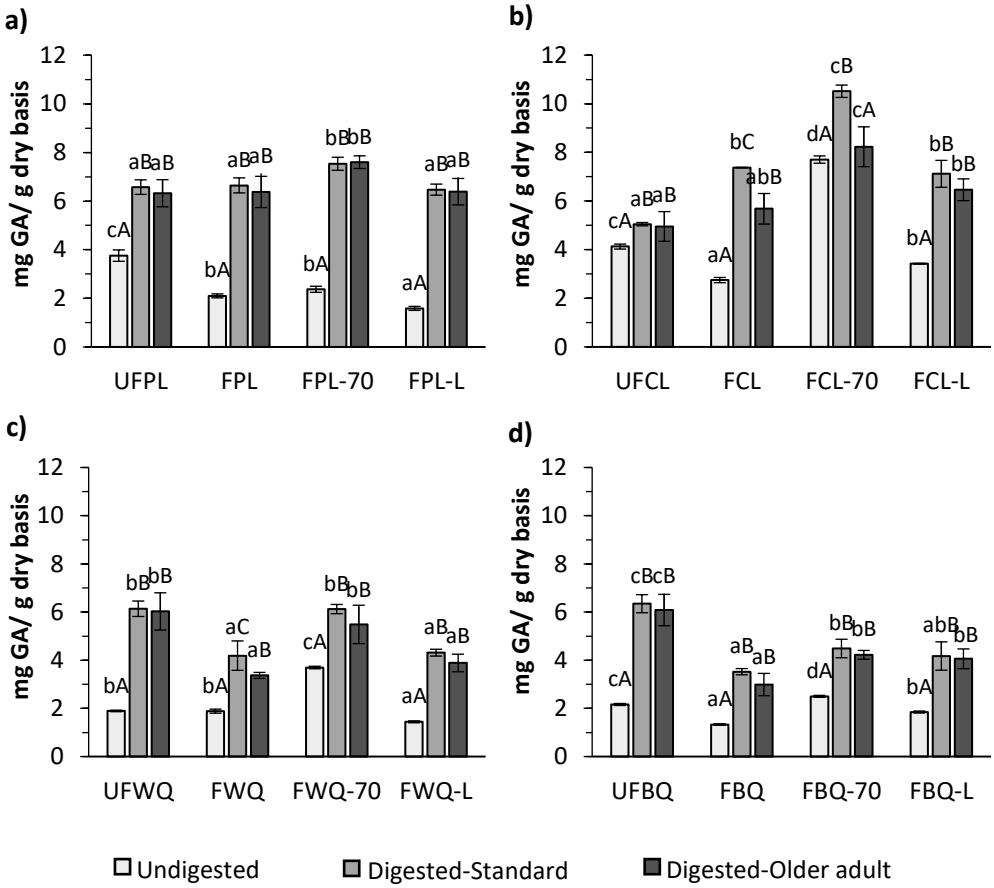

Figure 5.15. Total phenol content (mg gallic acid/g dry basis) in Pardina (**a**) and Castellana lentil (**b**) and white (**c**) and black quinoa (**d**) for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried

at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flour obtained with a standard or older adult *in vitro* digestion model. ^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between samples. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

When TPC in the bioaccessible fractions was measured using two digestive models, the TPC in the fermented dried samples remained largely unaltered. Due to the common altered gastrointestinal conditions that appear with ageing, fermented dried lentils at 70 °C are especially interesting in terms of their bioaccessible TPC.

Tungmunnithum et al. (2022) reported similar results in 10 bean varieties consumed in Thailand, with an increase in phenolic and flavonoid content associated with digestion. Phenol and flavonoid content rose with digestion but decreased during bean cooking. After gastrointestinal digestion, the TPC and total flavonoid content increased between 9% and 190%, and 4% and 266%, respectively, across different varieties. Physiological factors, such as digestive enzymes, bile salts, and pH, play a crucial role in the release of these compounds. Certain phenolic compounds are not present in free form in grains or seeds but are bound to the cell wall, creating macromolecular complexes. In addition to gastric digestion, a low pH causes an increase in polyphenols in their undissociated form, facilitating their release from the food matrix into the aqueous phase (Bohn, 2014; Li et al., 2022). However, the links between phenolic compounds and carbohydrates are reduced during intestinal digestion by the action of pancreatic enzymes, bile salts, and a neutral pH (6.9) (Cárdenas-Castro et al., 2020; Li et al., 2022).

The phenolic fraction's chromatographic analysis presented distinct profiles among the bioaccessible fractions based on both flour variety and digestive model, as shown in Table 5.23-5.26. Previously published studies (Sánchez-García et al.,

2023b, 2023a) have also performed the same chromatographic analysis on all samples before digestion. Furthermore, the chromatograms of the phenolic profile corresponding to unfermented, fermented, and fermented dried Pardina lentil samples after gastrointestinal digestion, both under the healthy adult (standard) and the older adult digestion model, can be found in the supplementary material (Figure S5.1–5.8). Upon comparison of substrates after *in vitro* digestion, the bioaccessible fractions of Castellana lentils demonstrated a greater abundance of vanillic and caffeic acids, whereas Pardina exhibited a great abundance of 4-O-caffeoylquinic and vanillic acids. White and black quinoa boasted higher amounts of gallic and vanillic acids, as well as quercitrin, compared to lentils. However, the quantities of these compounds differed depending on the treatment undergone by the flours. The bioaccessible portion of the unfermented flours contained lower levels of these compounds compared to their fermented counterparts, particularly those exposed to hot air drying. Vanillic and caffeic acids demonstrated a greater increase in the Castellana lentil, rising from 6.2 to 20 µg/g dry basis and from 3.4 to 10.8 µg/g dry basis, respectively. In contrast, vanillic acid increased from 7.6 to 20.7 µg/g dry basis in Pardina lentils. Furthermore, an increase in gallic acid was observed in both white and black quinoa samples fermented and dried at 70 °C. The increase in gallic acid was apparent and rose from 20 to 139 µg/g dry basis in white quinoa and from 30 to 42 µg/g dry basis in black quinoa. Consequently, SSF plus drying facilitates the liberation of specific phenolic acids and flavonoids, resulting in their incorporation into the water-soluble bioaccessible fraction during gastrointestinal digestion.

Table 5.23. Phenolic content (µg/g dry basis) in digested Pardina lentil for unfermented flour (UFPL), fermented grain (FPL), fermented dried at 70 °C (FPL-70) and fermented lyophilised (FPL-L) flour.

	Digested (Standard)				Digested (Older adult)			
	UFPL	**FPL**	**FPL-70**	**FPL-L**	**UFPL**	**FPL**	**FPL-70**	**FPL-L**
Phenolic acids								
Gallic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
p-Coumaric acid	4.5 ± 0.5^{cB}	2.87 ± 0.08^{bB}	3.19 ± 0.19^{bA}	2.14 ± 0.07^{aA}	2.2 ± 0.2^{aA}	2.33 ± 0.04^{aA}	3.02 ± 0.18^{bA}	2.0 ± 0.3^{aA}
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	88 ± 31^{bA}	49.5 ± 0.2^{aB}	42 ± 3^{aB}	60.6 ± 0.3^{bB}	86 ± 7^{cA}	34 ± 5^{aA}	32 ± 3^{aA}	46.9 ± 0.3^{bA}
4-Hydroxybenzoic acid	4.4 ± 0.2^{aA}	4.14 ± 0.15^{aB}	4.0 ± 0.5^{aB}	3.88 ± 0.13^{aB}	3.3 ± 0.3^{bA}	3.375 ± 0.010^{bA}	2.1 ± 0.3^{aA}	2.90 ± 0.17^{bA}
Vanillic acid	7.6 ± 0.5^{aA}	19.8 ± 0.6^{cB}	20.7 ± 0.5^{cB}	17.81 ± 0.12^{bB}	7.5 ± 0.7^{aA}	13.89 ± 0.12^{bA}	16.8 ± 0.7^{cA}	13.4 ± 1.3^{bA}
Ferulic acid	4.6 ± 1.2^{bA}	2.29 ± 0.02^{aB}	2.1 ± 1.3^{aA}	2.58 ± 0.09^{aA}	3.0 ± 0.2^{cA}	2.03 ± 0.03^{aA}	2.06 ± 0.03^{aA}	2.47 ± 0.11^{bA}
trans-Cinnamic acid	traces	3.02 ± 0.08^{aA}	9.5 ± 0.7^{bA}	2.19 ± 0.17^{aA}	n.d.	2.75 ± 0.02^{aA}	9.0 ± 0.6^{bA}	2.0 ± 0.3^{aA}
Flavonoids								
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	4.7 ± 0.4^{B}	n.d.	traces	traces	2.0 ± 0.4^{A}	traces	traces	traces
Apigenin-7-glucoside	0.62 ± 0.07^{A}	n.d.	traces	traces	2.80 ± 0.06^{cB}	2.28 ± 0.03^{b}	2.195 ± 0.007^{a}	2.67 ± 0.10^{c}
Quercetin	5.3 ± 0.4^{aB}	5.181 ± 0.006^{a}	5.0 ± 0.4^{a}	5.776 ± 0.008^{a}	0.78 ± 0.05^{A}	traces	traces	traces
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The results represent the mean of three repetitions with their standard deviation. ^{a,b,c} Different lowercase letters indicate significant differences between flours and ^{A,B} different capital letters indicate significant differences between digestion models ($p < 0.05$). n.d.: not detected. Traces: not quantifiable.

Table 5.24. Phenolic content (μg/g dry basis) in digested Castellana lentil for unfermented flour (UFCL), fermented grain (FCL), fermented dried at 70 °C (FCL-70) and fermented lyophilised (FCL-L) flour.

	Digested (Standard)				Digested (Older adult)			
	UFCL	**FCL**	**FCL-70**	**FCL-L**	**UFCL**	**FCL**	**FCL-70**	**FCL-L**
Phenolic acids								
Gallic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	3.4 ± 0.4^{aB}	5.5 ± 0.7^{bB}	10.8 ± 1.3^{cB}	5.9 ± 0.4^{bA}	2.50 ± 0.06^{aA}	3.3 ± 0.2^{abA}	4.4 ± 0.2^{bA}	6.6 ± 0.7^{cB}
p-Coumaric acid	6.2 ± 1.0^{cA}	1.91 ± 0.04^{aB}	3.0 ± 0.2^{bB}	1.74 ± 0.05^{aA}	6.4 ± 1.3^{bA}	1.637 ± 0.007^{aA}	1.80 ± 0.14^{aA}	1.86 ± 0.08^{aA}
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	n.d.	4.5 ± 1.4^{a}	5.5 ± 0.9^{a}	7.4 ± 0.4^{a}	n.d.	n.d.	n.d.	n.d.
Vanillic acid	6.2 ± 1.2^{a}	18 ± 3^{b}	20 ± 2^{b}	24 ± 2^{b}	traces	traces	traces	traces
Ferulic acid	traces	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trans-Cinnamic acid	n.d.	1.6 ± 0.3^{aA}	8.9 ± 0.9^{bB}	1.93 ± 0.03^{aA}	n.d.	2.25 ± 0.06^{aB}	5.8 ± 0.2^{cA}	3.3 ± 0.3^{bB}
Flavonoids								
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	traces	traces	traces	n.d.	traces	n.d.	n.d.	n.d.
Quercitrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Apigenin-7-glucoside	n.d.	2.61 ± 0.09^{aB}	5.4 ± 0.6^{bB}	3.23 ± 0.06^{aA}	n.d.	2.33 ± 0.03^{aA}	3.8 ± 0.4^{cA}	3.13 ± 0.15^{bA}
Quercetin	n.d.	n.d.	n.d.	n.d.	traces	traces	traces	traces
Naringenin	n.d.	traces	traces	traces	n.d.	traces	traces	traces
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The results represent the mean of three repetitions with their standard deviation. ^{a,b,c} Different lowercase letters indicate significant differences between flours and ^{A,B} different capital letters indicate significant differences between digestion models ($p < 0.05$). n.d.: not detected. Traces: not quantifiable.

Table 5.25. Phenolic content (µg/g dry basis) in digested white quinoa for unfermented flour (UFWQ), fermented seed (FWQ), fermented dried at 70 °C (FWQ-70) and fermented lyophilised (FWQ-L) flour.

	Digested (Standard)				Digested (Older adult)			
	UFWQ	**FWQ**	**FWQ-70**	**FWQ-L**	**UFWQ**	**FWQ**	**FWQ-70**	**FWQ-L**
Phenolic acids								
Gallic acid	20 ± 2^{a}	77 ± 7^{cA}	139 ± 13^{dB}	56 ± 3^{bA}	traces	68 ± 8^{bA}	75 ± 9^{bA}	43 ± 7^{aA}
Caffeic acid	6.7 ± 0.9^{b}	2.09 ± 0.09^{aB}	2.7 ± 0.6^{aB}	traces	traces	0.83 ± 0.06^{aA}	0.88 ± 0.02^{aA}	traces
p-Coumaric acid	2.9 ± 0.9^{A}	n.d.	traces	traces	6.8 ± 1.0^{B}	n.d.	traces	traces
Sinapic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	n.d.	traces	n.d.	n.d.	n.d.	traces	n.d.	n.d.
Vanillic acid	16 ± 2^{bB}	3.5 ± 0.4^{aA}	5.0 ± 0.4^{aA}	3.1 ± 0.3^{aA}	8.3 ± 0.9^{cA}	2.93 ± 0.02^{aA}	4.1448 ± 0.0014^{bA}	3.0 ± 0.4^{aA}
Ferulic acid	9.0 ± 1.0^{bA}	traces	1.68 ± 0.12^{aA}	traces	8.6 ± 0.7^{bA}	traces	1.699 ± 0.004^{aA}	traces
trans-Cinnamic acid	traces	1.3 ± 0.2^{bA}	2.95 ± 0.05^{cA}	0.74 ± 0.05^{aA}	traces	2.14 ± 0.08^{bB}	3.69 ± 0.02^{cB}	1.87 ± 0.10^{aB}
Flavonoids								
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin 3-glucoside	4.5 ± 1.0^{a}	3.162 ± 0.014^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	$6.2 \pm 0.3a^{bA}$	6.7 ± 0.9^{bB}	6.60 ± 0.08^{abA}	5.3 ± 0.4^{aA}	6.8 ± 1.7^{bA}	3.51 ± 0.10^{aA}	$5.9 \pm 0.7a^{bA}$	4.0 ± 1.1^{abA}
Apigenin-7-glucoside	2.23 ± 0.04^{a}	2.62 ± 0.08^{aA}	9.3 ± 0.4^{cB}	3.43 ± 0.14^{bB}	n.d.	2.33 ± 0.03^{bA}	8.40 ± 0.03^{cA}	1.99 ± 0.04^{aA}
Quercetin	traces	traces	traces	traces	traces	traces	traces	traces
Naringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d} Different lowercase letters indicate significant differences between flours and ^{A,B} different capital letters indicate significant differences between digestion models ($p < 0.05$). n.d.: not detected. Traces: not quantifiable.

Table 5.26. Phenolic content (μg/g dry basis) in digested black quinoa for unfermented flour (UFBQ), fermented seed (FBQ), fermented dried at 70 °C (FBQ-70) and fermented lyophilised (FBQ-L) flour.

	Digested (Standard)				Digested (Older adult)			
	UFBQ	**FBQ**	**FBQ-70**	**FBQ-L**	**UFBQ**	**FBQ**	**FBQ-70**	**FBQ-L**
Phenolic acids								
Gallic acid	30 ± 4^{aB}	21 ± 3^{aB}	42 ± 2^{bB}	39 ± 3^{bB}	15 ± 2^{aA}	16.03 ± 0.18^{aA}	29.81 ± 0.12^{bA}	27.7 ± 2.0^{bA}
Caffeic acid	2.08 ± 0.18^{A}	n.d.	n.d.	n.d.	1.87 ± 0.06^{A}	n.d.	n.d.	n.d.
p-Coumaric acid	traces	traces	traces	traces	1.97 ± 0.06^{b}	traces	traces	1.30 ± 0.07^{a}
Sinapic acid	15 ± 3^{bA}	1.29 ± 0.17^{aA}	traces	2.19 ± 0.03^{aB}	32 ± 3^{bB}	1.34 ± 0.11^{aA}	traces	1.64 ± 0.12^{aA}
4-O-Caffeoylquinic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vanillic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ferulic acid	4.9 ± 0.4^{A}	traces	traces	traces	8.79 ± 0.04^{B}	traces	traces	traces
trans-Cinnamic acid	traces	traces	traces	traces	traces	traces	traces	traces
Flavonoids								
Rutin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Epicatechin	traces	n.d.	n.d.	n.d.	traces	traces	traces	traces
Quercetin 3-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercitrin	10.0 ± 1.0^{bA}	3.7 ± 0.4^{aA}	5 ± 3^{abA}	8.71 ± 0.11^{abB}	11.5 ± 1.3^{bA}	6.95 ± 0.07^{aB}	6.030 ± 0.009^{aA}	5.4 ± 0.3^{aA}
Apigenin-7-glucoside	n.d.	2.23 ± 0.13^{aA}	3.6 ± 0.5^{bA}	3.92 ± 0.02^{bB}	n.d.	3.0 ± 0.3^{aA}	3.2 ± 0.4^{aA}	2.9 ± 0.3^{aA}
Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naringenin	n.d.	1.58 ± 0.18^{aA}	2.6 ± 0.3^{bA}	3.04 ± 0.08^{bB}	n.d.	2.77 ± 0.08^{aB}	2.33 ± 0.15^{aA}	2.26 ± 0.10^{aA}
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The results represent the mean of three repetitions with their standard deviation. ^{a,b} Different lowercase letters indicate significant differences between flours and ^{A,B} different capital letters indicate significant differences between digestion models ($p < 0.05$). n.d.: not detected. Traces: not quantifiable.

Older adult simulated conditions resulted in a significant reduction in the variety and number of phenolic compounds present in the bioaccessible fraction when compared to standard digestive conditions. However, chromatographic analysis did not detect all compounds in samples digested under the older adult model. In contrast, the same compounds were found in the bioaccessible fraction obtained using the standard model. This applies to vanillic and 4-hydroxybenzoic acids in the Castellana lentil. Phenolic compounds, found in various plant sources, can have preventive health benefits for humans. The extent of these benefits depends on the compounds' structure, such as their degree of glycosylation or acylation, molecular size, solubility, and conjugation with other phenols. These factors ultimately determine their absorption and metabolism (Ozcan et al., 2014). Vanillic acid (Ullah et al., 2021; Yalameha et al., 2023), caffeic acid (Agunloye et al., 2019; Alam et al., 2022) and gallic acid (Priscilla & Prince, 2009; Wianowska & Olszowy-Tomczyk, 2023) are widely recognised as common phenolic acids that exhibit diverse chemical and pharmacological properties, such as analgesic, anticancer, anti-inflammatory, antioxidant, antimicrobial, cardioprotective, and neuroprotective activities.

The antioxidant activity of the bioaccessible fraction was assessed through three assays: ABTS, FRAP, and DPPH. Table 5.27 a and b display the indexes for each assay and the antioxidant potency composite index (APCI). The ABTS-antioxidant activity increased following *in vitro* digestion, whereas no changes or slight decreases were observed in the FRAP and DPPH assays. Significant differences were found in the antioxidant activities of the bioaccessible fraction, with lower values when older adult conditions were used. The ABTS and DPPH antioxidant capabilities were reduced between 1% and 50%. However, the FRAP assay showed a decrease only in lentils, whereas quinoa showed an increase ranging from 10% to 70% for the digesta values of the older adult model compared to those of the standard model. (Gallego

et al., 2020) discovered similar results when evaluating the effect of cooking different legume pastes on antioxidant activity after gastrointestinal digestion using the DPPH, ABTS and FRAP methods. The study indicated a noteworthy improvement in the antioxidant activity of lentil pastes, reaching 12-fold greater levels than their original undigested content. However, they also found up to a four-fold reduction in pea paste using the DPPH method. The authors explained these differences were due to the activity of enzymes in the gastrointestinal system. These enzymes promote the breakdown of proteins and peptides, resulting in the release of amino acids and phenolic compounds, and the exposure of internal groups. These factors impact the amount, dimensions, and physicochemical features of these compounds and influence the antioxidant potential. Koehnlein et al. (2016) suggested that the high antioxidant capacity of cereals and legumes following gastrointestinal digestion may be due to the partial hydrolysis of total phenols and an increase in their content. Furthermore, the hydroxyl groups on the aromatic rings of the phenolic compounds may be deprotonated. Of all the treatments, flours that were fermented and dried 70 °C displayed the greatest antioxidant activity after gastrointestinal digestion. The fermented flours derived from Castellana lentil (FCL-70), and white quinoa (FWQ-70) demonstrated greater antioxidant capacity with an APCI exceeding 90% and 80%, respectively. Consequently, the results confirm the effectiveness of SSF followed by hot air drying (70 °C) in generating flours that boast an improved functionality of the bioaccessible fraction.

Table 5.27 (a). Antioxidant activity (mg trolox/g dry basis) by ABTS, DPPH and FRAP methods, and total phenol content (mg gallic acid/g dry basis) in undigested and digested Pardina and Castellana lentil and white and black quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flour, under standard and older adult simulated gastrointestinal conditions.

	Antioxidant Activity								
	ABTS			DPPH			FRAP		
	Undigested	Digested (Standard)	Digested (Older adult)	Undigested	Digested (Standard)	Digested (Older adult)	Undigested	Digested (Standard)	Digested (Older adult)
Pardina Lentil									
UFPL	9.5 ± 0.4^{dA}	11.5 ± 0.3^{aB}	11.6 ± 0.5^{abB}	2.07 ± 0.09^{cA}	2.29 ± 0.08^{cB}	2.51 ± 0.09^{cC}	7.6 ± 0.2^{bB}	4.1 ± 0.4^{cA}	3.79 ± 0.18^{cA}
FPL	5.7 ± 0.5^{cA}	12.8 ± 0.4^{bC}	11.4 ± 0.2^{abB}	0.64 ± 0.04^{bC}	0.53 ± 0.04^{aB}	0.44 ± 0.05^{aA}	0.31 ± 0.02^{aA}	1.8 ± 0.2^{aC}	1.35 ± 0.11^{aB}
FPL-70	3.91 ± 0.16^{bA}	13.7 ± 0.5^{bB}	12.5 ± 1.2^{bB}	0.516 ± 0.010^{aA}	0.88 ± 0.04^{bC}	0.76 ± 0.06^{bB}	0.351 ± 0.007^{aA}	2.60 ± 0.14^{bB}	2.38 ± 0.19^{bB}
FPL-L	3.20 ± 0.04^{aA}	11.8 ± 0.8^{aB}	11.0 ± 0.5^{aB}	0.502 ± 0.014^{aA}	0.83 ± 0.05^{bC}	0.70 ± 0.05^{bB}	0.31 ± 0.02^{aA}	2.54 ± 0.11^{bB}	2.3 ± 0.2^{bB}
Castellana Lentil									
UFCL	8.4 ± 0.4^{cA}	14.0 ± 1.4^{aB}	12.2 ± 1.5^{aB}	1.634 ± 0.015^{bA}	1.65 ± 0.05^{cA}	1.78 ± 0.16^{cA}	8.3 ± 0.2^{dB}	3.2 ± 0.4^{cA}	3.02 ± 0.06^{cA}
FCL	2.50 ± 0.09^{aA}	16.9 ± 1.3^{abC}	14.3 ± 1.4^{aB}	2.27 ± 0.13^{cB}	0.25 ± 0.04^{aA}	0.213 ± 0.017^{aA}	1.10 ± 0.03^{aC}	0.89 ± 0.02^{aB}	0.77 ± 0.05^{aA}
FCL-70	6.2 ± 0.2^{bA}	17.4 ± 1.8^{bB}	17.2 ± 1.5^{bB}	1.71 ± 0.02^{bA}	1.64 ± 0.10^{cA}	1.55 ± 0.10^{bA}	7.0 ± 0.3^{cB}	4.50 ± 0.15^{dA}	4.45 ± 0.11^{dA}
FCL-L	2.32 ± 0.16^{aA}	14.5 ± 1.7^{abB}	13.7 ± 1.7^{aB}	1.093 ± 0.016^{aC}	0.39 ± 0.04^{bB}	0.323 ± 0.014^{aA}	2.14 ± 0.05^{bC}	1.38 ± 0.07^{bB}	1.13 ± 0.13^{bA}
White Quinoa									
UFWQ	1.48 ± 0.08^{bA}	8.20 ± 1.14^{aB}	7.1 ± 0.6^{aB}	1.070 ± 0.005^{cC}	0.48 ± 0.04^{cB}	0.23 ± 0.02^{bA}	1.53 ± 0.05^{cB}	1.22 ± 0.14^{cA}	1.70 ± 0.03^{cB}
FWQ	1.87 ± 0.08^{cA}	10.1 ± 1.2^{abB}	9.1 ± 1.2^{aB}	1.334 ± 0.012^{dB}	0.127 ± 0.008^{aA}	0.119 ± 0.011^{aA}	0.47 ± 0.03^{aB}	0.34 ± 0.05^{aA}	0.53 ± 0.07^{aB}
FWQ-70	2.287 ± 0.006^{dA}	12.7 ± 1.3^{cB}	11.6 ± 1.8^{bB}	0.80 ± 0.04^{aB}	0.37 ± 0.02^{bA}	0.31 ± 0.04^{cA}	1.88 ± 0.09^{dB}	1.05 ± 0.04^{bA}	1.15 ± 0.16^{bA}
FWQ-L	1.12 ± 0.04^{aA}	10.8 ± 1.0b^{cC}	8.1 ± 0.8^{aB}	1.00 ± 0.03^{bB}	0.104 ± 0.009^{aA}	0.093 ± 0.013^{aA}	0.63 ± 0.03^{bB}	0.40 ± 0.02^{aA}	0.49 ± 0.08^{aA}
Black Quinoa									
UFBQ	2.48 ± 0.03^{cA}	10.1 ± 0.7^{aC}	7.3 ± 0.6^{bB}	0.88 ± 0.03^{bB}	0.69 ± 0.04^{dA}	0.63 ± 0.03^{dA}	2.74 ± 0.04^{dC}	1.7 ± 0.2^{cA}	2.1 ± 0.3^{bB}
FBQ	1.69 ± 0.10^{bA}	9.6 ± 0.4^{aC}	6.7 ± 0.7^{abB}	1.38 ± 0.02^{dB}	0.25 ± 0.02^{bA}	0.26 ± 0.03^{cA}	0.52 ± 0.02^{aA}	0.42 ± 0.03^{aA}	0.71 ± 0.09^{aB}
FBQ-70	1.68 ± 0.06^{bA}	10.2 ± 0.5^{aC}	7.4 ± 0.2^{bB}	0.666 ± 0.014^{aC}	0.36 ± 0.06^{cB}	0.20 ± 0.02^{bA}	1.26 ± 0.04^{cB}	0.66 ± 0.08^{bA}	0.74 ± 0.05^{aA}
FBQ-L	1.33 ± 0.05^{aA}	9.8 ± 1.2^{aC}	5.5 ± 1.0^{aB}	0.96 ± 0.06^{cB}	0.13 ± 0.02^{aA}	0.139 ± 0.009^{aA}	0.82 ± 0.05^{bB}	0.49 ± 0.08^{abA}	0.58 ± 0.09^{aA}

The results represent the mean of three repetitions with their standard deviation. ^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

Table 5.27 (b). Antioxidant activity (mg trolox/g dry basis) by ABTS, DPPH and FRAP method, and total phenol content (mg gallic acid/g dry basis) in undigested and digested Pardina and Castellana lentil and white and black quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flour, under standard and older adult simulated gastrointestinal conditions.

	Antioxidant Activity									APCI*		
	ABTS Index			DPPH Index			FRAP Index					
	Undigested	Digested (Standard)	Digested (Older adult)	Undigested	Digested (Standard)	Digested (Older adult)	Undigested	Digested (Standard)	Digested (Older adult)	Undigested	Standard	Older adult
Pardina Lentil												
UFPL	100.0	84.0	92.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	94.7	97.4
FPL	60.7	93.2	90.9	30.8	22.9	17.5	4.1	43.2	35.6	31.9	53.1	48.0
FPL-70	41.4	100.0	100.0	25.0	38.4	30.2	4.6	62.7	62.8	23.7	67.0	64.3
FPL-L	33.9	85.7	87.9	24.3	36.1	27.8	4.1	61.3	61.3	20.7	61.0	59.0
Castellana Lentil												
UFCL	100.0	80.7	70.5	72.0	100.0	100.0	100.0	70.2	67.9	90.7	83.6	79.5
FCL	29.9	97.2	82.7	100.0	15.0	11.9	13.4	19.8	17.4	47.7	44.0	37.3
FCL-70	73.9	100.0	100.0	75.1	99.3	87.1	85.3	100.0	100.0	78.1	99.8	95.7
FCL-L	27.7	83.5	79.5	48.2	23.4	18.1	25.9	30.7	25.3	33.9	45.9	41.0
White Quinoa												
UFWQ	64.7	64.5	61.5	80.2	100.0	75.0	81.5	100.0	100.0	75.5	88.2	78.8
FWQ	81.7	79.4	78.5	100.0	26.4	38.5	24.9	27.8	31.3	68.9	44.5	49.5
FWQ-70	100.0	100.0	100.0	60.3	76.2	100.0	100.0	86.2	67.7	86.8	87.5	89.2
FWQ-L	49.1	84.5	69.7	74.8	21.6	30.3	33.3	32.5	28.7	52.4	46.2	42.9
Black Quinoa												
UFBQ	100.0	99.0	99.4	63.6	100.0	100.0	100.0	100.0	100.0	87.9	99.7	99.8
FBQ	68.2	94.1	90.8	100.0	35.5	40.9	19.1	25.1	34.3	62.4	51.6	55.3
FBQ-70	67.8	100.0	100.0	48.3	52.4	31.0	46.2	39.8	35.5	54.1	64.1	55.5
FBQ-L	53.7	96.1	74.6	69.6	19.4	22.0	30.0	29.7	27.9	51.1	48.4	41.5

Values correspond to ABTS, DPPH and FRAP indexes, calculated among unfermented, fermented and fermented-dried at 70 °C and lyophilised samples of each plant food.
*APCI: Antioxidant potency composite index.

The effect of gastrointestinal conditions shows different trends depending on the pre-treatment of the food and the substrate itself, as well as the methodology used to measure the antioxidant activity. A significant reduction is only observed for DPPH in Pardina lentils and for ABTS in black quinoa after using the older adult model. However, white quinoa and Castellana lentils maintain or even increase the values reported by the control model. These data could help develop new products for this population group, because a high antioxidant capacity is necessary for good health.

3.2. Impact of GI conditions on the bioaccessibility of phytic acid and minerals of unfermented, fermented, and fermented dried lentils and quinoa

Minerals are inorganic substances found in all tissues and bodily fluids, which are vital for maintaining specific physicochemical processes essential for life (U. C. Gupta & Gupta, 2014; Soetan et al., 2010). They have structural functions involving the skeleton and soft tissues and regulatory functions including neuromuscular transmission, blood clotting, oxygen transport, and enzyme activity (National Research Council, 1989). Legumes and pseudocereals are excellent sources of minerals such as calcium, iron, zinc, potassium, and magnesium (Chakraverty et al., 2003; Martínez-Villaluenga et al., 2020). The mineral content of unfermented, fermented, and fermented dried samples (Mg, Ca, and Fe) was evaluated pre- and post-gastrointestinal digestion, as shown in Table 5.28. Undigested samples revealed that SSF and ensuing drying caused an increase in Mg and Ca contents, with increases in Mg ranging from 1% to 20% and Ca from 12% to 59%. Significant differences in Ca content were found in all samples, whereas significant differences in Mg content were found only in Castellana lentil and white quinoa samples dried at 70 °C and lyophilised. In contrast, SSF decreased the Fe content in all samples between 2% and 11%, with a significant difference in Pardina lentil and white quinoa samples.

Furthermore, the drying process increased the Fe content between 2% and 23% only in white and black quinoa samples, with significance in the fermented samples dried at 70 °C.

When comparing the undigested and digested samples, the findings indicate that the digestive process led to a decrease in mineral content (Mg, Ca, and Fe) in the bioaccessible fraction. Despite the digestion model used, the fermented and fermented dried samples exhibited higher Mg content than the unfermented samples in the 3% to 30% range. However, a notable difference was only observed in Castellana lentils and white quinoa. The fermented and fermented dried samples of Castellana lentil and white and black quinoa exhibited a significant increase in Ca content ranging from 18% to 124%. However, the fermented and fermented dried samples of Pardina lentil exhibited a decrease in Ca content. Regarding Fe content, both Castellana and Pardina lentils experienced a notable increase, ranging from 63% to 329% in their fermented and fermented dried samples. Furthermore, a decrease was observed in the fermented and fermented dried samples of white and black quinoa. Therefore, it can be inferred that mineral bioaccessibility is enhanced through the fermentation process.

When comparing digestion models, it was found that the older adult digestion model demonstrated a decrease in mineral content when evaluated against the standard. Despite individual cases of significant differences, no overall significant differences were observed between the digestion models.

Table 5.28. Mineral content (µg/g dry basis) in undigested and digested Pardina and Castellana lentil and white and black quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented-dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented-lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flour, under standard and older adult simulated gastrointestinal conditions.

	Magnesium (Mg)			Calcium (Ca)			Iron (Fe)		
	Undigested	**Digested (Standard)**	**Digested (Older adult)**	**Undigested**	**Digested (Standard)**	**Digested (Older adult)**	**Undigested**	**Digested (Standard)**	**Digested (Older adult)**
Pardina Lentil									
UFPL	112.6 ± 0.9^{aB}	76 ± 4^{aA}	71 ± 3^{aA}	62 ± 2^{aB}	43 ± 4^{cA}	44.1 ± 0.6^{bA}	11.3 ± 0.3^{cB}	1.60 ± 0.05^{aA}	1.05 ± 0.14^{aA}
FPL	125 ± 3^{bB}	78 ± 2^{aA}	70 ± 3^{aA}	90.5 ± 0.9^{cB}	22 ± 3^{bA}	15 ± 3^{aA}	10.05 ± 0.04^{bC}	3.168 ± 0.004^{cB}	2.45 ± 0.02^{bA}
FPL-70	112 ± 2^{aB}	74 ± 5^{aA}	75 ± 10^{aA}	79.6 ± 0.4^{bB}	12 ± 2^{aA}	12 ± 4^{aA}	8.92 ± 0.05^{aB}	2.6 ± 0.3^{bA}	2.2 ± 0.3^{bA}
FPL-L	109 ± 2^{aB}	71 ± 7^{aA}	76.4 ± 0.6^{aA}	80 ± 2^{bB}	44 ± 5^{cA}	52 ± 2^{cA}	8.9 ± 0.3^{aB}	2.60 ± 0.02^{bA}	2.78 ± 0.08^{cA}
Castellana Lentil									
UFCL	122 ± 2^{aB}	82 ± 7^{aA}	84.8 ± 0.9^{aA}	64.1 ± 0.9^{aB}	48 ± 6^{aA}	43 ± 4^{aA}	9.04 ± 0.07^{aB}	1.4 ± 0.3^{aA}	1.5 ± 0.3^{aA}
FCL	127 ± 4^{abB}	93 ± 5^{abA}	96 ± 2^{cA}	94.0 ± 1.0^{bB}	61 ± 6^{bA}	57 ± 9^{bA}	8.9 ± 0.2^{aC}	5.8 ± 1.0^{bB}	4.43 ± 0.03^{cA}
FCL-70	142 ± 4^{cC}	102 ± 2^{bB}	91 ± 2^{bA}	102 ± 5^{bB}	58 ± 3^{bA}	43 ± 6^{aA}	9.2 ± 0.3^{aC}	5.0 ± 0.2^{bB}	3.8 ± 0.2^{bA}
FCL-L	135 ± 3^{bcC}	107 ± 10^{bB}	92.5 ± 0.4^{bcA}	101 ± 6^{bB}	49 ± 5^{aA}	54 ± 5^{abA}	8.8 ± 0.2^{aB}	6.0 ± 1.4^{bA}	4.56 ± 0.12^{cA}
White Quinoa									
UFWQ	218 ± 2^{aB}	146 ± 3^{aA}	139 ± 6^{abA}	63 ± 2^{aB}	32.1 ± 1.5^{aA}	38 ± 3^{aA}	3.67 ± 0.02^{bB}	3.3 ± 0.2^{bA}	3.1 ± 0.6^{aA}
FWQ	232 ± 10^{aB}	163.7 ± 1.3^{bA}	157 ± 10^{bA}	81 ± 4^{bB}	72 ± 7^{cB}	47 ± 4^{cA}	3.45 ± 0.02^{aB}	2.4 ± 0.2^{aA}	2.0 ± 0.5^{aA}
FWQ-70	261 ± 3^{bB}	163 ± 7^{bA}	156 ± 3^{bA}	79 ± 3^{bC}	65 ± 6^{cB}	45 ± 3^{bA}	4.54 ± 0.07^{cB}	3.50 ± 0.04^{bA}	2.9 ± 0.5^{aA}
FWQ-L	248.3 ± 1.3^{bC}	166 ± 5^{bB}	133 ± 11^{aA}	78.4 ± 0.6^{bB}	49.8 ± 0.7^{bA}	54 ± 3^{cA}	3.78 ± 0.13^{bB}	2.9 ± 0.3^{abA}	2.2 ± 0.6^{aA}
Black Quinoa									
UFBQ	210 ± 8^{aB}	111 ± 10^{aA}	135 ± 6^{aA}	55 ± 2^{aB}	36 ± 4^{aA}	36 ± 5^{aA}	4.3 ± 0.3^{abB}	2.9 ± 0.4^{bA}	3.3 ± 0.2^{bA}
FBQ	212.0 ± 1.4^{aB}	136 ± 2^{abA}	135 ± 11^{aA}	63 ± 2^{bA}	56 ± 5^{bA}	60 ± 10^{cA}	3.86 ± 0.03^{aB}	1.9 ± 0.2^{aA}	1.54 ± 0.09^{aA}
FBQ-70	217 ± 12^{aB}	145 ± 2^{bA}	138.9 ± 0.7^{aA}	62 ± 2^{bA}	57 ± 12^{bA}	41 ± 4^{bA}	4.5 ± 0.2^{bB}	2.2 ± 0.6^{bA}	1.6 ± 0.3^{aA}
FBQ-L	220 ± 7^{aB}	130 ± 16^{abA}	144 ± 2^{aA}	66 ± 4^{bA}	58 ± 10^{bA}	42 ± 5^{bA}	4.4 ± 0.2^{abB}	1.7 ± 0.3^{aA}	1.8 ± 0.3^{aA}

Results represent the mean of three repetitions with their standard deviation. ^{a,b,c} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

Many legume grains, cereals, and pseudocereal seeds contain varying concentrations of phytic acid. Upon ingestion, it remains undigested in the human digestive system due to the lack of phytase enzyme. Phytic acid can bind to crucial micronutrients like iron, calcium, magnesium, and zinc, reducing their absorption during gastrointestinal digestion (Ojo, 2020). Processes such as SSF have been used to reduce this anti-nutrient. This study analyses the effects of SSF and drying on the bioaccessibility of phytic acid in unfermented, fermented, and fermented dried samples of Castellana and Pardina lentils and white and black quinoa. The analysis was conducted using standard and older adult *in vitro* digestion models, as illustrated in Figure 5.16. A marked reduction in the phytate content of approximately 90% can be observed in undigested fermented and fermented dried samples of Castellana lentil, white and black quinoa as compared to their unfermented counterparts. Furthermore, there is no significant effect on Pardina lentils. These findings indicate that the decrease in this anti-nutritional factor is due to the activation of the endogenous phytase present in each substrate, as discussed previously (Sánchez-García et al., 2023b, 2023a). When the undigested and digested samples were compared, a significant reduction in phytic acid release was observed after gastrointestinal digestion. The reductions ranged from 70% to 80% in unfermented lentil samples (Pardina and Castellana) and quinoa (white and black) regarding their initial content (undigested).

For both digested and fermented dried samples, black quinoa saw a reduction of approximately 40%, whereas Pardina lentils saw a reduction of approximately 80%. In contrast, the reduction in Castellana lentils and white quinoa was 100%, which was due to their minimal phytic acid content in undigested samples rather than the simulated gastrointestinal digestion's physiological conditions. Therefore, a reduction in phytic acid in fermented and dehydrated fermented samples may be

associated with increased levels of Mg, Ca, and Fe following gastrointestinal digestion. Chawla et al. (2017) evaluated the impact of SSF in black-eyed pea seed flour using an *Aspergillus oryzae* strain on the mineral bioavailability of iron and zinc. They determined that after 96 h of fermentation, iron and zinc, increased from 17.3% to 30.2% and from 14.4% to 29.6%, respectively. The authors attributed the improved mineral bioaccessibility to the degradation of anti-nutrient compounds, including phytic acid.

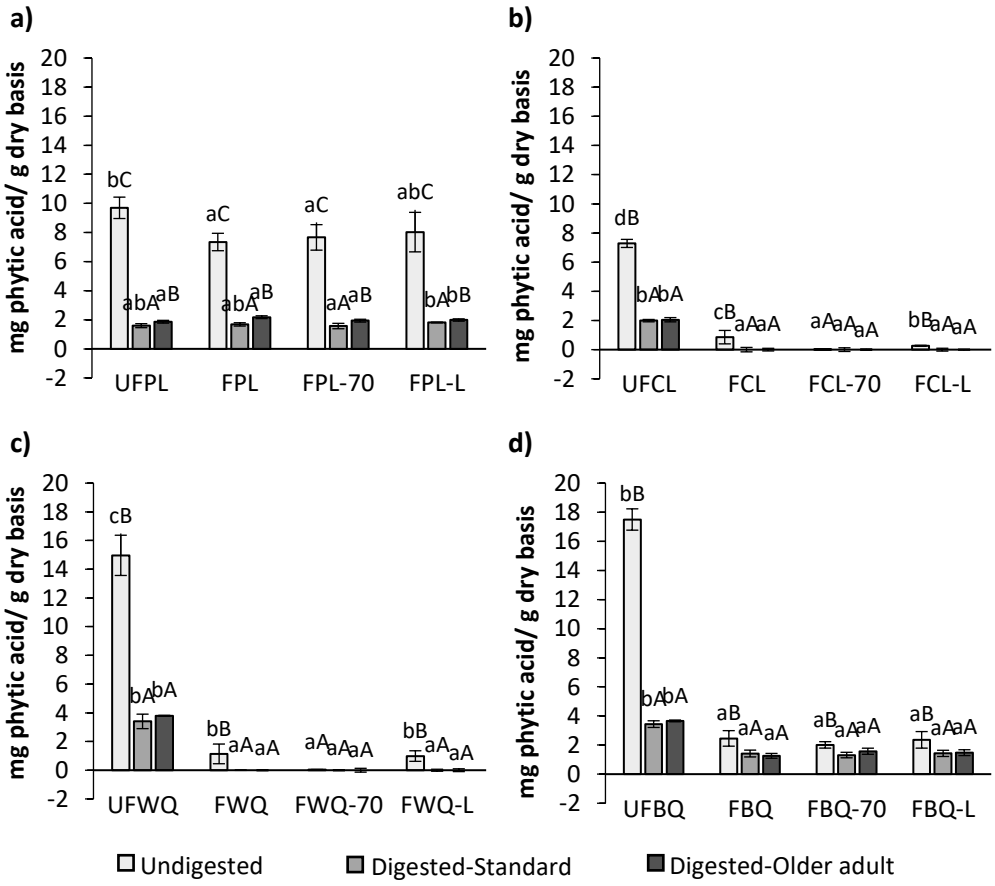

Figure 5.16. Phytic acid content (mg/g dry basis) in Pardina (**a**) and Castellana lentil (**b**) and white (**c**) and black quinoa (**d**) for unfermented flour (UFPL, UFCL, UFWQ,

UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flour obtained with a standard and older adult *in vitro* digestion model. [a,b,c] Different lowercase letters indicate significant differences ($p < 0.05$) between samples. [A,B,C] Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

When comparing digestion models, a significant difference was observed between the standard model and the older adult model for Pardina lentils. However, no significant differences were found for Castellana lentils, white quinoa and black quinoa. Therefore, there is no significant effect of the occurrence of digestive disorders with age. Couzy et al. (Couzy et al., 1998) studied zinc absorption in older and younger subjects (with similar zinc status) using serum concentration curve (SCC). They administered soy milk fortified with 50 mg of zinc containing three levels of phytic acid: 0, 0.13, and 0.26 g/200 mL. They found that phytic acid reduced zinc absorption as the concentration of phytic acid increased in the beverage. Furthermore, they indicated there were no differences between the older and younger subjects.

4. Conclusions

SSF, coupled with drying at 70°C, had a positive impact on the bioaccessibility of phenolic compounds and antioxidant activity, albeit to various degrees depending on the substrate. The profile of phenolic compounds following gastrointestinal digestion showed an increase in vanillic and caffeic acids in Castellana lentils and in vanillic acid in Pardina lentils, reaching approximately three times the levels of the unfermented samples. There was a significant increase in gallic acid of up to 7 and 1.4 times more than in the unfermented analogue in white and black quinoa, respectively. Regarding antioxidant activity, the Castellana lentil and white quinoa flours fermented and

dried at 70 °C showed the highest APCI (>90% and >80%, respectively) after digestion, thus having a higher capacity to neutralise free radicals than the other samples. Fermented and fermented dried samples (at 70°C and lyophilised) displayed a mineral bioaccessible content that was higher than the unfermented samples. This, together with the low phytic acid content present in fermented dried samples, renders such flours attractive for developing functional products with superior bioaccessibility than unfermented flours. Finally, typical age-related digestive conditions did not appear to affect the mineral bioavailability of Fe, Mg, and Ca in lentils and quinoa flours. However, these conditions reduced the phenolic profile and antioxidant activity of digesta when compared to the results obtained in the standard model.

Fermented Castellana and white quinoa flours are the optimal choice for product development, specifically catering to this population group to maximise health benefits. Furthermore, it is essential to evaluate the techno-functional properties of fermented flours to determine their compatibility with different food applications. Moreover, it is crucial to perform scale-up tests of the fermentation process to facilitate the technological transfer of this process to the food industry.

SUPPLEMENTARY MATERIAL

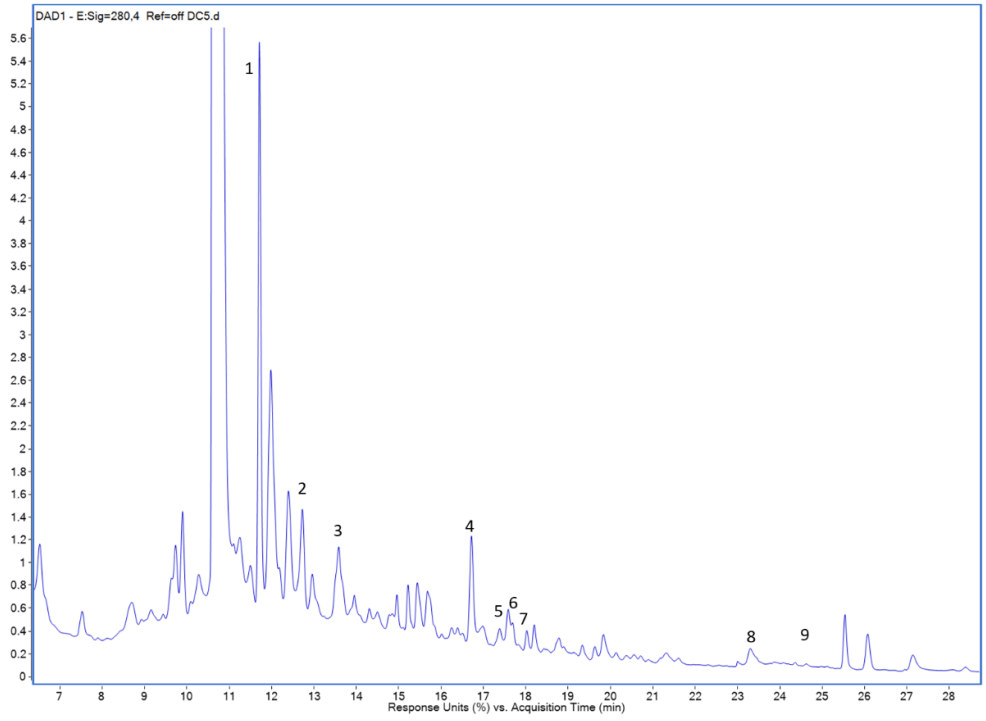

Figure S5.1. Chromatographic profile of Unfermented Pardina Lentil (UFPL) digested (standard) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

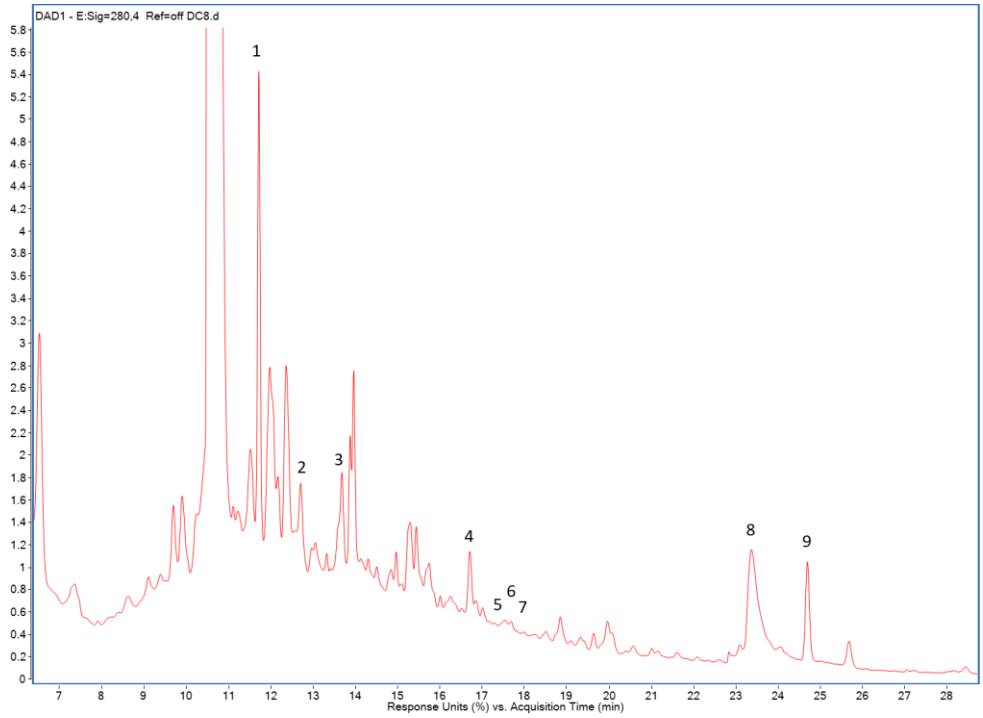

Figure S5.2. Chromatographic profile of Fermented Pardina Lentil (FPL) digested (standard) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

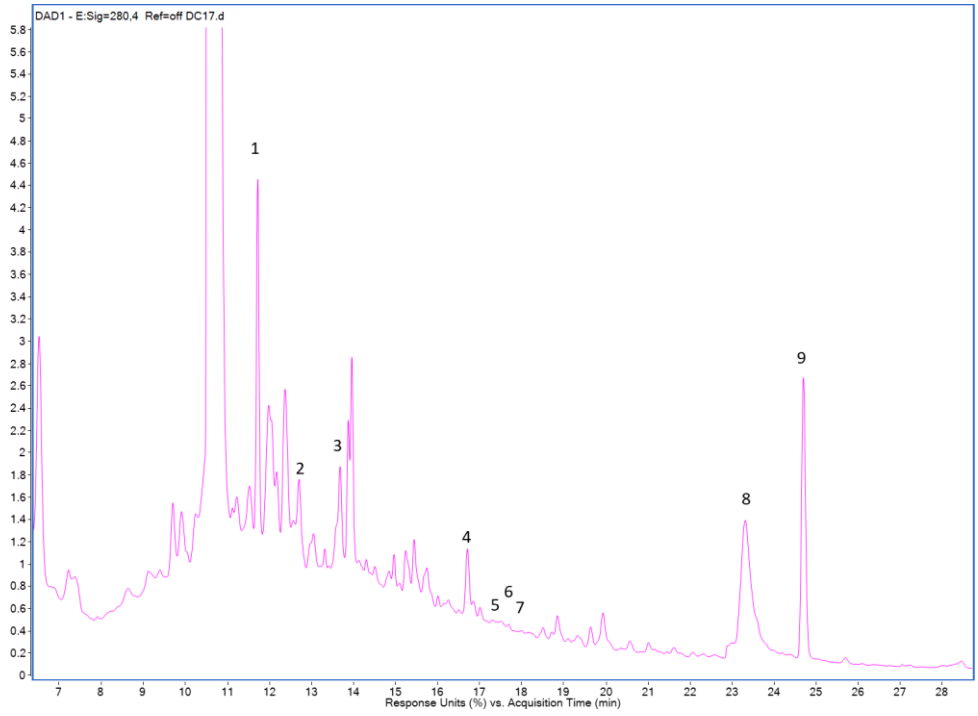

Figure S5.3. Chromatographic profile of Fermented-dried at 70 °C Pardina Lentil (FPL-70) digested (standard) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

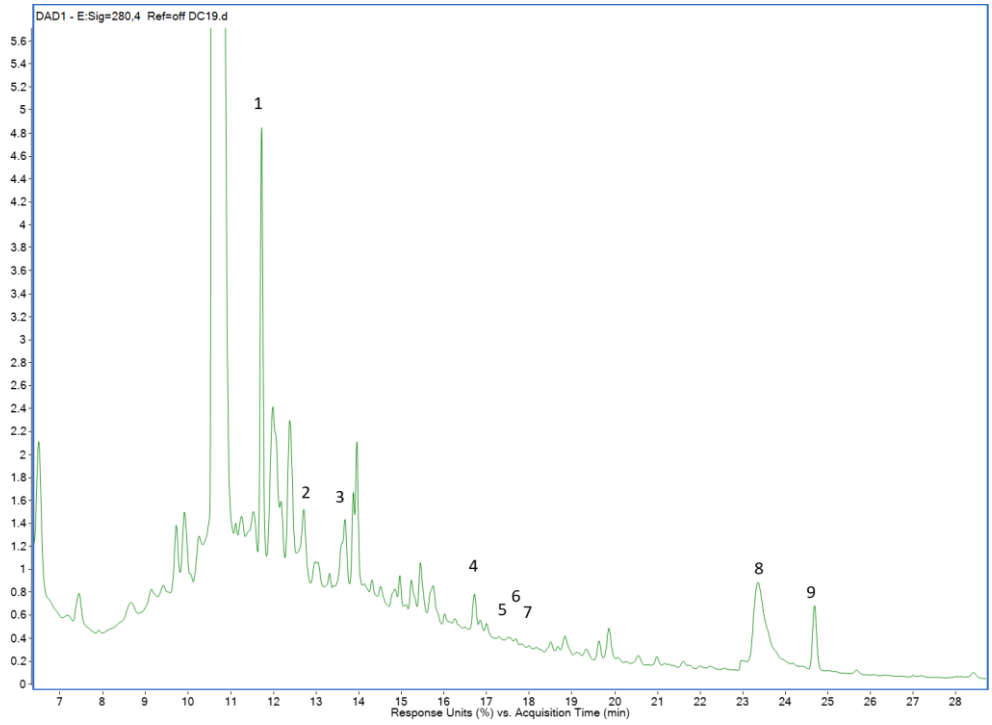

Figure S5.4. Chromatographic profile of Fermented-lyophilised Pardina Lentil (FPL-L) digested (standard) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

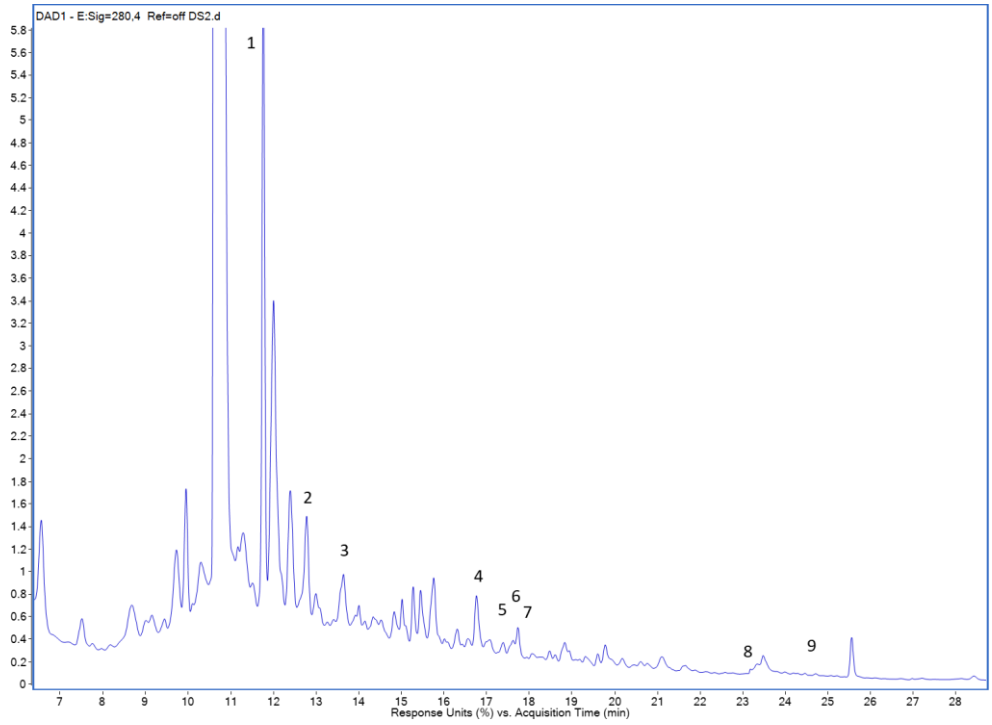

Figure S5.5. Chromatographic profile of Unfermented Pardina Lentil (UFPL) digested (Older adult) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

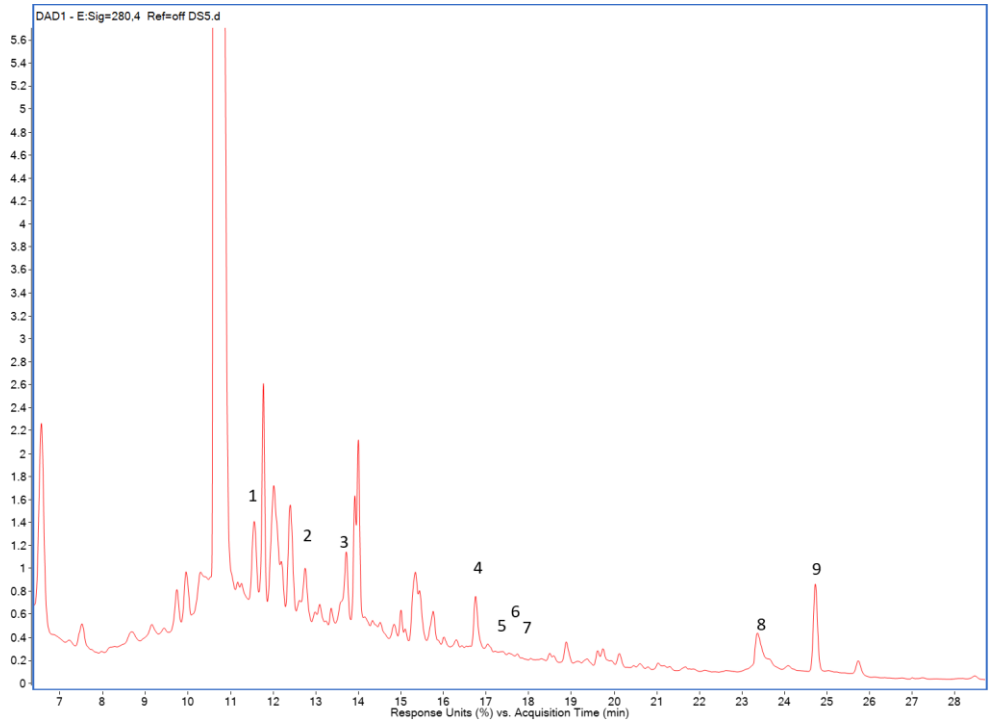

Figure S5.6. Chromatographic profile of Fermented Pardina Lentil (FPL) digested (Older adult) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

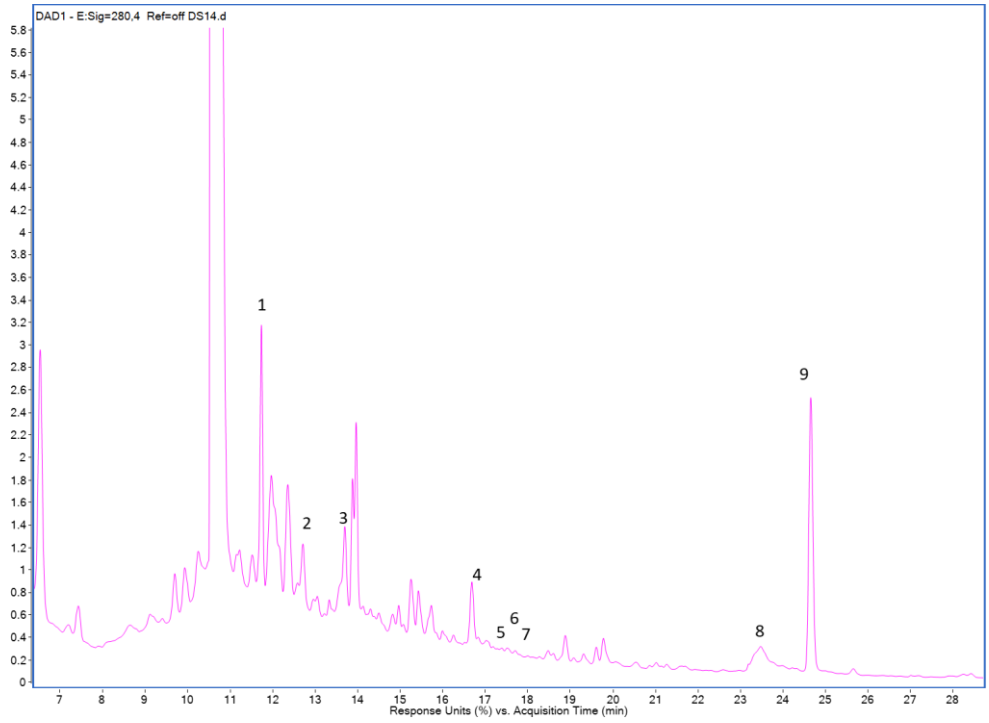

Figure S5.7. Chromatographic profile of Fermented-dried at 70 °C Pardina lentil (FPL-70) digested (Older adult) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

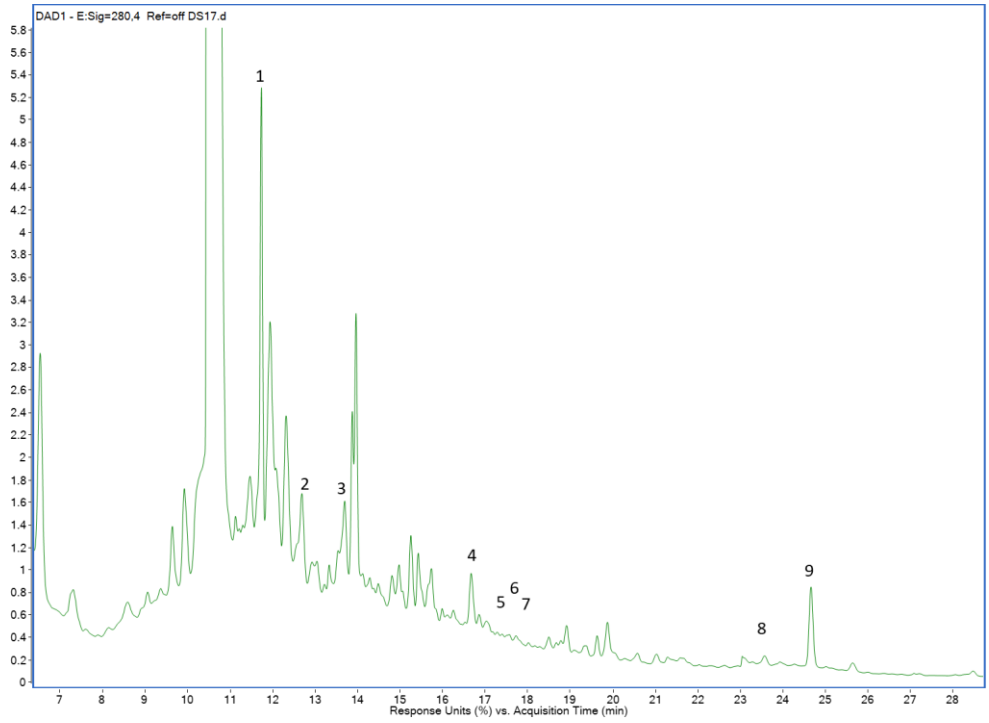

Figure S5.8. Chromatographic profile of Fermented-lyophilised Pardina Lentil (FPL-L) digested (Older adult) extracted at 280 nm. 1: 4-O-Caffeoylquinic; 2: 4-Hydroxybenzoic acid; 3: Vanillic acid; 4: p-Coumaric acid; 5: Ferulic acid; 6: Quercitrin; 7: Apigenin-7-glucoside; 8: Quercetin; 9: trans-Cinnamic acid. Note: not all the compounds are quantified at 280 nm.

ARTÍCULO 6

ARTÍCULO 6: Protein digestibility and ACE inhibitory activity of fermented flours in older adults and standard gastrointestinal simulation.

ABSTRACT

Consumption of essential amino acids responsible for muscle protein synthesis is important in preventing sarcopenia among older individuals. This population may experience gastrointestinal disorders that inhibit protein digestibility, making it crucial to address. Therefore, solid-state fermentation (SSF) using *Pleurotus ostreatus* and air drying has been suggested as a means of improving the protein digestibility of lentils and quinoa. SSF combined with air drying at 70 °C resulted in a slight increase in protein hydrolysis compared to unfermented samples. SSF was found to boost the proportion of small peptides to 35%. Following digestion, SSF and drying yielded bioactive peptides of 1.4 and 0.45 kDa, with a range of 11% to 28%, respectively, and peptides <0.19 kDa making up 60% of the total. SSF promoted valine, leucine, and isoleucine generation; however, hot air drying reduced free amino acids due to the amino acid-reducing sugar bonding but was never lower than the initial content of its unfermented counterpart. Furthermore, SSF and drying at 70 °C improved the release of hydrophobic amino acids (>70 mg/g dry basis) and negatively charged amino acids (>20 mg/g dry basis) in lentils during digestion. The SSF samples exhibited lower angiotensin Converting Enzyme (ACE) inhibitory activity, ≤35%, compared to unfermented flours after digestion. However, the ACE inhibitory activity increased in SSF-dried samples, in part because of melanoidins generated during drying. Finally, lower values of protein digestibility and thus smaller peptides, amino acid profile, and ACE inhibitory activity of fermented flours were found in the older adult digestion model.

Keywords: quinoa, lentils, *Pleurotus ostreatus*, proteolysis, peptide, amino acids.

1. Introduction

Protein comprises 20 different amino acids linked through peptide bonds. To exert health benefits, dietary protein should be hydrolysed by enzymes in the small intestine into free amino acids, dipeptides, or tripeptides (G. Wu, 2016). Protein intake is relevant throughout life, from gestation to old age. Dietary protein recommendations consider essential amino acids, conditionally essential amino acids, and nitrogen required for various functions in tissue growth, repair, and maintenance (Henley et al., 2010). The Recommended Daily Allowance (RDA) for protein in g per kg of body weight per day for different population groups is as follows: (i) infants aged 0.3–0.5 and 0.75–1 years is 1.52 and 1.50, respectively, (ii) children aged 1–3 and 4–8 years is 1.10 and 0.95, respectively, (iii) adolescents aged 9–13 and 14–18 years is 0.95 and 0.85, respectively, (iv) and adults ≥19 years is 0.80 (Wu, 2016). Higher RDAs in infants and children are due to their increased protein requirements during their growth stage (G. Wu, 2016). In contrast, the minimum protein amount required to prevent muscle mass decline in most adults is 0.8 grams per kg of body weight per day. However, this amount could be insufficient for maintaining muscle health in older adults (Gaffney-Stomberg et al., 2009; Landi et al., 2016; Wolfe et al., 2008). Among protein fractions, small peptide chains, also called bioactive fractions, can exert additional functional properties (Sánchez & Vázquez, 2017). Potential bioactivities of these compounds include antioxidant, anti-inflammatory, antidiabetic, antithrombotic, antihypertensive, anticancer, antimicrobial, antiobesity, opioid, and mineral binding properties, as well as cholesterol-lowering (hypocholesterolaemia) and immunomodulatory effects (Peighambardoust et al., 2021; Sánchez & Vázquez, 2017; Shahidi & Zhong, 2008). Consumption of dietary sources with antihypertensive activity is especially relevant for preventing cardiovascular disease.

Digestive disturbances often occur with ageing, leading to changes in the oral cavity, such as tooth loss, the use of dentures, gingivitis, and decreased saliva production (Brownie, 2006). This results in larger particle sizes and different viscosities during chewing, which considerably influences digestion. Furthermore, reduced enzyme secretion, changes in pH gradients, luminal electrolyte composition, motility, and bile secretion could lead to maldigestion and protein malabsorption, increasing the predisposition to develop diseases such as sarcopenia (Hernández-Olivas et al., 2022; S. Lee et al., 2023). The current RDA does not consider changes that occur with ageing, such as a decline in muscle mass, increased fat mass, changes in eating habits and physical activity, and the most common diseases (Gaffney-Stomberg et al., 2009). Evidence supports that intakes above the RDA can improve muscle mass, strength, and function in older adults. In addition, other factors, such as immune health, blood pressure, wound healing, and bone health, can be improved by increasing protein consumption above RDA (Landi et al., 2016; Wolfe et al., 2008). Therefore, it is recommended that this population group includes high-quality proteins in their diet, with a high content of essential amino acids, from animal (lean meat and dairy products) or vegetable (such as soya beans, lentils and peanuts) sources, and in sufficient quantities (Landi et al., 2016). Wall et al. (2014) indicated that 30–40 g of high-quality protein after physical activity and at regular intervals promotes better muscle protein synthesis rates. In contrast, when animal and plant proteins are compared, the digestibility of plant proteins is reduced due to the presence of anti-nutritional factors. However, once plant protein sources are free of these anti-nutritional compounds, they can have a digestibility rate comparable to animal proteins (Ewy et al., 2022; van Vliet et al., 2015). Therefore, using the solid-state fermentation (SSF) process for legumes and cereals can be a strategy to increase nutrient bioaccessibility by decreasing anti-nutritional factors and partially

hydrolysing macromolecules such as proteins into free peptides and amino acids, as well as generating *novo* amino acids (Nkhata et al., 2018; J. Wang et al., 2023). This study aimed to determine the effect of SSF and subsequent drying of lentil and quinoa flours on *in vitro* protein digestibility and functionality (soluble protein, amino acid profile and angiotensin Converting Enzyme (ACE) inhibitory activity).

2. Materials and methods

2.1. Materials

The raw materials were purchased from local shops in Valencia (Spain). Lentils (*Lens culinaris*) of the Castellana and Pardina varieties of the Hacendado® brand and quinoa (*Chenopodium quinoa Wild*) of the black and white varieties of the Nut&me and Hacendado® brands were used, respectively. The *Pleurotus ostreatus* strain was purchased from the Spanish Type Culture Collection (CECT20311).

Pancreatin from porcine pancreas (8 × USP), bile bovine (dried, unfractionated), pepsin from porcine gastric mucosa (≥3200 U/mg), p-toluene-sulfonyl-L-arginine methyl ester (TAME, T4626), analytical grade salts (sodium chloride, sodium bicarbonate, calcium chloride, potassium chloride, potassium dihydrogen phosphate, magnesium chloride hexahydrate, and ammonium carbonate), concentrated hydrochloric acid, sodium hydroxide, sodium phosphate dibasic, potassium phosphate monobasic, trifluoroacetic acid, acetonitrile (HPLC grade), formic acid, hydrogen peroxide, hydrobromic acid, Lithium hydroxide monohydrate, trichloroacetic acid, EDTA, urea, tyrosine, ACE from rabbit lung (2.0 units/mg protein) (A6778-25UN), N-Hippuric-His-Leu hydrate (HHL), ethyl acetate, glucose, mycopeptone were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Malt extract and agar were obtained from Scharlau (Barcelona, Spain). For HPLC analysis,

cytochrome C (12.5 kDa), insulin (5.7 kDa), bacitracin (1.45 kDa), Gly-Gly-Tyr-Arg (0.45 kDa), and triglycine (0.19 kDa) were obtained from Sigma-Aldrich as analytical standards (HPLC grade). The Waters AccQ-Tag kit and AccQ-Tag ultra eluent A were obtained from Waters Corporation (Massachusetts, USA).

2.2. Fungal solid-state fermentation and flour production

The fermentation process was performed according to the methodology described by Sánchez-García et al. (2023). First, the starter culture was prepared by growing *P. ostreatus* mycelium on a small portion of substrate (10 g) with 65% moisture, incubated at 28 °C for 14 days. The fermentation process was then performed by inoculating a portion of the starter culture into a glass jar containing 35 g of lentil grains (Pardina or Castellana) and quinoa seeds (white or black) at 65% moisture and incubated under the same conditions as the starter culture.

Fermented lentil grains and quinoa seeds were dried by hot air at 70 °C using a convective dryer (Pol-Eko-Aparatura, CLW 750 TOP+, Kokoszycka, Poland) with an air speed of 10.5 ± 0.2 m/s and an air humidity of 8.7 ± 1.2%, during 3.5–4 h to obtain a target product moisture content of 7%. Freeze drying was performed as a reference standard using a freeze dryer (Telstar, Lyoquest-55, Terrassa, Spain) at -45 °C and 0.8 mBar for 48 h. Then, both the unfermented and fermented dried samples were milled using a food processor (Vorwerk, Thermomix® TM6-1, Wuppertal, Germany), applying 10000 rpm at 15-s intervals for 1 min.

2.3. Simulated *in vitro* gastrointestinal digestion

Two *in vitro* digestion models were used to digest unfermented, fermented, and fermented dried samples: the healthy adult model (standard) (Brodkorb et al., 2019;

Minekus et al., 2014) and the older adult model (Menard et al., 2023). The gastrointestinal conditions used in each *in vitro* digestion model are shown in Table 5.29. Enzyme activities were calculated before performing the experiments following the supplementary information in the INFOGEST protocol (Brodkorb et al., 2019). Simulated salivary fluid, gastric fluid, and intestinal fluid were prepared fresh daily considering the enzyme concentration, bile salts, and pH of each digestive stage of the standard and older adult digestion models. *In vitro* digestion was performed as described below:

Oral stage: The sample was mixed with SSF containing the enzyme concentration according to the digestion model (Table 5.29) in a 1:1 ratio (5 g of sample and 5 mL of SSF). The pH was adjusted to 7 with NaOH 1 M and mixed at 25 rpm using an Intelli-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia) and incubated in a thermostated cabin (JP Selecta SA, 3000957, Barcelona, Spain) at 37 °C for 2 min.

Gastric stage: The oral stage sample was mixed with 10 mL of SGF containing the enzyme concentration and the pH adjustment corresponding to each digestion model (Table 5.29) with HCl 1 M. The contents were mixed at 55 rpm and incubated at 37 °C for 2 h.

Intestinal stage: The gastric stage chyme was mixed with 20 mL of SIF containing the concentration of enzymes and bile salts according to each digestion model (Table 5.29). The pH was adjusted to 7 with NaOH 1 M; the contents were mixed at 55 rpm and incubated at 37 °C for 2 h. When gastrointestinal digestion ended, enzyme activity was inhibited by adjusting the pH to 5 with HCl 1M and placing the samples in an ice bath. The samples were then centrifuged at 8000 ×*g* for 10 min and aliquots of the bioaccessible fraction were taken for the different analytical determinations.

Table 5.29. Gastrointestinal conditions established for an *in vitro* digestion model for a healthy adult (standard) (Brodkorb et al., 2019; Minekus et al., 2014) and older adult (Menard et al., 2023).

Digestive stage	Digestion models	
	Healthy adult (standard)	**Older adult**
Oral stage	Amylase (75 U/mL) pH 7 2 min	**Amylase (112.5 U/mL)** pH 7 2 min
Gastric stage	Pepsin (2000 U/mL) pH 3 2 h	**Pepsin (1200 U/mL)** **pH 3.7** 2h
Intestinal stage	Pancreatin (100 U/mL) Bile salts (10 mM) pH 7 2 h	**Pancreatin (80 U/mL)** **Bile salts (7 mM)** pH 7 2 h

Modifications in the older adult model compared to the standard model are highlighted in bold.

2.4. Analytical determinations

Total protein content

The total protein content was assessed using the Kjeldahl method, according to the procedures described in the Association of Official Analytical Chemists methodologies (AOAC, 2000). The results were expressed in g/100 g dry basis.

Trichloroacetic acid (TCA)-soluble protein

The TCA-soluble protein of the samples before and after *in vitro* digestion was determined following the methodology described by Hernández-Olivas et al. (2022) and Gallego et al. (2020). For undigested samples, extraction was performed by mixing 100 mg of sample with a TCA solution to a final concentration of 12% and incubated at 4 °C for 15 min. The samples were then centrifuged at 4200 ×*g* for 10

min. For digested samples, determination was performed on the bioaccessible fraction.

The extract/digest was diluted with 50 mM EDTA and 8M UREA buffer (pH 10), and absorbance was measured by ultraviolet spectrophotometry (Beckman Coulter, DU®730, CA, USA) at 280 nm. A calibration curve was used for quantification using tyrosine as a standard. The results were expressed as g of TCA-soluble protein/100 g protein.

Bioactive peptide determination

Bioactive peptide fractions were determined by size exclusion chromatography (SEC) on samples before and after *in vitro* digestion. A double extraction of the protein was performed by mixing 5 g of sample with 45 mL of distilled water. The pH of the sample was adjusted to 11 and then centrifuged at 10000 ×*g* at 4 °C for 20 min. The supernatants were mixed, and pH was adjusted to the isoelectric point (4.5), maintained with gentle shaking for 1.5 h at 4 °C and centrifuged at 10000 ×*g* at 4 °C for 20 min. The pellet was dissolved in 50 mM phosphate buffer, pH = 7. The extracts were immediately analysed or were stored at -40 °C (Akillioğlu & Karakaya, 2009). The extracts obtained and the bioaccessible fraction of the digested samples were filtered with a 0.2 µm PVDF hydrophilic filter.

The filtered extracts/digests were analysed using a HPLC-Alliance 2695, with a 2996 photodiode array detector (Waters, USA) fitted with an Agilent Bio SEC-3 3 µm, 100A column (7.8 x 300 mm) (Agilent, Palo Alto, CA, USA) according to the methodology described by Zhuang et al. (2009). The mobile phase used was acetonitrile (ACN) and bi-distilled water at a ratio of 1:1 (v/v) with 1 mL/L trifluoroacetic acid. The column operating temperature, flow rate, and injection

volume were 25 °C, 0.5 mL/min, and 20 µL, respectively. Compounds were identified at a wavelength of 220 nm. A molecular weight calibration curve was prepared according to the mean retention times of the following reference standards: cytochrome C (12.5 KDa), insulin (5.7 KDa), bacitracyn (1.4 kDa), Gly-Gly-Tyr-Arg (0.45 KDa), and triglycine (0.19 KDa). The results were expressed as g/100 g protein.

Amino acid profile

The extraction of amino acids from the undigested samples was performed by acid hydrolysis and alkaline hydrolysis according to the methodology described by T. Lee et al. (2022) and Sousa et al. (2023) with some modifications. To perform acid hydrolysis, 0.2 g of sample was mixed with 10 mL of 6 M HCl and heated at 110 °C for 24 h. The extracts were cooled to room temperature and filtered with a 0.2 µm cellulose acetate filter. An aliquot of 200 µL of the acid extract was then evaporated in a vacuum oven (JP Selecta, 0493583, Barcelona, Spain) at 100 °C for 1 h and reconstituted with 40 µL of 20 mM HCl. For alkaline extraction, 0.2 g of sample was mixed with 10 mL of 4.3 M $LiOH{\cdot}H_2O$ in a sealed tube under a nitrogen atmosphere at 120 °C for 16 h. The extracts were cooled to room temperature and filtered with a 0.2 µm cellulose acetate filter. The bioaccessible fraction of digested samples was also filtered with a 0.2 µm cellulose acetate filter. Then an aliquot of the extract/digest was taken and diluted with 40 µL of internal standard (alpha-aminobutyric acid) and bi-distilled water to 1 mL.

To perform the derivatisation process, the Waters AccQ-Tag kit was used. A 10 µL aliquot of the diluted extract/digest containing the internal standard was placed in an Eppendorf tube and 70 µL of AccQ-Flour borate buffer was added and briefly mixed in a vortex. Subsequently, 20 µL of reconstituted AccQ-Flour reagent was added and immediately mixed in a vortex for several seconds. The contents stood for

1 min at room temperature and then transferred to an autosampler vial limited volume insert. The vial was capped and heated in a heating block at 55 °C for 10 min.

Derivatized samples were analysed using a 1200 Series Rapid Resolution HPLC coupled to a Series diode array detector (Agilent, Palo Alto, CA, USA). Amino acid separation was performed using an AccQ-Tag column (3.9 x 150 mm) at 37 °C, with a 1 mL/min flow rate and injection volume of 5 µL. Mobile phase A was Eluent A (90:10, bi-distilled water: AccQ-Tag Eluent A) and mobile phase B was ACN (60:40, ACN: bi-distilled water). The following gradient program was established: 0 min, 100% A; 0.5 min, 98% A; 12 min, 95% A; 15 min, 93% A; 19 min, 90% A; 25 min, 67% A; 33 min, 67% A; 34 min, 0% A; 37 min, 0% A; 38 min, 100 % A; and 42 min, 100% A. Unknown compounds were identified by comparing chromatographic retention times of the samples with reference standards at a wavelength of 250 nm. To quantify the identified compounds, a standard curve was used, and the results were expressed as g/100 g protein.

Angiotensin-converting enzyme inhibitory activity (ACE ia (%))

ACE ia (%) was determined for samples before and after *in vitro* digestion according to the methodology described by Akillioğlu & Karakaya (2009) and Hernández-Olivas et al. (2022). For undigested samples, a double extraction was performed as described in the bioactive peptide determination. For digested samples, determination was performed on the bioaccessible fraction.

The ACE reagent (25 mU/mL) and the Hip-His-Leu substrate (5 mM) were separately dissolved in 0.15 M Tris base buffer containing 0.3 M NaCl, and the pH was adjusted to 8.3. Three controls were included: (i) 100 µL ACE + 40 µL distilled water, (ii) 140 µL distilled water, and (iii) 100 µL distilled water + 40 µL sample extract/digest,

together with the samples (100 µL ACE + 40 µL sample extract/digest), and then incubated at 37 °C for 5 min. An amount of 100 µL of substrate was added to each tube and the incubation process was continued for 30 min at the same temperature. Then 150 µL of 1 M HCl was added to stop the reaction and 1 mL of ethyl acetate was added and vigorously mixed on a vortex mixer. The samples were centrifuged at 1200 ×*g* for 10 min, and 750 µL of the supernatant was taken and placed in clean tubes. The ethyl acetate reagent contained in the supernatant was evaporated by light stirring at 80 °C. The solid hippuric acid remaining in the tubes was dissolved in 1 mL of distilled water and the absorbance was recorded at 228 nm. The ACE ai (%) was calculated according to Equation 5.8:

$$ACE\ ia\ (\%) = 100 - \left\{100 \times \frac{C - D}{A - B}\right\} \qquad (5.8)$$

Where A, B, C, and D are the absorbance of ACE + distilled water, distilled water, ACE + sample extract/digest, and distilled water + sample extract/digest, respectively.

2.5. Statistical analysis

The samples were analysed in triplicate, and the results were reported as mean ± standard deviation. The results obtained were statistically analysed using Statgraphics Centurion-XV software. Analysis of variance (One-way ANOVA) with 95% confidence interval ($p < 0.05$) was performed to determine the effect of processing (SSF and drying) between different samples after gastrointestinal digestion and the effect of the *in vitro* digestion model.

3. Results and discussion

3.1. Proteolysis of unfermented, fermented, and fermented died lentil and quinoa under standard and older adult GI conditions.

The extent of proteolysis in unfermented, fermented, and fermented dried samples was evaluated after gastric and intestinal stages. Thus, TCA-soluble protein, corresponding to small peptides and free amino acids, was measured and expressed as g tyrosine/100g protein. SSF slightly increased the total protein content of the substrates studied. Concretely, an increase of 23.8% to 24.1% and of 26.4% to 26.9%, was found for Pardina and Castellana lentils, respectively, whereas an increase from 13.9% to 16% was given for white quinoa. For black quinoa, a decrease from 14.3% to 12% occurred. The effect of SSF on the nutritional profile of the substrate is detailed and discussed in Sánchez-García et al. (2023b, 2023a).

As observed (Figure 5.17), protein hydrolysis starts in the gastric stage (values from 5 to 30 g tyrosine/100 g protein) and progresses until reaching from 40 to 90 g tyrosine/100 g protein, depending on the sample. Therefore, proteolysis was higher in white and black quinoa, with average proteolysis values of approximately 80 g of TCA-soluble protein/100 g protein, compared to both lentil varieties, which exhibited proteolysis approximately 50 g TCA-soluble protein/100 g protein at the end of GI digestion. According to other studies, cereals, and pseudocereals appear more digestible compared to legumes. Higher levels of proteolysis have been reported for quinoa, varying in the range of 80%–100% (Graf et al., 2015; Hernández-Olivas et al., 2021; Sobota et al., 2020), whereas percentages ranging from 40%–75% have been reported for lentils (Alrosan et al., 2021; Asensio-Grau et al., 2020; Barbana & Boye, 2013; Hernández-Olivas et al., 2021). However, SSF with hot air drying at 70 °C only slightly increased protein hydrolysis compared to unfermented samples. Increases of

up to 20% were observed compared to unfermented digested samples at the intestinal stage. Consistent with the findings reported by Asensio-Grau et al. (2020), these increases in proteolysis could be due to the partial hydrolysis of protein by fungal lytic mechanisms during fermentation.

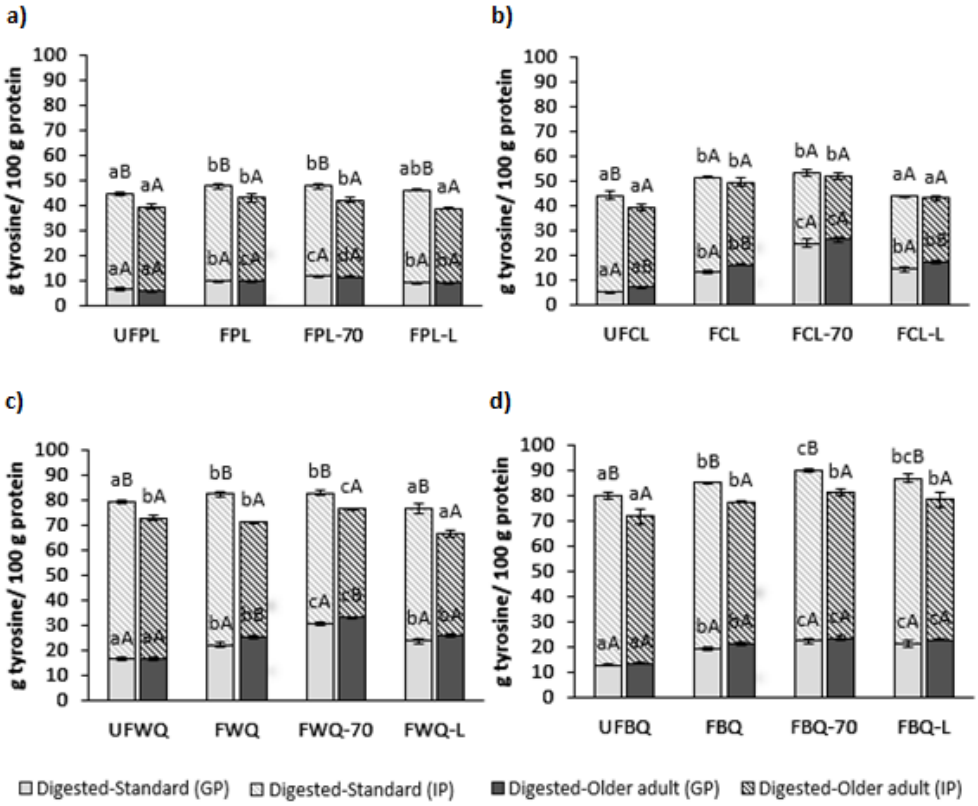

Figure 5.17. Soluble protein fraction in TCA (g tyrosine/100 g protein) in Pardina (**a**) and Castellana (**b**) lentil and white (**c**) and black (**d**) quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flours obtained with a standard or older adult *in vitro* digestion model. [a,b,c] Different lowercase letters indicate significant differences ($p < 0.05$) between samples. [A,B] Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

Regarding the impact of the altered GI conditions that appear with ageing, suboptimal gastric conditions did not result in lower protein digestibility. However, at the end of gastrointestinal digestion, lower values were achieved under older adult conditions, corresponding to approximately 10% less than the standard model for the varieties of Pardina lentils and white and black quinoa. In the case of the Castellana lentil, a 5% decrease is found, although this difference is not statistically significant between the two digestion models. Hernández-Olivas et al. (2021) discovered that lentils and quinoa had much lower proteolysis, simulating gastrointestinal conditions in older adults. However, the authors also simulated more severe conditions similar to those found in this population group, such as longer digestion times and achlorhydria/hyposecretion. This led to a more profound impact on proteolysis, with reductions of up to 50% compared to the 10% reduction observed in this study.

3.2. Peptide molecular mass distribution of unfermented, fermented, and fermented dried lentils and quinoa before and after gastrointestinal digestion.

The SSF process of lentils and quinoa and the subsequent digestion of these samples showed significant changes in percentages of molecular mass distribution of peptides (Figure 5.18). Focusing on the undigested samples, the unfermented samples of the lentil and quinoa varieties mainly comprised larger peptides (>12.5 kDa) accounting for >90% of the total peptidic fraction. SSF decreased the percentage of peptides >12.5 kDa, 65%–85%; however, an increase of 12.5 kDa peptides was observed (15% to 35%). Fermented samples of Castellana lentil (FCL) and white quinoa (FWQ) exhibited the highest proportions of 12.5 kDa peptides, representing a total of 35%. During the fermentation process, the proteolytic activity of the *P.*

ostreatus fungus performed the breakdown of the protein into smaller peptides. Similar results were found in fermented red kidney beans and koji production from defatted soybean meal using other fungi, such as *Rhizopus oligosporus* and *Aspergillus oryzae*, respectively (Sun et al., 2023; Y. Zhao et al., 2018). In kidney beans, a peptide concentration <10 kDa of 157 mg/g was observed after 35 h of fermentation, compared to its unfermented counterpart (20 mg/g). Furthermore, in the koji samples, the smaller peptides (<3 kDa) increased from approximately 60% to >80% when the fermentation reached 40 h, whereas the larger peptides (>10 kDa) decreased significantly ($p < 0.05$).

Subsequently, when the fermented samples were subjected to a hot air drying process at 70 °C, larger peptides (>12.5 kDa) were obtained (80%–90%), whereas 12.5 kDa peptides represented 10% to 20% of the total peptide fraction. Fermented dried samples at 70 °C of Castellana lentil (FCL-70) and white quinoa (FWQ-70) showed a higher percentage of 12.5 kDa peptides (20%) than their other varieties (10%). Similar results were observed during the drying process of fermented samples using freeze drying.

Regarding the digestion process, an increase in the proportion of smaller peptides (<0.19, 0.45, and 1.4 kDa) can be observed for all substrates and their respective treatments (unfermented, fermented and fermented dried) under the standard digestion model, as expected by the efficient action of pancreatic proteases including trypsin and chymotrypsin (Corrigan & Brodkorb, 2020). Digesta of unfermented samples exhibited a higher percentage of peptides ≥12.5 kDa, reaching up to 27% between the two peptide sizes, in contrast to digesta of their fermented and fermented dried counterparts at 70 °C, which exhibited a noticeable decrease in the presence of these larger peptides (≥12.5 kDa). SSF and drying facilitate the

breakdown of proteins into smaller fragments during digestion, resulting in peptides of 1.4 and 0.45 kDa. These peptides ranged from 11% to 28% in fermented samples, compared to a range of 8% to 17% in unfermented samples. These findings suggest that bioprocessed samples exhibit improved protein digestibility during gastrointestinal digestion. Furthermore, digests of fermented and fermented dried samples of white quinoa (FWQ and FWQ-70) and Pardina lentils (FPL and FPL-70) were notable for having a higher proportion of peptides with sizes of 1.4 and 0.45 kDa compared to their unfermented counterparts. On the other hand, the proportion of peptides <0.19 kDa, which represented over 60% of the total peptide fraction, corresponds to amino acids generated during gastrointestinal digestion.

When comparing the digestion models, the digestive conditions of older adults revealed a higher percentage of larger peptides (≥12.5 kDa), which reached 37%. This is in contrast to the standard model that observed a maximum of 30% of larger peptides. However, sometimes a higher percentage of peptide sizes of 1.4 and 0.45 kDa was observed under the older adult digestion model, although this implied a decrease in the proportion of smaller peptides (<0.19 kDa) corresponding to amino acids. Pardina lentil and black quinoa were the substrates that showed lower proportions of peptides <0.19 kDa under the older adult digestion model compared to the standard model. In contrast, lentils and white quinoa exhibited similar or even higher peptide ratios <0.19 kDa than the standard model. The generation of larger peptides after gastrointestinal digestion in the older adult model is attributed to a reduction in enzyme concentration in the gastrointestinal tract and pH variations, factors that could influence the reduction of protein degradation and thus decrease their digestibility.

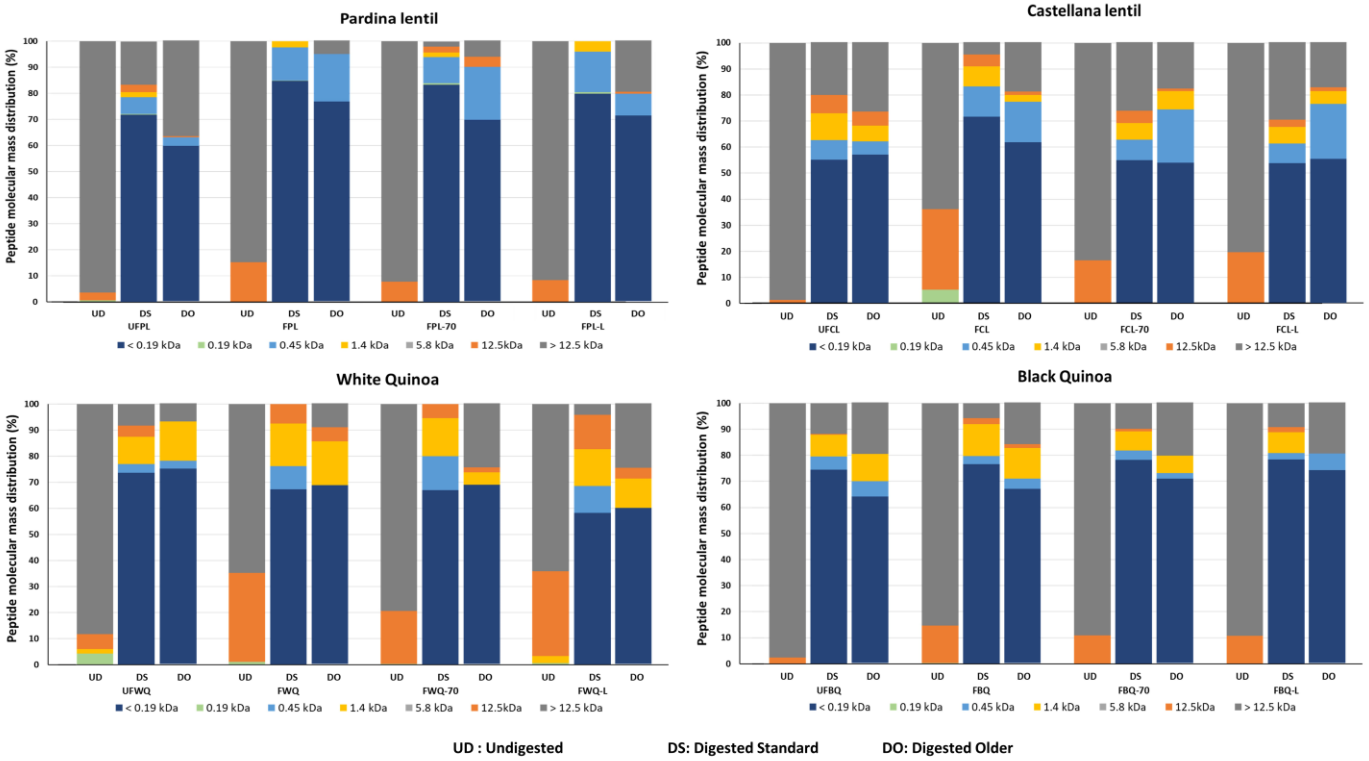

Figure 5.18. Peptide molecular mass distribution (%) in Pardina and Castellana lentil and white and black quinoa for unfermented flour (UFPL, UFCL, UFWQ, and UFBQ), fermented grain/seed (FPL, FCL, FWQ, and FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, and FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, and FBQ-L) flours obtained with a standard or older adult *in vitro* digestion model.

3.3. Amino acid profile of unfermented, fermented, and fermented dried lentils and quinoa before and after gastrointestinal digestion.

High proteolysis alone is not enough to guarantee good health for older adults, as the biological value depends on the post-digestion amino acid profile. Providing the body with a high content of essential amino acids (EAAs) is vitally important in the muscle protein system, counteracting the effect of sarcopenia during ageing in older adults (Volpi et al., 2003; Walston, 2012). Tables 5.30, 5.31, 5.32, and 5.33 show individual amino acid contents (mg amino acids/g dry basis) before and after digestion for Pardina lentil, Castellana lentil, white quinoa, and black quinoa, respectively.

When the amino acid profile of undigested unfermented samples was compared, both lentil varieties were characterised by high Glu content, followed by Asp, Arg, Lys, and Leu, as well as low Met and Cys. In contrast, the amino acid profiles of white and black quinoa were highlighted by a higher abundance of Glu, followed by Asp, Gly, Arg, Lys, and Leu, although in lower amounts than in lentils. However, quinoas are up to four times richer in Met than lentils. According to previous studies, no differences in amino acid concentration were found in lentils (Dhull et al., 2023) and quinoa (Dakhili et al., 2019). High concentrations of Leu and Lys contribute to muscle improvement. Leu has been reported to initiate the rate-limiting translation initiation step of muscle protein synthesis (Crozier et al., 2005). High doses of Lys have been associated with muscle tissue development (Liao et al., 2015). Furthermore, Met is involved in protein synthesis and has been recognised as a limiting amino acid implicated in human malnutrition, so its dietary intake plays a key role (Neubauer & Landecker, 2021).

Table 5.30. Amino acids profile (mg/g dry basis) of undigested and digested Pardina lentil for unfermented flour (UFPL), fermented grains (FPL), fermented dried at 70 °C (FPL-70) and fermented lyophilised (FPL-L) flour.

	Undigested				Digested (Standard)				Digested (Older adult)			
	UFPL	**FPL**	**FPL-70**	**FPL-L**	**UFPL**	**FPL**	**FPL-70**	**FPL-L**	**UFPL**	**FPL**	**FPL-70**	**FPL-L**
Aspartic acid (Asp)	29.97^{bC}	29.33^{abB}	28.20^{abB}	26.00^{aB}	4.60^{aB}	4.76^{aA}	4.97^{aA}	4.67^{aA}	3.57^{aA}	4.82^{dA}	4.20^{cA}	3.80^{bA}
Serine (Ser)	10.47^{aA}	12.13^{aA}	10.77^{aA}	10.33^{aA}	13.82^{aB}	15.64^{aC}	15.54^{aB}	14.40^{aB}	10.93^{aAB}	13.55^{cB}	12.38^{bA}	12.08^{bA}
Glutamic acid (Glu)	42.69^{bB}	40.43^{abB}	38.45^{abB}	35.80^{aB}	13.45^{aA}	16.73^{bA}	17.33^{bA}	14.08^{aA}	11.05^{aA}	15.57^{dA}	14.63^{cA}	12.35^{bA}
Glycine (Gly)	10.75^{aB}	14.78^{bC}	10.69^{aB}	9.64^{aB}	6.19^{aA}	8.33^{cB}	7.59b^{cA}	6.97^{abA}	5.01^{aA}	6.91^{cA}	6.22b^{cA}	5.75^{abA}
Histidine (His)	6.37^{aA}	6.57^{aA}	5.29^{aA}	5.18^{aA}	17.52^{bC}	16.43^{abB}	15.93^{aC}	15.71^{aB}	15.18^{bcB}	15.38^{cB}	13.61^{aB}	14.40^{abB}
Arginine (Arg)	19.21^{bA}	17.69^{aA}	15.32^{aA}	15.10^{aA}	24.74^{aC}	24.59^{aB}	23.77^{aC}	24.13^{aB}	20.84^{abB}	22.64^{cB}	20.25^{aB}	21.85^{bcB}
Threonine (Thr)	7.02^{aB}	6.58^{aA}	6.85^{aAB}	6.23^{aA}	5.19^{aA}	7.04^{bA}	6.86^{bB}	6.29^{abA}	5.28^{aA}	5.65^{bA}	5.09^{aA}	5.94^{cA}
Alanine (Ala)	7.96^{aB}	8.11^{aB}	7.97^{aB}	7.34^{aA}	6.34^{aAB}	7.14^{aA}	7.31^{aAB}	6.82^{aA}	5.45^{aA}	7.08^{cA}	6.39^{bA}	6.15^{bA}
Proline (Pro)	8.44^{aB}	9.02^{aB}	8.22^{aB}	7.62^{aB}	2.00^{aA}	2.98^{cA}	2.86^{cA}	2.46^{bA}	1.76^{aA}	2.73^{dA}	2.49^{cA}	2.20^{bA}
Cystine (Cys)	1.12^{bA}	0.94^{aA}	0.87^{aA}	0.85^{aA}	1.41^{aA}	1.66^{bC}	1.58^{abB}	1.54^{abC}	1.17^{aA}	1.35^{aB}	1.37^{aB}	1.24^{aB}
Tyrosine (Tyr)	4.75^{bA}	4.66^{abA}	4.58^{abA}	4.07^{aA}	14.35^{aC}	15.07^{aC}	14.64^{aC}	14.48^{aC}	11.31^{aB}	13.04^{bC}	11.72^{aB}	12.55^{bB}
Valine (Val)	10.39^{aB}	12.19^{bB}	9.59^{aAB}	8.58^{aA}	8.55^{aA}	10.17^{bA}	10.31^{bB}	9.55^{abA}	7.43^{aA}	8.99^{cA}	8.14^{bA}	8.54^{bcA}
Methionine (Met)	0.73^{aA}	1.01^{cA}	0.80^{bA}	0.69^{aA}	2.13^{aC}	1.88^{aB}	1.76^{aC}	1.69^{aC}	1.58^{cB}	1.30^{aA}	1.36^{abB}	1.39^{bB}
Lysine (Lys)	16.76^{cA}	15.81^{bA}	13.68^{aA}	13.56^{aA}	22.40^{aB}	22.07^{aC}	20.96^{aC}	21.30^{aB}	18.38^{abA}	20.10^{cB}	17.61^{aB}	19.22^{bcB}
Isoleucine (Ile)	8.66^{bB}	9.42^{cAB}	7.63^{abA}	6.72^{aA}	8.35^{aB}	9.61^{bB}	9.49^{bB}	8.93^{abB}	6.83^{aA}	8.71^{cA}	7.98^{bA}	8.02^{bAB}
Leucine (Leu)	16.82^{aC}	18.24^{bC}	16.18^{aB}	15.26^{aA}	14.16^{aB}	14.97^{aB}	14.76^{aAB}	14.76^{aA}	11.43^{aA}	13.73^{cA}	12.63^{bA}	13.50^{bcA}
Phenylalanine (Phe)	11.08^{abB}	12.15^{bB}	10.88^{abB}	10.09^{aB}	3.72^{aA}	3.56^{aA}	3.53^{aA}	3.41^{aA}	2.73^{aA}	3.16^{aA}	2.94^{aA}	3.09^{aA}
Tryptophan (Trp)	2.41^{bA}	2.86^{cA}	2.15^{aA}	1.96^{aA}	20.13^{aC}	21.77^{aC}	21.79^{aC}	21.60^{aB}	16.93^{aB}	20.04^{cB}	18.71^{bB}	19.71^{bcB}
EAA/NEAA ratio	0.59^{aA}	0.62^{aA}	0.58^{aA}	0.58^{aA}	1.18^{aB}	1.11^{aB}	1.10^{aB}	1.15^{aB}	1.21^{aC}	1.11^{aB}	1.11^{aB}	1.20^{aC}
HAA	72.37^{abAB}	78.60^{bA}	68.87^{abA}	63.17^{aA}	81.13^{aB}	88.80^{bB}	88.02^{bB}	85.25^{bB}	66.62^{aA}	80.13^{cA}	73.74^{bA}	76.38^{bcB}
PCAA	42.34^{bA}	40.07^{abA}	34.29^{aA}	33.83^{aA}	64.67^{aC}	63.09^{aC}	60.65^{aC}	61.14^{aB}	54.40^{abB}	58.12^{cB}	51.47^{aB}	55.48^{bcB}
NCAA	72.66^{bB}	69.76^{abB}	66.65^{abB}	61.80^{aB}	18.06^{aA}	21.50^{bA}	22.30^{bA}	18.75^{aA}	14.63^{aA}	20.40^{dA}	18.82^{cA}	16.15^{bA}
AAA	18.24^{bcA}	19.67^{cA}	17.61^{abA}	16.11^{aA}	38.19^{aC}	40.40^{aC}	39.96^{aC}	39.50^{aB}	30.98^{aB}	36.24^{cB}	33.37^{abB}	35.34^{bcB}

The results represent the mean of three repetitions. ^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models. Essential amino acids/Nonessential amino acids ratio: EAA/NEAA ratio; Hydrophobic amino acids (HAA) = Ala, Pro, Cys, Tyr, Val, Met, Ile, Leu, Phe, and Trp; Positively charged amino acids (PCAA) = His, Arg, and Lys; Negatively charged amino acids (NCAA) = Asp and Glu; Aromatic amino acids (AAA) = Tyr, Phe, and Trp.

Table 5.31. Amino acids profile (mg/g dry basis) of undigested and digested Castellana lentil for unfermented flour (UFCL), fermented grains (FCL), fermented dried at 70 °C (FCL-70) and fermented lyophilised (FCL-L) flour.

	Undigested				Digested (Standard)				Digested (Older adult)			
	UFCL	**FCL**	**FCL-70**	**FCL-L**	**UFCL**	**FCL**	**FCL-70**	**FCL-L**	**UFCL**	**FCL**	**FCL-70**	**FCL-L**
Aspartic acid (Asp)	30.48^{abC}	36.70^{bB}	29.47^{aB}	28.41^{aB}	4.39^{aB}	5.58^{aA}	5.35^{aA}	4.78^{aA}	3.53^{aA}	5.39^{bA}	5.58^{bA}	5.02^{bA}
Serine (Ser)	11.01^{abA}	13.88^{bA}	9.80^{aA}	10.83^{abA}	11.10^{aA}	15.61^{bA}	11.93^{aB}	11.56^{aA}	11.64^{aA}	13.56^{aA}	11.55^{aB}	12.10^{aA}
Glutamic acid (Glu)	41.85^{aB}	42.69^{aB}	35.93^{aB}	35.93^{aB}	12.52^{aA}	19.95^{bA}	15.14^{aA}	13.85^{aA}	11.42^{aA}	18.12^{cA}	16.05^{bA}	15.34^{bA}
Glycine (Gly)	11.86^{aC}	19.91^{bC}	13.81^{aB}	13.22^{aB}	3.88^{bB}	4.37^{bB}	2.18^{aA}	1.70^{aA}	0.95^{aA}	1.75^{aA}	1.98^{bA}	2.04^{bA}
Histidine (His)	$7.19b^{cA}$	8.18^{cA}	5.26^{aA}	5.77^{abA}	8.32^{aB}	10.98^{bC}	7.80^{aB}	8.41^{aB}	9.86^{bC}	9.47^{bB}	7.60^{aB}	9.19^{bB}
Arginine (Arg)	18.45^{cA}	21.20^{dA}	14.01^{aA}	15.73^{bA}	18.88^{aA}	24.27^{bA}	18.92^{aB}	18.74^{aA}	22.89^{cB}	21.61^{bcA}	18.42^{aB}	19.28^{abA}
Threonine (Thr)	6.99^{aC}	9.38^{bB}	7.91^{abB}	8.45^{abB}	5.07^{aB}	7.71^{bAB}	5.60^{aA}	5.39^{aA}	4.39^{aA}	6.07^{bA}	5.06^{abA}	5.51^{bA}
Alanine (Ala)	8.39^{aB}	10.95^{bB}	8.83^{abB}	8.40^{aB}	5.86^{aA}	7.76^{bAB}	6.45^{aA}	6.25^{aA}	5.31^{aA}	6.89^{bA}	6.51^{bA}	6.26^{bA}
Proline (Pro)	8.63^{aB}	10.43^{bB}	8.68^{abB}	8.53^{aB}	2.00^{aA}	3.53^{cA}	3.14^{bcA}	2.81^{bA}	2.11^{aA}	3.31^{bA}	3.26^{bA}	3.04^{bA}
Cystine (Cys)	1.17^{aA}	1.75^{bA}	1.10^{aB}	1.05^{aA}	1.78^{bB}	1.86^{bA}	1.38^{aA}	1.31^{aA}	1.23^{aA}	1.59^{bA}	1.28^{aA}	1.27^{aA}
Tyrosine (Tyr)	5.10^{aA}	5.66^{aA}	4.52^{aA}	4.43^{aA}	11.27^{aB}	15.18^{bB}	12.15^{aC}	11.38^{aB}	12.95^{cC}	12.79^{bcB}	11.40^{abB}	10.86^{aB}
Valine (Val)	10.82^{aB}	13.42^{bB}	10.62^{aB}	10.00^{aA}	8.21^{aA}	11.40^{bAB}	8.98^{aA}	8.55^{aA}	8.01^{aA}	9.54^{bA}	8.65^{abA}	8.53^{abA}
Methionine (Met)	0.63^{aA}	1.66^{cB}	0.94^{bA}	0.90^{bA}	2.75^{cC}	1.49^{bB}	1.04^{aA}	1.29^{abA}	1.77^{cB}	1.10^{abA}	0.90^{aA}	1.12^{bA}
Lysine (Lys)	16.99^{bB}	17.04^{bA}	10.90^{aA}	11.94^{aA}	15.03^{bA}	18.55^{cA}	11.91^{aA}	13.74^{abA}	16.54^{bB}	16.57^{bA}	11.76^{aA}	12.99^{aA}
Isoleucine (Ile)	10.04^{aB}	10.14^{aA}	8.97^{aB}	8.63^{aA}	7.68^{aA}	10.73^{bA}	8.55^{aAB}	8.30^{aA}	8.14^{aA}	9.34^{bA}	8.20^{abA}	8.26^{abA}
Leucine (Leu)	17.47^{aC}	19.72^{bA}	15.93^{aB}	15.37^{aA}	13.89^{aA}	16.85^{bA}	13.87^{aA}	13.20^{aA}	15.19^{aB}	14.76^{aA}	13.69^{aA}	14.57^{aA}
Phenylalanine (Phe)	11.38^{aC}	14.26^{bA}	10.75^{aB}	10.50^{aA}	8.55^{aA}	12.55^{bA}	9.71^{aA}	9.90^{aA}	10.77^{aB}	10.84^{aA}	9.76^{aA}	10.45^{aA}
Tryptophan (Trp)	2.43^{aA}	3.09^{bA}	1.94^{aA}	2.36^{aA}	3.82^{cC}	3.47^{bcA}	3.00^{abB}	2.83^{aA}	3.14^{aB}	2.75^{aA}	2.80^{aB}	2.71^{aA}
EAA/NEAA ratio	0.61^{aA}	0.59^{aA}	0.58^{aA}	0.58^{aA}	1.02^{aB}	0.96^{abB}	0.92^{aB}	0.99^{aB}	1.08^{aB}	0.95^{aB}	0.90^{aB}	0.98^{aB}
HAA	76.05^{aB}	91.08^{bA}	72.29^{aB}	70.17^{aA}	65.81^{aA}	84.82^{cA}	68.26^{bAB}	65.83^{aA}	68.61^{aA}	72.91^{bA}	66.47^{aA}	67.07^{aA}
PCAA	42.63^{bA}	46.43^{bA}	30.16^{aA}	33.44^{aA}	42.24^{aA}	53.79^{bA}	38.62^{aB}	40.89^{aA}	49.30^{cB}	47.64^{bcA}	37.78^{aB}	41.46^{abA}
NCAA	72.33^{abC}	79.39^{bB}	65.40^{aB}	64.34^{aB}	16.91^{aB}	25.54^{cA}	20.48^{bA}	18.63^{abA}	14.95^{aA}	23.51^{cA}	21.63^{bcA}	20.35^{bA}
AAA	18.91^{aA}	23.01^{bA}	17.22^{aA}	17.28^{aA}	23.65^{aB}	31.20^{bB}	24.86^{aC}	24.11^{aB}	26.86^{aC}	26.38^{aAB}	23.97^{aB}	24.01^{aB}

The results represent the mean of three repetitions. ^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models. Essential amino acids/Nonessential amino acids ratio: EAA/NEAA ratio; Hydrophobic amino acids (HAA) = Ala, Pro, Cys, Tyr, Val, Met, Ile, Leu, Phe, and Trp; Positively charged amino acids (PCAA) = His, Arg, and Lys; Negatively charged amino acids (NCAA) = Asp and Glu; Aromatic amino acids (AAA) = Tyr, Phe, and Trp.

Table 5.32. Amino acids profile (mg/g dry basis) of undigested and digested white quinoa for unfermented flour (UFWQ), fermented seeds (FWQ), fermented dried at 70 °C (FWQ-70) and fermented lyophilised (FWQ-L) flour.

	Undigested				Digested (Standard)				Digested (Older adult)			
	UFWQ	**FWQ**	**FWQ-70**	**FWQ-L**	**UFWQ**	**FWQ**	**FWQ-70**	**FWQ-L**	**UFWQ**	**FWQ**	**FWQ-70**	**FWQ-L**
Aspartic acid (Asp)	14.28^{aB}	23.47^{bC}	18.08^{abC}	16.55^{abC}	4.03^{abA}	4.83^{bcB}	5.25^{cB}	3.56^{aB}	3.59^{bA}	3.51^{bA}	4.03^{bA}	2.37^{aA}
Serine (Ser)	6.52^{aA}	10.98^{bA}	7.14^{aA}	6.89^{aA}	10.05^{aB}	11.34^{aA}	11.19^{aB}	9.54^{aB}	8.55^{abAB}	8.62^{bA}	8.83^{bAB}	7.19^{aA}
Glutamic acid (Glu)	24.31^{abC}	32.55^{bC}	20.47^{aC}	19.80^{aC}	11.57^{aB}	12.12^{aB}	11.72^{aB}	10.72^{aB}	8.84^{bA}	9.00^{bA}	7.74^{abA}	6.41^{aA}
Glycine (Gly)	11.99^{abB}	16.03^{bB}	11.44^{aB}	10.53^{aB}	3.32^{aA}	4.54^{aA}	3.92^{aA}	3.37^{aA}	2.24^{aA}	2.92^{bcA}	3.43^{cA}	2.69^{abA}
Histidine (His)	3.60^{aA}	4.75^{bA}	2.66^{aA}	2.89^{aA}	8.54^{bC}	8.38^{bC}	7.88^{bC}	7.07^{aC}	7.22^{cB}	6.59^{bcB}	6.29^{abB}	5.60^{aB}
Arginine (Arg)	13.43^{abA}	16.88^{bA}	10.19^{aA}	9.89^{aA}	19.83^{bB}	19.16^{bB}	18.75^{bC}	16.81^{aC}	19.06^{cB}	16.63^{bA}	15.38^{abB}	14.65^{aB}
Threonine (Thr)	4.90^{aB}	8.86^{bB}	6.33^{abB}	6.15^{abB}	4.50^{aAB}	5.49^{bA}	5.57^{bAB}	4.36^{aAB}	3.83^{aA}	4.16^{aA}	4.10^{aA}	3.66^{aA}
Alanine (Ala)	6.23^{aB}	12.12^{bC}	8.06^{aC}	7.68^{aB}	5.33^{aAB}	7.13^{bB}	7.10^{bB}	5.86^{aAB}	4.62^{aA}	5.50^{bA}	5.61^{bA}	4.38^{aA}
Proline (Pro)	5.89^{aB}	7.90^{aB}	6.30^{aB}	5.73^{aB}	1.71^{aA}	2.32^{bA}	2.60^{bA}	1.64^{aA}	1.46^{abA}	1.67^{bcA}	1.88^{cA}	1.23^{aA}
Cystine (Cys)	1.81^{bB}	1.38^{abAB}	1.19^{aAB}	1.12^{aA}	1.45^{bB}	1.56^{bB}	1.46^{bB}	1.21^{aA}	0.93^{aA}	1.07^{bA}	1.00a^{bA}	0.99^{aA}
Tyrosine (Tyr)	3.56^{aA}	2.49^{aA}	3.25^{aA}	3.06^{aA}	9.21^{aB}	9.18^{aC}	9.25^{aB}	8.38^{aB}	8.94^{bB}	7.56^{aB}	8.10a^{bB}	8.04^{aB}
Valine (Val)	6.02^{aA}	10.63^{bC}	8.27^{abB}	7.29^{abB}	7.21^{aA}	8.52^{bB}	8.55^{bB}	6.91^{aB}	6.13^{bA}	6.51^{bcA}	6.63^{cA}	5.16^{aA}
Methionine (Met)	2.95^{aA}	2.18^{aB}	2.83^{aB}	2.61^{aB}	2.51^{cA}	1.03^{aA}	1.29^{bA}	1.17^{abA}	2.21^{bA}	0.61^{aA}	1.06^{aA}	1.02^{aA}
Lysine (Lys)	9.13^{abA}	11.84^{bA}	7.31^{aA}	7.42^{aA}	12.82^{aB}	12.60^{aA}	11.38^{aB}	11.15^{aC}	11.87^{bB}	10.23^{aA}	9.02^{aA}	8.70^{aB}
Isoleucine (Ile)	4.75^{aA}	8.14^{bB}	6.96^{abAB}	5.95^{abB}	6.55^{aC}	7.62^{bB}	7.82^{bB}	6.39^{aB}	5.83^{bB}	5.84^{bA}	6.04^{bA}	4.84^{aA}
Leucine (Leu)	9.76^{aA}	15.61^{bC}	12.02^{abA}	11.24^{abB}	12.52^{aC}	13.16^{aB}	13.48^{aA}	12.33^{aB}	10.65^{abB}	10.40^{abA}	10.91^{bA}	9.67^{aA}
Phenylalanine (Phe)	6.11^{aA}	9.33^{bB}	7.16^{abA}	6.68^{aA}	8.01^{aB}	8.19^{aAB}	8.71^{aA}	7.83^{aA}	7.46^{aB}	7.16^{aA}	7.48^{aA}	7.09^{aA}
Tryptophan (Trp)	1.91^{abA}	2.03^{bB}	1.73^{aA}	1.76^{aA}	3.69^{cC}	2.61^{aC}	3.06^{bC}	2.58^{aC}	3.11^{dB}	1.58^{aA}	2.47^{cB}	2.11^{bB}
EAA/NEAA ratio	0.56^{aA}	0.59^{aA}	0.64^{aA}	0.64^{aA}	1.00^{aB}	0.94^{aB}	0.95^{aB}	0.98^{aB}	1.00^{aB}	0.94^{aB}	0.96^{aB}	1.00^{aB}
HAA	48.99^{aA}	71.82^{cC}	57.76^{bB}	53.12^{bB}	58.20^{abB}	61.32^{bB}	63.32^{bB}	54.31^{aB}	51.35^{cA}	47.90^{bA}	51.18^{cA}	44.53^{aA}
PCAA	26.16^{bA}	33.47^{cA}	20.17^{aA}	20.20^{aA}	41.19^{bB}	40.13^{bB}	38.01^{abC}	35.03^{aC}	38.16^{bB}	33.46^{bA}	30.69^{abB}	28.96^{aB}
NCAA	38.59^{aC}	56.01^{bC}	38.55^{aC}	36.35^{aC}	15.59^{abB}	16.95^{bB}	16.97^{bB}	14.28^{aB}	12.43^{bA}	12.51^{bA}	11.77^{bA}	8.78^{aA}
AAA	11.59^{aA}	13.86^{aA}	12.13^{aA}	11.51^{aA}	20.91^{aB}	19.98^{aC}	21.02^{aC}	18.79^{aB}	19.50^{cB}	16.31^{aB}	18.05^{bB}	17.23^{abB}

The results represent the mean of three repetitions. ^{a,b,c,d} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models. Essential amino acids/Nonessential amino acids ratio: EAA/NEAA ratio; Hydrophobic amino acids (HAA) = Ala, Pro, Cys, Tyr, Val, Met, Ile, Leu, Phe, and Trp; Positively charged amino acids (PCAA) = His, Arg, and Lys; Negatively charged amino acids (NCAA) = Asp and Glu; Aromatic amino acids (AAA) = Tyr, Phe, and Trp.

Table 5.33. Amino acids profile (mg/g dry basis) of undigested and digested black quinoa for unfermented flour (UFBQ), fermented seeds (FBQ), fermented dried at 70 °C (FBQ-70) and fermented lyophilised (FBQ-L) flour.

	Undigested				Digested (Standard)				Digested (Older adult)			
	UFBQ	**FBQ**	**FBQ-70**	**FBQ-L**	**UFBQ**	**FBQ**	**FBQ-70**	**FBQ-L**	**UFBQ**	**FBQ**	**FBQ-70**	**FBQ-L**
Aspartic acid (Asp)	12.66^{aC}	12.77^{aB}	13.52^{aB}	12.72^{aB}	4.01^{aB}	3.78^{aA}	3.86^{aA}	3.90^{aA}	3.18^{aA}	3.31^{bA}	3.78^{bA}	3.56^{abA}
Serine (Ser)	5.52^{aA}	7.06^{aA}	5.80^{aA}	5.85^{aA}	10.68^{bC}	10.00^{aB}	10.38^{abC}	10.15^{aB}	8.47^{aB}	8.92^{abAB}	9.22^{bB}	9.48^{bB}
Glutamic acid (Glu)	19.84^{cC}	15.47^{aC}	16.37^{bB}	16.37^{bC}	11.98^{bB}	10.26^{aB}	9.00^{aA}	9.85^{aB}	9.45^{bA}	8.77^{aA}	8.86^{aA}	9.06^{abA}
Glycine (Gly)	10.20^{abB}	11.30^{bB}	9.75^{abB}	9.48^{aB}	5.50^{aA}	4.84^{aA}	4.77^{aA}	5.41^{aA}	5.64^{bA}	4.73^{aA}	4.29^{aA}	4.85^{aA}
Histidine (His)	3.12^{aA}	3.48^{aA}	2.72^{aA}	2.83^{aA}	6.81^{aB}	5.95^{aB}	5.93^{aB}	6.65^{aB}	7.05^{bB}	5.64^{aB}	5.64^{aB}	5.64^{aB}
Arginine (Arg)	10.81^{bA}	8.64^{aA}	8.25^{aA}	8.42^{aA}	18.58^{bC}	15.75^{aC}	15.61^{aC}	15.65^{aB}	16.26^{bB}	13.92^{aB}	13.72^{aB}	14.52^{aB}
Threonine (Thr)	4.15^{aB}	4.29^{aC}	4.51^{aB}	4.56^{aB}	4.19^{cB}	3.32^{bB}	3.65^{bcA}	2.44^{aA}	2.02^{aA}	2.22^{aA}	3.49^{bA}	3.17^{bA}
Alanine (Ala)	5.33^{aB}	5.88^{aA}	5.63^{aAB}	5.61^{aA}	5.53^{aB}	5.53^{aA}	5.91^{aB}	6.00^{aA}	4.74^{aA}	5.20^{abA}	5.40^{bA}	5.73^{bA}
Proline (Pro)	5.24^{bB}	4.85^{abB}	4.81^{abB}	4.66^{aB}	2.20^{bA}	1.93^{aA}	2.24^{bA}	2.10^{abA}	2.09^{bA}	1.83^{aA}	2.13^{bA}	2.10^{bA}
Cystine (Cys)	1.04^{bA}	0.83^{abA}	0.74^{aA}	0.82^{abA}	1.61^{aB}	1.44^{aC}	1.48^{aC}	1.44^{aB}	1.29^{abAB}	1.16^{aB}	1.27^{abB}	1.40^{bB}
Tyrosine (Tyr)	2.94^{aA}	2.81^{aA}	2.52^{aA}	2.64^{aA}	10.64^{bC}	8.94^{aC}	9.21^{aC}	9.44^{aB}	8.95^{cB}	7.29^{aB}	7.85^{abB}	8.69^{bcB}
Valine (Val)	5.84^{aA}	7.35^{cAB}	6.54^{bA}	6.21^{abA}	7.97^{aC}	7.58^{aB}	8.13^{aC}	7.96^{aB}	6.74^{aB}	6.86^{aA}	7.22^{abB}	7.58^{Bb}
Methionine (Met)	2.36^{bA}	1.25^{aC}	1.44^{aB}	2.16^{bC}	2.58^{cA}	0.98^{aB}	1.33^{bAB}	1.31^{bB}	2.38^{cA}	0.45^{aA}	1.06^{bA}	1.00^{bA}
Lysine (Lys)	8.00^{cA}	6.95^{bA}	6.03^{aA}	6.29^{aA}	13.46^{bB}	11.81^{aC}	11.48^{aC}	11.58^{aB}	12.35^{bB}	10.43^{aB}	9.94^{aB}	10.68^{aB}
Isoleucine (Ile)	4.82^{aA}	5.61^{bA}	5.44^{bA}	5.05^{aA}	7.28^{aC}	6.73^{aC}	7.23^{aC}	6.96^{aB}	6.29^{aB}	6.00^{aB}	6.29^{aB}	6.56^{aB}
Leucine (Leu)	8.74^{aA}	9.48^{bA}	9.45^{bA}	9.32^{abA}	12.97^{bC}	11.18^{aB}	11.63^{abB}	11.46^{aB}	11.14^{bB}	9.64^{aA}	10.80^{bB}	11.51^{bB}
Phenylalanine (Phe)	5.24^{aA}	5.97^{aA}	5.62^{aA}	5.49^{aA}	8.26^{bC}	6.90^{aA}	7.27^{aB}	6.98^{aB}	7.03^{bB}	6.08^{aA}	6.62^{bB}	7.07^{bB}
Tryptophan (Trp)	1.23^{aA}	1.53^{bA}	1.29^{aA}	1.18^{aA}	3.83^{cC}	2.43^{aC}	2.98^{bC}	3.08^{bB}	3.33^{cB}	1.73^{aB}	2.63^{bB}	2.67^{bB}
EAA/NEAA ratio	0.59^{aA}	0.66^{aA}	0.64^{aA}	0.65^{aA}	0.95^{aB}	0.91^{aB}	0.95^{aB}	0.91^{aB}	0.97^{aC}	0.89^{aB}	0.95^{aB}	0.94^{aB}
HAA	42.77^{aA}	45.56^{aA}	43.48^{aA}	43.13^{aA}	62.87^{bC}	53.65^{aB}	57.40^{abC}	56.73^{aB}	53.98^{bB}	46.25^{aA}	51.27^{bB}	54.31^{bB}
PCAA	21.93^{cA}	19.07^{bA}	17.00^{aA}	17.54^{abA}	38.86^{bC}	33.51^{aC}	33.02^{aC}	33.87^{aB}	35.65^{bB}	29.99^{aB}	29.30^{aB}	30.84^{aB}
NCAA	32.50^{bC}	28.24^{aB}	29.88^{abB}	29.09^{aB}	15.99^{bB}	14.04^{aA}	12.85^{aA}	13.75^{aA}	12.63^{aA}	12.08^{aA}	12.64^{aA}	12.62^{aA}
AAA	9.41^{aA}	10.31^{aA}	9.43^{aA}	9.31^{aA}	22.72^{bC}	18.27^{aC}	19.45^{aC}	19.51^{aB}	19.31^{cB}	15.09^{aB}	17.09^{bB}	18.43^{bcB}

The results represent the mean of three repetitions. ^{a,b,c} Different lowercase letters indicate significant differences ($p < 0.05$) between flours. ^{A,B,C} Different capital letters indicate significant differences ($p < 0.05$) between digestion models. Essential amino acids/Nonessential amino acids ratio: EAA/NEAA ratio; Hydrophobic amino acids (HAA) = Ala, Pro, Cys, Tyr, Val, Met, Ile, Leu, Phe, and Trp; Positively charged amino acids (PCAA) = His, Arg, and Lys; Negatively charged amino acids (NCAA) = Asp and Glu; Aromatic amino acids (AAA) = Tyr, Phe, and Trp.

Fermentation with *P. ostreatus* significantly modified the amino acid profile. The impact of fungal fermentation on the amino acid profile demonstrates a more notable effect on Castellana lentils and white quinoa than on Pardina lentils and black quinoa. Castellana lentils and white quinoa experienced an increase in most amino acids up to twice their initial content, consequently enhancing their biological value. However, the Pardina lentil and black quinoa increased amino acids, such as Val, Ile, Leu, and Trp. These results in all substrates would favour the three branched-chain amino acids (Val, Leu, and Ile) necessary for muscle protein synthesis in older people. Furthermore, an increase in Gly could improve metabolic regulation, antioxidant reactions, and neurological function (W. Wang et al., 2013). Although the effect of *P. ostreatus* varies depending on the substrate. Bolaniran et al. (2019) and Dairo et al. (2017) also observed an increase in most of the same amino acids with the same inoculum to ferment air potato bulbils and rice husk. The amino acids Gly, Arg, and Phe increased the most, and these authors also observed a significant increase in two of the branched-chain amino acids (Leu and Val) after fermentation. Regarding the ratio of essential amino acids and nonessential amino acids ratio (EAA/NEAA ratio), both lentil and quinoa varieties showed a ratio of approximately 0.60, therefore, there was no effect of SSF or drying at 70 °C. Both lentil and quinoa varieties have a higher content of hydrophobic amino acids (HAA) and negatively charged amino acids (NCAA), with lentils higher than the quinoas; however, significant changes were observed after fermentation. HAA increased in the four fermented substrates, whereas NCAA increased in Castellana lentils and fermented white quinoa but decreased in Pardina lentils and black quinoa. Both groups of amino acids have been related to antioxidant activities. In the case of HAA, authors have demonstrated a high correlation between the concentration of hydrophobic amino acids and the level of $DPPH^{\bullet}$ scavenging activity (Pownall et al., 2010). Furthermore,

the NCAA could donate its excess electrons during free radical reaction, resulting in strong antioxidant properties (Onuh et al., 2014).

Regarding the effect of drying of the fermented samples, hot air drying at 70 °C decreases the amino acid profile content in the two varieties of lentils and quinoa by up to 40%. These decreases may be manifested by the Maillard reaction that can occur at 70 °C. This reaction is triggered by the presence of reducing sugars that bind to EAAs, causing the loss of the latter (Belitz et al., 2009a; Chumroenphat et al., 2021). Although the type of amino acids and reducing sugars affect the quantity and quality of Maillard products, Lys is particularly affected by this reaction (Yokoyama et al., 2021). In this study, Lys is also significantly affected, reducing its initial concentration in all fermented and dried substrates at 70 °C. However, despite the reduction of amino acids, they mostly never drop below the levels of unfermented samples. Furthermore, this decrease due to the Maillard reaction can generate other compounds (Amadori compounds, considered aroma precursors) which are currently receiving great interest due to the health-promoting properties attributed to them for their high antioxidant capacity and their antimicrobial and cytotoxic properties (Golon et al., 2014). In lentils and quinoa, after fermentation and drying, volatile compounds increased significantly (Sánchez-García et al., 2024). Similar results were found after lyophilisation in all samples. A decrease in amino acid content was also found when the samples were subjected to the lyophilisation process; the reduction was even greater than that observed when drying at 70 °C. Similar results were found by Chumroenphat et al. (2021) using turmeric samples dried by freeze drying (FD), hot air (HD) at 50 °C, and sun dried (SD) at 35–45 °C, decreasing the amino acid content in the following order: SD > FD > HD.

The amino acid profile under standard and older adult GI conditions is also shown in Tables 5.30 to 5.33. The Pardina lentil was the only substrate that exhibited an EAA/NEAA ratio greater than 1 in all samples (unfermented, fermented and fermented dried), whereas the Castellana lentil, white quinoa, and black quinoa showed values close to 1. A higher ratio of EAA/NEAA results in a greater bioaccessibility of essential amino acids. SSF and drying at 70 °C increased the content of HAA and NCAA in the Pardina and Castellana lentil samples, as well as white quinoa, compared to their unfermented digested counterparts. This finding was not observed in black quinoa. These increases were more favourable in the two lentil varieties, with contents >70 mg/g dry basis in HAA and >20 mg/g dry basis in NCAA. Whereas white quinoa showed contents >60 mg/g dry basis in HAA and >17 mg/g dry basis in NCAA. These two groups of amino acids (HAA and NCAA) are associated with high antioxidant capacities. Ketnawa et al. (2018) showed that high amounts of HAA in the bioaccessible fraction may enhance interactions with lipids in foods and enhance antioxidant entry into target organs through hydrophobic interactions with membrane lipid bilayers. Focusing on individual amino acids, fermented samples of Castellana lentils and white quinoa showed an enhanced content of amino acids such as Phe, Ala, and Thr in the bioaccessible fraction, which is more significant in Castellana lentils than white quinoa. This increase is beneficial because these compounds play a key role in the preservation of cognitive function in older adults (Kinoshita et al., 2021). However, drying at 70 °C negatively affects these amino acids by decreasing their content in Castellana lentils, although it does not appear to affect white quinoa in the same way. Furthermore, SSF and drying at 70 °C increases the content of branched-chain amino acids (Val, Leu, and Ile) in bioaccessible fractions of Pardina and Castellana lentils, as well as in white quinoa,

compared to their digested unfermented analogues. The release of these amino acids contributes to protein synthesis and muscle regeneration in adults.

Finally, the digestive conditions of the older adult affected protein digestibility, reducing ≤20% in HAA and NCAA in both the lentil and quinoa varieties. Furthermore, by analysing the complete amino acid profile, significant differences between the digestion models can be observed on all substrates, except in the fermented and fermented dried samples of lentils (FCL, FCL-70, and FCL-L). These results are consistent with those obtained for proteolysis, where no significant differences were observed on these samples (Figure 5.17).

3.4. Angiotensin I-converting enzyme (ACE) inhibition of unfermented, fermented and fermented died lentil and quinoa under standard and older adult GI conditions.

The ACE plays a critical role in controlling blood pressure regulation. It mainly catalyses the conversion of angiotensin I to angiotensin II. Therefore, angiotensin II has a dual role in increasing blood pressure; it acts as a vasoconstrictor and degrades a vasodilator known as bradykinin, thus contributing to increased blood pressure (Los et al., 2018; Rui et al., 2012). ACE inhibitory activity (%) was evaluated in unfermented, fermented, and fermented dried samples before and after gastrointestinal digestion (Figure 5.19). In undigested samples, both SSF and hot air drying significantly increased the ACE inhibitory capacity of the two lentil and quinoa varieties. These increases ranged from 6% (unfermented samples) to 18% (fermented and fermented dried samples). The generation of bioactive peptides during fermentation could be responsible for the observed antihypertensive activity. These results have been discussed previously (Sánchez-García et al., 2023b, 2023a).

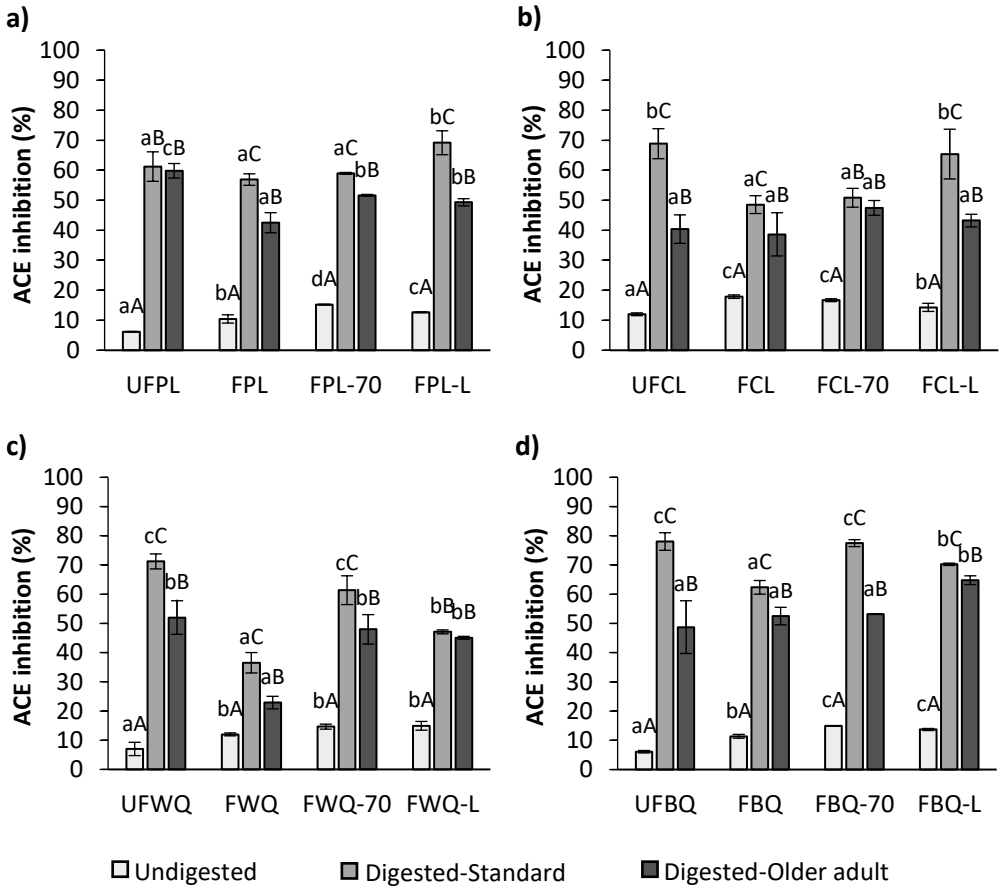

Figure 5.19. ACE inhibitory activity (%) in Pardina (**a**) and Castellana (**b**) lentil and white (**c**) and black (**d**) quinoa for unfermented flour (UFPL, UFCL, UFWQ, UFBQ), fermented grain/seed (FPL, FCL, FWQ, FBQ), fermented dried at 70 °C (FPL-70, FCL-70, FWQ-70, FBQ-70) and fermented lyophilised (FPL-L, FCL-L, FWQ-L, FBQ-L) flours obtained with a standard or older adult *in vitro* digestion model. [a,b,c,d] Different lowercase letters indicate significant differences ($p < 0.05$) between samples. [A,B] Different capital letters indicate significant differences ($p < 0.05$) between digestion models.

RESULTADOS Y DISCUSIÓN

Simulated *in vitro* gastrointestinal digestion significantly ($p < 0.05$) increased the percentage of ACE inhibition in the bioaccessible fraction regardless of the sample and digestion model. All unfermented, fermented, and fermented dried samples achieved high ACE inhibition after gastrointestinal digestion between 30% and 80%, compared to undigested samples that exhibited ACE inhibition percentages between 6% and 18%. Despite increases in ACE inhibitory activity in undigested fermented and fermented dried samples (≤18%), they did not show higher ACE inhibitory activity after gastrointestinal digestion than unfermented samples. SSF decreases the ACE inhibitory activity between 4% and 35% in both lentil and quinoa varieties. This reduction was more remarkable in Castellana lentil and white quinoa, with ACE inhibitory activities of 48% and 36%, respectively, in contrast to their unfermented counterparts, which displayed an ACE inhibitory activity of approximately 70%. To the best of the authors' knowledge, no information is available on the evaluation of ACE inhibitory activity after *in vitro* gastrointestinal digestion of fermented products with *P. ostreatus*. However, Vermeirssen et al. (2003) found results with the same trends in pea protein isolate fermented with *Lactobacillus helveticus* and *Saccharomyces cerevisiae*. Fermentation increased the ACE inhibitory activity from 17% (unfermented pea) to 40% (fermented pea). Then, both samples reached levels between 90% and 100% after gastrointestinal digestion, with lower ACE inhibitory activity in fermented pea. During fermentation, enzymes from microorganisms initially hydrolyse the dietary protein into smaller peptides; then these peptides are further degraded during gastrointestinal digestion. Therefore, hydrolysis above the optimal degree could degrade more ACE inhibitory peptides than could be formed, reducing the overall ACE inhibitory activity (Vermeirssen et al., 2003). In contrast, hot air drying increased the ACE inhibitory activity of the fermented samples to levels comparable to those of the digested unfermented samples (60%–80%). The only

exception was the fermented dried Castellana lentil (70 °C), which exhibited a significantly lower percentage than its unfermented counterpart. These increases could be explained by the formation of Maillard reaction products resulting from the heat treatment applied to stabilise the samples after SSF. Maillard reaction products, such as melanoidins generated during roasting of coffee, have been reported to increase *in vitro* ACE inhibitory activity. It was also indicated that as heat treatment intensifies, the ACE inhibitory activity increases (Rufián-Henares & Morales, 2007).

When comparing digestion models, the simulated conditions of the older adult digestion model significantly reduced the ACE inhibitory activity in the bioaccessible fraction of the samples compared to the standard model. These decreases ranged from 10% to 30%, depending on the type of sample. The enzymatic action of pepsin and pancreatin during gastrointestinal digestion, together with the suboptimal pH during the gastric stage, could influence the release of peptides, reducing the ACE inhibitory capacity. Hernández-Olivas et al. (2022) reported that altered gastric digestion conditions (pepsin concentration 1500 U/mL and pH 6) resulted in a drastic reduction in ACE inhibitory activity in four different types of meat (chicken, turkey, pork, and beef). Furthermore, they indicated that altered conditions of intestinal digestion (pancreatin 50 U/mL, bile salts 5 mM and 4 h of digestion) significantly reduced this parameter in beef.

4. Conclusions

SSF and drying at 70 °C significantly increased proteolysis in both lentil and quinoa varieties, reaching an increase of up to 20% at the end of gastrointestinal digestion. Furthermore, SSF increases the percentage of small peptides (12.5 kDa), reaching up to 35%, due to the proteolytic activity of the fungus *P. ostreatus*. After gastrointestinal digestion, SSF with drying (70 °C) promotes the increase in bioactive

peptides of 1.4 and 0.45 kDa between 11% and 28%, as well as a high percentage of peptides <0.19 kDa, which correspond to amino acids, reaching 60%. The amino acid profile was enriched in both lentil and quinoa varieties due to SSF, favouring the increase in branched-chain amino acids (Val, Leu, and Lle) essential for muscle protein synthesis in older adults. However, hot air drying at 70 °C negatively affected amino acid content, reducing it by up to 40%, although it did not decrease below the initial content of their unfermented counterparts. However, SSF and hot air drying at 70 °C increased the content of amino acid groups HAA and NCAA after gastrointestinal digestion. It was more favourable in Castellana and Pardina lentils, with increases of up to 16% in HAA and 36% in NCAA. Fermentation decreased ACE inhibitory activity by up to 35% compared to the digested unfermented samples. However, hot air drying at 70 °C of fermented samples increased the ACE inhibitory activity by up to 60%–80%, due to melanoidins generated during drying. Finally, older adult gastrointestinal conditions slightly compromised vegetal protein digestibility in all samples, with a 10% reduction in proteolysis and up to 20% in certain amino acid groups (HAA and NCAA). Furthermore, a higher percentage of larger peptides (≥12.5 kDa) and a 30% reduction in ACE inhibitory activity were obtained compared to the digesta under standard digestion conditions.

Fermented Castellana and Pardina lentil flours would be the most suitable options for developing products intended for the older adult population, aiming to enhance protein digestibility due to their amino acid content. Additionally, it is imperative to assess the techno-functional properties of fermented flours to ascertain their compatibility with diverse food applications. Furthermore, conducting scale-up tests of the fermentation process is crucial to facilitate the technological transfer of this process to the food industry.

CONCLUSIONES DEL CAPÍTULO II

- La FES y el secado a 70 °C aumentaron significativamente el contenido fenólico total tras la digestión GI en lenteja Pardina y Castellana, incrementando hasta 7.5 y 10.5 mg ácido gálico/g base seca, respectivamente, mientras que en las quinoas inclusive se presentaron disminuciones.
- La FES y el secado a 70 °C aumentaron el contenido de ácido vanílico y cafeico en la lenteja Castellana, y el ácido vanílico en la lenteja Pardina, hasta tres veces más el contenido de sus homólogos sin fermentar digeridos. La quinoa blanca incrementó hasta 7 veces más el contenido de ácido gálico.
- La fracción bioaccesible de las harinas de lenteja Castellana y quinoa blanca fermentadas-secas a 70 °C presentaron el índice de actividad antioxidante (APCI) más alto, 99% y 88%, respectivamente.
- La FES y el secado a 70 °C mejora la bioaccesibilidad mineral de todas las muestras en comparación con sus análogos sin fermentar. La lenteja Castellana se destacó con incrementos de hasta el 16% para el Mg, el 33% para el Ca, y el 329% para el Fe.
- La reducción de la liberación del ácido fítico después de la digestión GI, resultado de un menor contenido de este antinutriente en las muestras durante la FES, contribuyen a una mayor bioaccesibilidad de minerales.
- La FES y el secado a 70 °C después de la digestión gastrointestinal favorece el aumento de péptidos bioactivos de 1.4 y 0.45 kDa entre el 11% y el 28%, así como un alto porcentaje de péptidos <0.19 kDa, que corresponden a aminoácidos, alcanzando el 60%.
- La FES y el secado a 70 °C incrementó la proteólisis hasta en un 20% con respecto a las muestras digeridas sin fermentar al final de la digestión GI.

- La FES aumenta el porcentaje de péptidos más pequeños (12.5 kDa), alcanzando hasta un 35%, debido a la actividad proteolítica del hongo *P. ostreatus*.
- La FES incrementa el contenido del perfil de aminoácidos en lenteja Castellana y quinoa blanca, hasta 2 veces el contenido de las muestras sin fermentar. En lenteja Pardina y quinoa negra incrementaron solo en Val, Leu, Ile y Trp.
- La FES favorece el incremento de aminoácidos de cadena ramificada (Val, Leu e Ile) necesarios para la síntesis de proteínas musculares en los adultos mayores.
- El secado a 70 °C disminuye el contenido de aminoácidos hasta en un 40% debido a la unión aminoácido-azúcar reductor, pero no descienden por debajo del contenido inicial de sus homólogos sin fermentar.
- La FES y el secado a 70 °C aumentan el contenido de aminoácidos hidrofóbicos (HAA) y aminoácidos cargados negativamente (NCAA) en la fracción bioaccesible. Estos incrementos fueron más favorables en las dos variedades de lenteja, con incrementos de hasta el 16% en HAA y el 36% en NCAA con respecto a sus contrapartes sin fermentar digeridos.
- La FES disminuye la actividad inhibitoria de la ECA hasta 35 puntos porcentuales en comparación con las muestras sin fermentar, debido a una mayor hidrólisis de la proteína durante la FES y luego durante la digestión gastrointestinal.
- La actividad inhibidora de la ECA aumentó en las muestras fermentadas-secas a 70 °C hasta valores comparables con sus homólogos sin fermentar digeridos (60–80%), debido a las melanoidinas generadas durante el secado.
- Las condiciones digestivas comunes que aparecen con el envejecimiento no parecieron comprometer la bioaccesibilidad mineral de Fe, Mg y Ca de las harinas fermentadas, pero sí el perfil fenólico y la actividad antioxidante de la digesta, en comparación con el modelo estándar.

- El modelo de digestión del adulto mayor afectó la digestibilidad de las proteínas, reduciendo la proteólisis en un 10% y hasta un 20% en los grupos de aminoácidos (HAA y NCAA). Además, la actividad inhibidora de la ECA también se redujo en un 30% en comparación con el modelo de digestión estándar.
- Las harinas fermentadas de lenteja Castellana y quinoa blanca serían la opción más adecuada para el desarrollo de productos que mejoren la bioaccesibilidad de las propiedades antioxidantes, mientras que las lentejas Castellana y Pardina podrían mejorar la digestibilidad de las proteínas.

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6. PERSPECTIVAS A FUTURO

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Los resultados obtenidos en esta tesis doctoral demuestran que la fermentación en estado sólido (FES) de lenteja y quinoa con el hongo *P. ostreatus* y su posterior secado por aire caliente a 70 °C, permiten obtener harinas con perfil nutricional y funcional mejorados, concretamente con cambios notables tanto en la fracción de fibra como de proteína total, así como en la digestibilidad de ésta última. De igual forma, cabe destacar el aumento que la FES produce en la bioaccesibilidad de minerales como el hierro, calcio y magnesio. Asimismo, es importante señalar que las harinas fermentadas también presentaron una digestibilidad mayor cuando se simularon las típicas alteraciones gastrointestinales atribuibles al adulto mayor.

Partiendo de esta información, y dado que la harina quinoa blanca fermentada presentó un notable aumento en el contenido de fibra dietética durante la FES, sería de interés evaluar la capacidad prebiótica de estas harinas a través de estudios de fermentación colónica. Los modelos actuales ya sean estático o dinámicos, permiten evaluar el impacto del consumo de estas harinas tanto en población sana como en condiciones disbióticas del adulto mayor.

Por otro lado, y con objeto de considerar la inclusión de estas harinas en la formulación de alimentos ricos en proteína vegetal, sería necesario abordar el análisis de las propiedades tecnofuncionales para identificar en qué tipos de alimentos podrían ser incorporadas con mayor éxito. La nueva información generada, conjuntamente con los aromas diferenciados de las harinas fermentadas, podrían ayudar a diversificar el tipo de alimentos a los cuales incorporar harinas de legumbres y pseudocereales.

La creación de nuevos productos dirigidos tanto a la población en general como a grupos específicos que puedan estar en riesgo de deficiencia proteica, como es el caso de los adultos mayores, se podría abordar a través de herramientas de co-creación con la participación de todos los agentes de la cadena de valor y en particular con los potenciales consumidores teniendo en cuenta sus intereses, expectativas, preferencias y necesidades alimentarias.

Finalmente, realizar pruebas de escalado del proceso de fermentación en estado sólido sería una propuesta interesante, con la finalidad de llevar a cabo una transferencia tecnológica de este proceso a la industria alimentaria, lo que podría llevar eventualmente a la introducción en el mercado de un nuevo producto a base de legumbres o pseudocereales con propiedades mejoradas.