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APLICACIÓN DE MALTODEXTRINA RESISTENTE EN ZUMO DE NARANJA PASTEURIZADO: ANÁLISIS DE PROPIEDADES FISICOQUÍMICAS, COMPUESTOS BIOACTIVOS, DIGESTIÓN IN VITRO E IMPACTO SENSORIAL

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PhD IN FOOD SCIENCE, TECHNOLOGY AND MANAGEMENT

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APPLICATION OF RESISTANT MALTODEXTRIN IN PASTEURIZED ORANGE JUICE: ANALYSIS OF PHYSICOCHEMICAL PROPERTIES, BIOACTIVE COMPOUNDS, IN VITRO DIGESTION AND SENSORY IMPACT

Tesis presentada para optar al Grado de Doctor por Elías Arilla Codoñer

Thesis submitted for the Degree of Doctor by Elías Arilla Codoñer

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CERTIFY

That the PhD thesis entitled ¨Application of resistant maltodextrin in pasteurized orange juice: analysis of physicochemical properties, bioactive compounds, *in vitro* digestion, and sensory impact¨ has been developed by Elías Arilla Codoñer under our supervision at the Food Technology Deparment at the Universitat Politècnica de València, to obtain the PhD degree in Food Science, Technology and Management at the Universitat Politècnica de València.

Valencia, April 2024,

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ABSTRACT

Adding prebiotics to orange juice emerge as a natural way to innovate and stimulate the fruit juice market. Resistant maltodextrin (RMD) has proved to exert health benefits in clinical trials. Most scientific background on prebiotics focus on their health effects in the human body, but studying how prebiotics impact the food in which it is added is barely addressed. This work aims to explore the different outcomes of RMD addition to orange juice before pasteurization. For this reason, different studies were conducted. All the research was performed using orange juice with pulp (2.5%) and without pulp, and with increasing RMD concentrations: 0, 2.5, 5 and 7.5%.

First, a study addressing the impact of RMD addition in the physicochemical properties of pasteurized orange juice was performed. Due to its good solubility in water, RMD influenced key properties such as ºBrix, acidity, density, turbidity, rheology, and color, being the changes more noticeable with higher RMD concentrations. This work proved the feasibility of adding RMD in a wide range of concentrations to orange juice.

Then, research focusing on RMD addition before pasteurization process to orange juice and its effect on the bioactive compounds and their *in vitro* bioaccesibility was carried out. RMD showed a protective effect of all bioactive compounds from thermal degradation, namely phenols, carotenoids, ascorbic acid, and vitamin C (ascorbic acid + dehydroascorbic acid), with higher RMD levels offering greater protection. Pulp-added samples presented higher values of all bioactive compounds than pulp-free samples, so its incorporation improved the antioxidant capacity of orange juice. Also, RMD improved phenols and vitamin C bioaccesibility while decreased it for carotenoids and ascorbic acid. Orange pulp presence increased ascorbic acid and vitamin C bioaccesibility but decreased it for phenols and carotenoids. As a result, RMD addition and orange pulp incorporation slightly decreased the bioaccesible antioxidant capacity of orange juice. Despite the bioaccesibility decrease, in absolute terms, the total amount of bioactive compounds that remained available to be absorbed were higher in pulp-added and RMD-added samples. This work demonstrated that, besides its prebiotic effect, RMD could have interesting applications in the food technology field leading to health-related benefits.

Next, a sensory evaluation with expert panelists and the measurement of main physicochemical properties along with the analysis of the aromatic profile of samples was performed. RMD addition improved almost all sensory attributes, leading to higher overall rating scores than RMD-free samples. The physicochemical impact of adding

RMD to orange juice followed the same trend as it was previously studied. Moreover, RMD addition to orange juice did not significantly alter its aromatic profile, whereas orange pulp presence played a much more decisive role by increasing 1-terpinen-4-ol, octanal, nonanal, decanal and α-pinene, and decreasing limonene and β-myrcene. Therefore, RMD could be added to orange juice to upgrade its organoleptic acceptability.

In the final work, the stability of the bioactive compounds and the antioxidant capacity of pasteurized orange juice with RMD along storage time was assessed. Determinations were performed at 0, 15, 45, 75, 105 and 170 days of storage. At the beginning, RMD showed a protective effect in all bioactive compounds, especially phenols and carotenoids. Despite orange pulp could be added to naturally increase the bioactive compounds in foodstuff, in this case pulp-free samples presented a greater antioxidant capacity than pulp-added samples. However, the evolution over time showed that the protective effect of RMD was more pronounced in the pulp-added samples than in the pulp-free samples, suggesting an interaction between RMD and pulp. This study enlightens the potential use of RMD to better preserve the health-related compound of thermally treated foodstuff.

This studies collectively demonstrate RMD’s role in boosting the health-related benefits of orange juice and its organoleptic acceptability. By exploring the interactions between RMD, orange juice and orange pulp, this work shows a promising pathway for innovating in the fruit juice market. The strategic addition of prebiotics like RMD in the food technology sector could potentially set a new standard for developing functional beverages.

RESUMEN

Añadir prebióticos al zumo de naranja surge como una forma natural de innovar y estimular el mercado de los zumos de frutas. Se ha demostrado que la maltodextrina resistente (RMD) ejerce beneficios para la salud en ensayos clínicos. La mayoría de los antecedentes científicos sobre los prebióticos se centran en sus efectos sobre la salud en el cuerpo humano, pero apenas se aborda el estudio de cómo los prebióticos impactan en los alimentos en los que se agregan. Este trabajo tiene como objetivo explorar los diferentes resultados de la adición de RMD al zumo de naranja antes de la pasteurización. Por este motivo se realizaron diferentes estudios. Toda la investigación se realizó utilizando zumo de naranja con pulpa (2,5%) y sin pulpa, y con concentraciones crecientes de RMD: 0, 2,5, 5 y 7,5%.

Primero, se realizó un estudio que abordó el impacto de la adición de RMD en las propiedades fisicoquímicas del zumo de naranja pasteurizado. Debido a su buena solubilidad en agua, el RMD influyó en propiedades clave como ºBrix, acidez, densidad, turbidez, reología y color, siendo los cambios más notorios con concentraciones más altas de RMD. Este trabajo demostró la viabilidad de agregar RMD en una amplia gama de concentraciones al zumo de naranja.

Luego, se llevó a cabo una investigación centrada en la adición de RMD antes del proceso de pasteurización al zumo de naranja y su efecto sobre los compuestos bioactivos y su bioaccesibilidad *in vitro*. La RMD mostró un efecto protector de todos los compuestos bioactivos frente a la degradación térmica, a saber, fenoles, carotenoides, ácido ascórbico y vitamina C (ácido ascórbico + ácido dehidroascórbico), y los niveles más altos de RMD ofreciendo una mayor protección. Las muestras con pulpa añadida presentaron valores más altos de todos los compuestos bioactivos que las muestras sin pulpa, por lo que su incorporación mejoró la capacidad antioxidante del zumo de naranja. Además, la RMD mejoró la bioaccesibilidad de los fenoles y la vitamina C, mientras que la disminuyó para los carotenoides y el ácido ascórbico. La presencia de pulpa de naranja aumentó la bioaccesibilidad del ácido ascórbico y la vitamina C, pero la disminuyó para los fenoles y carotenoides. Como resultado, la adición de RMD y la incorporación de pulpa de naranja disminuyeron ligeramente la capacidad antioxidante bioaccesible del zumo de naranja. A pesar de la disminución de la bioaccesibilidad, en términos absolutos, la cantidad total de compuestos bioactivos que permanecieron disponibles para ser absorbidos fue mayor en las muestras a las que se les añadió pulpa y RMD. Este trabajo demostró que, además de su efecto prebiótico, la RMD podría tener aplicaciones interesantes en el campo de la tecnología alimentaria, lo que conduciría a beneficios relacionados con la salud.

A continuación, se realizó una evaluación sensorial con panelistas expertos y la medición de las principales propiedades fisicoquímicas junto con el análisis del perfil aromático de las muestras. La adición de RMD mejoró casi todos los atributos sensoriales, lo que dio lugar a puntuaciones de calificación general más altas que las muestras sin RMD. El impacto fisicoquímico de añadir RMD al zumo de naranja siguió la misma tendencia estudiada anteriormente. Además, la adición de RMD al zumo de naranja no alteró significativamente su perfil aromático, mientras que la presencia de pulpa de naranja jugó un papel mucho más decisivo al aumentar el 1-terpinen-4-ol, el octanal, el nonanal, el decanal y el α-pineno, y disminuir el limoneno y el β-mirceno. Por lo tanto, se podría agregar RMD al zumo de naranja para mejorar su aceptabilidad organoléptica.

En el trabajo final se evaluó la estabilidad de los compuestos bioactivos y la capacidad antioxidante del zumo de naranja pasteurizado con RMD a lo largo del tiempo de almacenamiento. Las determinaciones se realizaron en los días 0, 15, 45, 75, 105 y 170 de almacenamiento. Al inicio, la RMD mostró un efecto protector en todos los compuestos bioactivos, especialmente fenoles y carotenoides. A pesar de que se podría añadir pulpa de naranja para aumentar de forma natural los compuestos bioactivos de los alimentos, en este caso las muestras sin pulpa presentaron una mayor capacidad antioxidante que las muestras con pulpa añadida. Sin embargo, la evolución a lo largo del tiempo mostró que el efecto protector de la RMD fue más pronunciado en las muestras con pulpa añadida que en las muestras sin pulpa, lo que sugiere una interacción entre el RMD y la pulpa. Este estudio ilustra el uso potencial de la RMD para preservar mejor los compuestos saludables de los alimentos tratados térmicamente.

Estos estudios demuestran colectivamente el papel de la RMD en el aumento de los beneficios saludables del zumo de naranja y su aceptabilidad organoléptica. Al explorar las interacciones entre RMD, zumo de naranja y pulpa de naranja, este trabajo muestra un camino prometedor para innovar en el mercado de zumos de frutas. La incorporación estratégica de prebióticos como la RMD en el sector de la tecnología alimentaria podría establecer un nuevo estándar para el desarrollo de bebidas funcionales.

RESUM

Afegir prebiòtics al suc de taronja sorgeix com una forma natural d'innovar i estimular el mercat dels sucs de fruites. S'ha demostrat que la maltodextrina resistent (RMD) exerceix beneficis per a la salut en assajos clínics. La majoria dels antecedents científics sobre els prebiòtics se centren en els seus efectes sobre la salut en el cos humà, però a penes s'aborda l'estudi de com els prebiòtics impacten en els aliments en els quals s'agreguen. Este treball té com a objectiu explorar els diferents resultats de l'addició de RMD al suc de taronja abans de la pasteurització. Per este motiu es van realitzar diferents estudis. Tota la investigació es va realitzar utilitzant suc de taronja amb polpa (2,5%) i sense polpa, i amb concentracions creixents de RMD: 0, 2,5, 5 i 7,5%.

Primer, es va realitzar un estudi que va abordar l'impacte de l'addició de RMD en les propietats fisicoquímiques del suc de taronja pasteuritzat. A causa de la seua bona solubilitat en aigua, el RMD va influir en propietats clau com ºBrix, acidesa, densitat, terbolesa, reologia i color, sent els canvis més notoris amb concentracions més altes de RMD. Este treball va demostrar la viabilitat d'agregar RMD en una àmplia gamma de concentracions al suc de taronja.

Després, es va dur a terme una investigació centrada en l'addició de RMD abans del procés de pasteurització al suc de taronja i el seu efecte sobre els compostos bioactius i el seu bioaccessibilitat *in vitro*. La RMD va mostrar un efecte protector de tots els compostos bioactius enfront de la degradació tèrmica, a saber, fenols, carotenoides, àcid ascòrbic i vitamina C (àcid ascòrbic + àcid dehidroascòrbic), i els nivells més alts de RMD oferint una major protecció. Les mostres amb polpa afegida van presentar valors més alts de tots els compostos bioactius que les mostres sense polpa, per la qual cosa la seua incorporació va millorar la capacitat antioxidant del suc de taronja. A més, la RMD va millorar la bioaccessibilitat dels fenols i la vitamina C, mentres que la va disminuir per als carotenoides i l'àcid ascòrbic. La presència de polpa de taronja va augmentar la bioaccessibilitat de l'àcid ascòrbic i la vitamina C, però la va disminuir per als fenols i carotenoides. Com a resultat, l'addició de RMD i la incorporació de polpa de taronja van disminuir lleugerament la capacitat antioxidant bioaccessible del suc de taronja. Malgrat la disminució de la bioaccessibilitat, en termes absoluts, la quantitat total de compostos bioactius que van romandre disponibles per a ser absorbits va ser major en les mostres a les quals se'ls va afegir polpa i RMD. Este treball va demostrar que, a més del seu efecte prebiòtic, la RMD podria tindre aplicacions interessants en el camp de la tecnologia alimentària, la qual cosa conduiria a beneficis relacionats amb la salut.

A continuació, es va realitzar una avaluació sensorial amb panelistes experts i el mesurament de les principals propietats fisicoquímiques juntament amb l'anàlisi del perfil aromàtic de les mostres. L'addició de RMD va millorar quasi tots els atributs sensorials, la qual cosa va donar lloc a puntuacions de qualificació general més altes que les mostres sense RMD. L'impacte fisicoquímic d'afegir RMD al suc de taronja va seguir la mateixa tendència estudiada anteriorment. A més, l'addició de RMD al suc de taronja no va alterar significativament el seu perfil aromàtic, mentres que la presència de polpa de taronja va jugar un paper molt més decisiu en augmentar el 1-terpinen-4-ol, el octanal, el nonanal, el decanal i el α-pinè, i disminuir el limonè i el β-mircè. Per tant, es podria agregar RMD al suc de taronja per a millorar la seua acceptabilitat organolèptica.

En el treball final es va avaluar l'estabilitat dels compostos bioactius i la capacitat antioxidant del suc de taronja pasteuritzat amb RMD al llarg del temps d'emmagatzematge. Les determinacions es van realitzar en els dies 0, 15, 45, 75, 105 i 170 d'emmagatzematge. A l'inici, la RMD va mostrar un efecte protector en tots els compostos bioactius, especialment fenols i carotenoides. A pesar que es podria afegir polpa de taronja per a augmentar de manera natural els compostos bioactius dels aliments, en este cas les mostres sense polpa van presentar una major capacitat antioxidant que les mostres amb polpa afegida. No obstant això, l'evolució al llarg del temps va mostrar que l'efecte protector de la RMD va ser més pronunciat en les mostres amb polpa afegida que en les mostres sense polpa, la qual cosa suggereix una interacció entre el RMD i la polpa. Este estudi il·lustra l'ús potencial de la RMD per a preservar millor els compostos saludables dels aliments tractats tèrmicament.

Estos estudis demostren col·lectivament el paper de la RMD en l'augment dels beneficis saludables del suc de taronja i la seua acceptabilitat organolèptica. En explorar les interaccions entre la RMD, el suc de taronja i la polpa de taronja, este treball mostra un camí prometedor per a innovar en el mercat de sucs de fruites. La incorporació estratègica de prebiòtics com la RMD en el sector de la tecnologia alimentària podria establir un nou estàndard per al desenvolupament de begudes funcionals.

CHAPTER 1. INTRODUCTION AND WORK JUSTIFICATION

1.1. FRUIT JUICE: FROM DEFINITION TO PROCESSING

Fruit juice definition

Fruit juice is defined as the ¨unfermented but fermentable liquid obtained from edible parts of sound appropriately mature and fresh fruit, or from fruit maintained in sound condition by suitable means. This includes postharvest surface treatments applied in accordance with the Codex General Standards (FAO, 2005). In a general sense, fruit juice can be defined as an extract, or an extracted fluid content of cells or tissues, made by mechanically squeezing or pressing out the natural liquid contained in ripe fruits without using any heat or solvent (Rajauria y Tiwari, 2018). This liquid must remain unfermented and retain the essential qualities of the fruit. Fruit juices are recognized for their natural sweetness, vitamins, and minerals, making them a popular choice in diets worldwide (Caswell, 2009). The definition encompasses a variety of fruits, with each juice offering unique nutritional benefits and sensory attributes.

Fruit juices and certain similar products intended for human consumption are subjected to specific Community rules under Council Directive 2001/112/EC regarding the composition, reserved names, manufacturing, and labelling characteristics. The terms used to describe fruit juices and related products are defined as follows (Mihalev *et al*., 2018):

- Fruit juice, obtained by mechanical processes from fresh fruit, as previously described.
- Concentrated fruit juice, obtained by the physical removal of a specific proportion of water content, usually by evaporation.
- Fruit juice from concentrate, made by reconstituting concentrated fruit juice with water. The original juice concentrate is obtained by removing water from the fresh juice through a concentration process, usually by evaporation.
- Water extracted fruit juice, obtained by diffusion with water of pulpy whole fruit such as acai berry where the juice cannot be extracted by mechanical processes only, or from dehydrated whole fruit such as dried plum.
- Dehydrated/powdered fruit juice, obtained by the physical removal of virtually all the water content, using drying techniques as spray drying.

The use of juice concentrates makes industrial sense. Juice concentration is accomplished to serve three primary purposes: firstly, to reduce water activity of the juice product which lengthens its shelf life; secondly, to minimize packaging, storage, and transport costs; and thirdly, to stabilize or simplify the handling on the final juice product (Adnan *et al*., 2018). However, concentrating fruit juice alters its natural nutritional and sensory characteristics, diminishing resemblance to the original product even after dilution (Dasenaki & Thomaidis, 2019). For this reason, from a consumer's perspective, it is preferable to consume direct fruit juices that retains the natural characteristics of the fruit to a greater extent.

Fruit juice nutritional importance

Fruit juices are important in the human diet for their nutritional richness and for hydration. Accordingly, they are a right fit for consumers of all age groups and are highly accepted because of their appealing and diverse sensory profiles, ranging from textures, flavors, aromas, and colors (Nonglait *et al*., 2022). However, there is a growing belief that fruit juices, due to their high content of natural occurring sugars, mainly fructose, may have the same adverse health effects than sugar-sweetened beverages (Ruxton & Myers, 2021). Up to date, it remains unclear whether they lead to the same metabolic consequences if consumed in equal doses (Pepin *et al*., 2019). Moreover, this comparison does not seem to be fair because it excludes health-related compounds naturally present in fruit juices. For example, fruit juices are an important bioavailable source of polyphenols and other bioactive compounds, which have been linked with health benefits (Ruxton & Myers, 2021; Miles & Calder, 2021). In addition, juices made from citrus fruits are particularly high in several compounds with antioxidants effects such as vitamin C, flavonoids, carotenoids, essential oil (terpenes and limonoids) and more (Saini *et al*., 2022). For this reason, fruit juices are a valuable component of dietary global patterns. Therefore, a moderate consumption of fruit juices within a balanced diet could be considered as desirable for their potential health effects.

1.2. OVERVIEW OF THE FRUIT JUICES SPANISH MARKET

Global view of the fruit juice market

The fruit juice market encompasses a wide range of products that can be storage in ambient, chilled, and even frozen conditions, only including fruit/vegetable 100% juice content, whether single flavor products or mixtures of different flavors. This market is measured both in terms of retail sales value and volume, and it excludes products like syrups and nectars, which contain less fruit/vegetable juice content and are usually included in the soft drinks segment. The global juice market, including Europe, is experiencing growth, mainly due to consumer preferences for healthier lifestyles (Priyadarshini & Priyadarshini, 2018). As of 2023, the global juice market is valued at $116 billion and is projected to experience an annual growth rate of 3.65% (CAGR 2023-2027) (Statista, n.d.a). When compared globally, the United States takes the lead in generating the highest revenue, reaching $26.1 billion. In Spain, the revenue generated in the juices market in 2023 reached €1,317 million and is expected to grow annually by 1.79% from 2023 to 2027 (Statista, n.d.b).

Deeping into the Spanish fruit juice market

Despite the positive expectations for the fruit juice market, it is important to take a closer look to deeply understand the lights and shadows of this segment. For example, Alimarket reported a continued decrease in the consumption of ambient juices, mainly due to the transfer towards high added value categories (Castillo, 2019). From 2017 to 2018, a decline of 3.6% in volume and 2.1% in value was reported. In relation to volume, all categories decreased except fortified juices. In terms of value, only the fortified, the fruit and vegetable mix juices and the chilled managed to improve (Castillo, 2019). Similar conclusions were drawn by Kantar World Panel from its analysis of the domestic market for 2018, with a decrease in the ambient category of 7.7% in volume and 5.7% in value, which drags down the entire sector (-6.8% in liters and -3.6% in €), without the advance of chilled products being able to prevent it (Castillo, 2019). Table 1 shows the top-10 fruit juice companies in Spain in last years (2021 and 2022).

Table 1. Top-10 fruit juice companies in Spain in years 2021 and 2022, according to their produced volume and sales. Source: Castillo, 2019.

Company	% Own Brands	Volume, 2021 (million liters)	Volume, 2022 (million liters)	Sales, 2021 (millions of euros)	Sales, 2022 (millions of euros)
AMC Natural Drinks Group	4%	570	800	550	628
Grupo García Carrión	63%	403,1	399,8	500	574,8
Refresco Iberia, S.A.U.	0%	110	195	40	50
Juver Alimentación, S.L.	63%	180	185	118,9	120,6
Agrozumos, S.A.	0%	130	130	56,3	66,4
Nufri Grupo	0%	140	115	80	80
Quirante Fruits, S.L.	0%	90	90	74,6	75
Grupo Indulleida	0%	100	80	60	60
Agricultura y Conservas, S.A.	0%	60	70	40,5	44
Miguel Parra e Hijos, S.A.	0%	70	70	21	22

AMC Natural Drinks Group and Grupo García Carrión are the leading companies in the fruit juice market in Spain in both volume and revenue. It is important to consider that these companies mainly market their own brands: Via Nature in the case of AMC Natural Drinks Group and Don Simón in the case of Grupo García Carrión. This is very relevant since these businesses rely mostly in the brand value (InfoRETAIL, 2021), and therefore market with higher prices their beverages. That explains its high sales figures. However, the private label business is of great importance for the Spanish market (Sgroi & Salamone, 2022). Several reasons explain the private label phenomenon. For example, Spain has experienced economic fluctuations and periods of austerity (crisis of 2008 and more recently during the COVID-19 pandemic), that have made Spanish consumers more price-sensitive (McKinsey & Company, 2022). Also, over the years, there has been a significant improvement in the quality of the private label products. Accordingly, Spanish consumers have increasingly perceived these products as equal or superior to branded ones, which is not always the case in other markets where private label items might still be seen primarily as low-cost alternatives (McKinsey & Company, 2020; Discount Retail Consulting, 2021). And finally, retailers have tailored their private label offerings to be efficiently aligned with local consumer preferences, while global own brand often take a more one-size-fits-all approach.

Refresco as a beverage and fruit juice company

Refresco Iberia, which is currently the third biggest company in terms of volume in the Spanish juice market (Table 1), does not own any brand. From a global point of view, Refresco is a leading global beverage company, renowned for its extensive capabilities in the development, production, and distribution of a wide range of non-alcoholic beverages. Refresco's history as a company began in 1999 when Menken Beverages, the predecessor of Refresco, was founded with a management buy-out of Menken Drinks and Refrescos de Sur Europa S.A. from a major Dutch dairy group (Refresco, n.d.). The company officially launched on March 30, 2000, when Menken Beverages acquired Krings Fruchtsaft in Germany. Since then, Refresco has pursued aggressive growth through the implementation of a buy and build strategy. Starting from its inception until 2016, the organization expanded by acquiring several companies and facilities throughout Europe. In 2018, Refresco made its largest acquisition by taking over Cott's bottling operation, which significantly transformed it into the world's largest independent bottler for retailers and leading brands (GlobeNewswire, 2018). More recently, in 2022, Refresco ventured into the Australian market by acquiring Tru Blu Beverages, establishing a third geographical platform for further growth (GlobeNewswire, 2022a).

As a multinational entity, Refresco operates with a significant presence across the world, showcasing its diverse and expansive reach in the beverage industry. They combine retail brand production and contract manufacturing for premium brand beverage companies into an efficient multi-tenant production platform that benefits from scale advantages and additional services. Their production platforms cover more than 70 manufacturing plants in Europe, the United States, Canada, Mexico, and Australia (Figure 1) (Refresco, n.d.). This extensive network allows the company to serve both local and international markets efficiently, making it a key partner for many leading retail and beverage brands around the world.

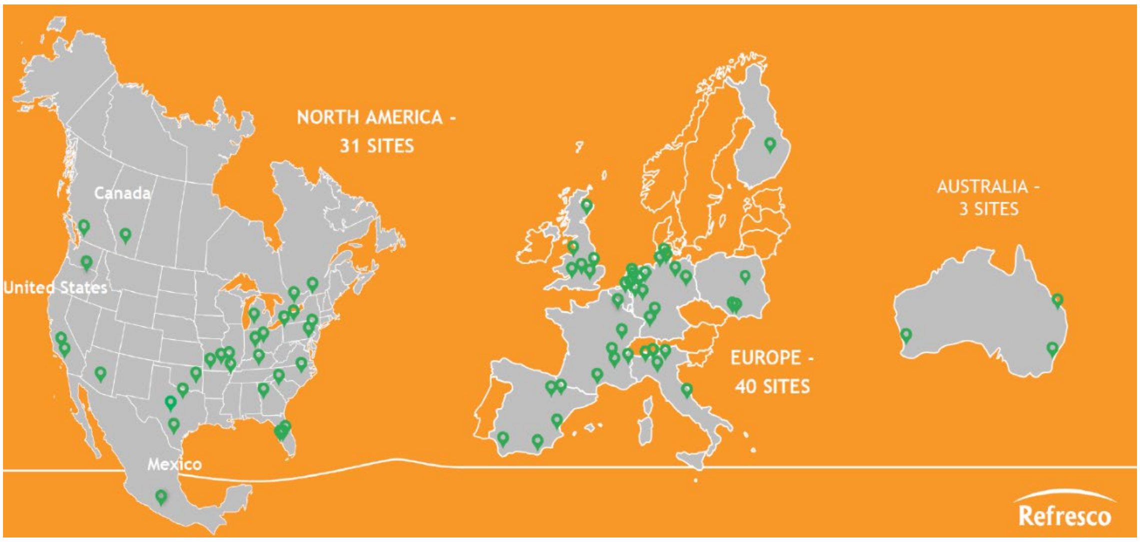

Figure 1. Geographic representation of Refresco's production platforms around the world. Source: Refresco, n.d.

Orange juice market

The fruit juice market is characterized by its wide array of flavors. However, although the range of flavors continues to expand, the orange juice is still the most consumed fruit juice worldwide due to its widespread availability, appealing taste, and perceived health benefits (Priyadarshini & Priyadarshini, 2018; Castillo, 2019; Neves *et al*., 2020; IMARC, 2023; Statista, n.d.c). These factors combined make orange juice a staple in many diets globally.

In 2023, the worldwide revenue in the orange juice market amounts to $30.6 billion and is projected to grow annually by 2.19% (CAGR 2023-2027) (Statista, n.d.c). In Spain, the orange juice market generated a revenue of €411.6 million and is expected to grow by 2.29% (CAGR 2023-2027) (Statista, n.d.d). As a counterpoint to these data, a decline in the consumption of orange juice in developed countries and increase in consumption in emerging economies has been reported over the last years (Neves *et al.*, 2020). Also, the shift from juices from concentrate to not-from-concentrate (NFC) juices was highlighted, indicating a preference for freshness and quality (Neves *et al*., 2020). These insights underline the evolving nature of the orange juice market, driven by health-conscious consumer choices and market innovations.

The orange juice market in Spain has been subject to various influences. On one hand, despite orange production is the largest citrus category within the European Union accounting for over 55% of total citrus production, the overall European production of oranges has declined in the 2022-2023 period. This has been primarily attributed to reduced orange production in Spain and Italy, that negatively impact the volume of available orange for processing into juice (Citrus Industry, 2023). On the other hand, the overall juice market in Spain, including orange juice, registered a CAGR of -3.31% from 2016 to 2021. The marked saw its strongest performance in 2021, growing by 6.37% over the previous year, but it experienced a significant decline in 2020 (GlobalData, 2023). This could be explained because the COVID-19 hit and the subsequent quarantine period, social distancing across nations and reduced social gathering that impacted the demand of ready to eat foods (Data Bridge Market Research, 2020).

1.3. UNDERSTANDING THE ORANGE JUICE INDUSTRY

Orange juice in the diet

Historically, orange juice has evolved from a luxury item into a staple of the breakfast table (Hyman, 2013), appreciated for its refreshing taste and for deliver health-promoted compounds such as carotenoids, phenols, ascorbic acid, and vitamin C (ascorbic + dehydroascorbic acid). These bioactive compounds contribute to protect against oxidative stress (Pisoschi *et al*., 2021). Oxidative stress is a condition that leads to cellular and tissue damage and is associated with health issues, including cancer, cardiovascular diseases, and neurodegenerative disorders (Hajam *et al*., 2022). Thus, orange juice emerges as an easy-to-obtain source of antioxidants which, together with a varied and balanced diet and a healthy lifestyle, could help reduce the risk of suffering from certain diseases.

Orange juice: with or without pulp?

The presence of pulp in orange juice plays a role in the mouthfeel, enhancing the body, thickness, and pulpy sensation (Rega *et al*., 2004). Therefore, it affects the overall sensory consumer satisfaction. Besides texture perception, consumer preference for pulp-added or pulp-free orange juice could be influenced by factors such as age, personal taste, and perceptions of health benefits. So, some consumers opt for smooth, pulp-free juice for its ease of consumption, while others seek out pulpy juice for its closer resemblance to the fresh juice, believing it to be more natural and less processed.

Orange pulp is considered a by-product of orange juice production, which was underutilized until a few years ago. Nowadays, citrus by-products have gained attention because represent a sustainable resource for producing high-value products such as bioethanol, bioactive compounds (hesperidin), and biomaterials (nanocellulose) (Cypriano *et al*., 2018), underscoring the importance of reutilizing industrial by-products. Moreover, the orange pulp itself contains a great amount of antioxidant compounds, such as phenols, flavonoids, and ascorbic acid (Al-Juhaimi, 2014; Saleh *et al*., 2021). This enlightens the potential of orange pulp in developing functional foods that naturally contribute to health benefits, thereby supporting health and sustainability in food production.

NFC orange juice: something unique from Refresco Iberia

Despite the large scale of the entire Refresco company, the Iberia division has something that differences from the rest: the citrus business, primarily dedicated to orange processing. This specialization has positioned Refresco Iberia as a leading producer of NFC orange juice. Typically, to produce juices and fruit-based beverages, companies use fruit juice concentrates because of their industrial efficiency, but they are less desirable from a consumer point of view. However, this is not the case for the orange juice at Refresco Iberia thanks to the availability of machinery, processing capabilities and expertise in orange processing. The production of NFC orange juice at Refresco Iberia started when the company bought the manufacturing plant placed in Oliva (Valencia, Spain) in 2003, as part of its European expansion. Before, the Oliva plant was owned by an originally Valencian company named Interfruit Vital S.A., now extinct.

The production of orange juice at industrial scale

It is important to have a general idea of how orange juice is industrially produced. Figure 2 schematizes the orange processing and its different outcomes: NFC orange juice, orange juice concentrated, pulp, peel oil and the remaining material (mainly wet peel, rag, and seeds) that is commonly used for animal feed. Since this work focuses on orange juice, only orange juice production will be explained.

Upon arrival at the processing facility, oranges are unloaded and prewashed to get rid of dust, dirt and pesticide residues before any leaves and stems still attached are removed. Subsequently, a manual inspection for pregrading is conducted to eliminate any fruit deemed unsuitable (rotten and visibly damaged fruit). Sound fruit is then transported to storage bins, while any damaged fruit is directly sent to the feed mill. The fruit is thoroughly washed immediately prior to the extraction process. The wash water may include a mild disinfectant to help reduce microbial presence on the fruit's surface. The, the fruit passes over a series of grading tables for final visual inspection. Before juice extraction, the fruit is sorted on a sizing table, which divides it into different streams according to fruit diameter. A sizing table consists of rotating rollers over which the fruit passes. Initially, smaller fruits fall through the gaps onto a conveyor leading to an extractor tailored to their size range. As fruits progress and the gaps widen, larger fruits find their path to corresponding extractors designed for their size.

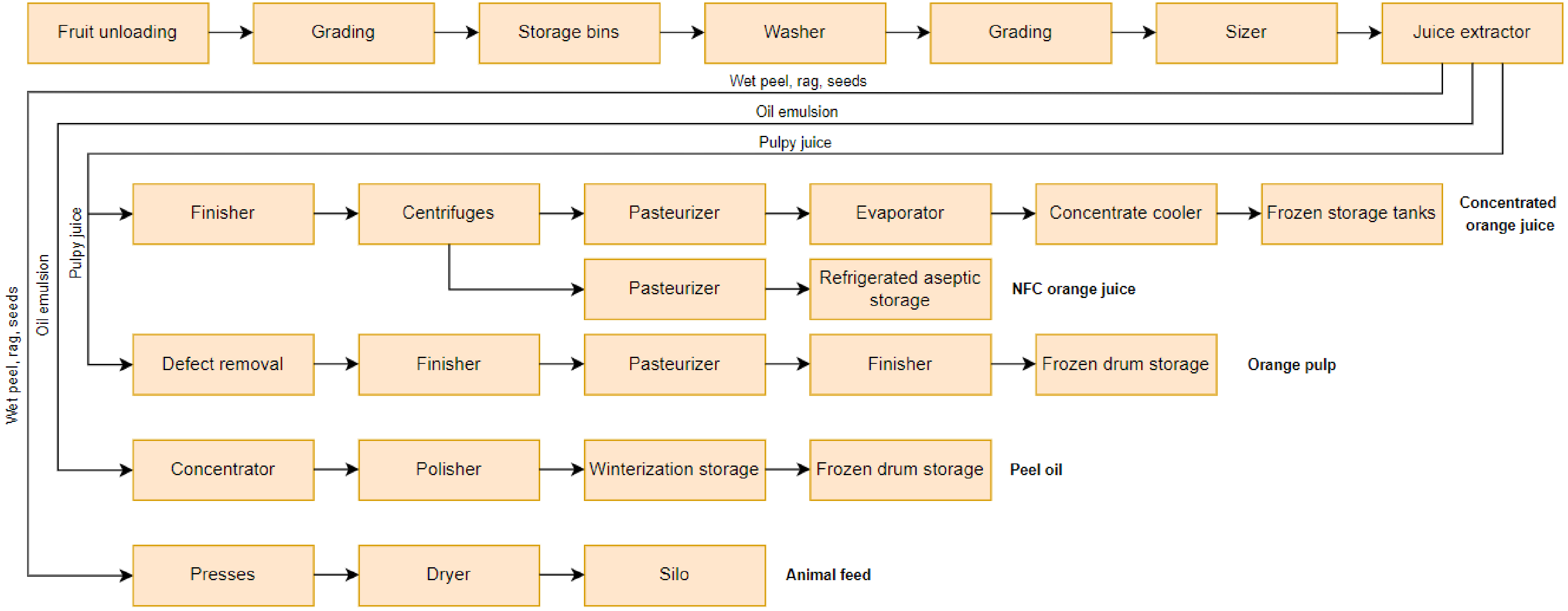

Figure 2. Flow chart with all typical processing steps found in an orange processing plant.

Deeping into the extraction process, two types of extractor systems are used: the squeezer-type and the reamer-type. Squeezer-type extractors (Figure 3) are the most common worldwide, being John Bean Technologies (JBT) the major brand. In fact, it is the system used at Refresco Iberia, using JBT extractors. For this reason, only the squeezer-type extractors are being discussed in this work.

Figure 3. Photography of the JBT squeezer-type extractor used at Refresco Iberia for orange juice production.

The extractor head (Figure 4) consists of two parts: an upper and a lower cup, equipped with metal fingers that interlock as the upper cap descends onto the lower one. A cutter, integrated into the lower cup, creates an opening through the orange's skin, enabling the inner parts to be extracted. This cutter is also a component of the perforated strainer tube, often called the pre-finisher, facilitating the juice extraction process.

Under the pressure applied during extraction, the peel breaks up and moves upward through the extractor cups' fingers. Pulpy juice is then collected through a strainer tube into a manifold, while the core material exits via the orifice tube at the bottom. During the final extraction step, oil is squeezed from the peel, with peel fragments being washed in recycled water to further extract the oil, which is then collected as a water emulsion. The squeezer-type extractor efficiently separates the fruit into four main product streams in one step: juice, oil, peel fragments and core material. This ensures minimal contact between juice and oil, as well as juice and peel.

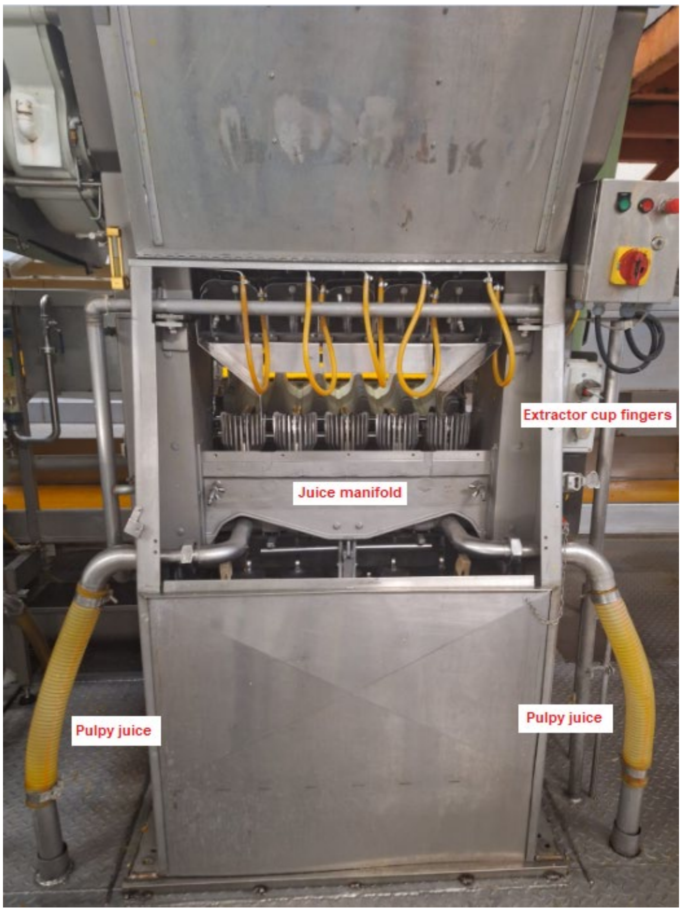

Figure 4. Photography of the extractor head of the JBT squeezer-type extractor used to produce pulpy orange juice, and other outcomes such as peel, oil emulsion and core material, at Refresco Iberia.

After the extraction process, juice undergoes clarification (Figure 5). The oil emulsion proceeds to peel oil recovery, where it is separated using centrifugation. Meanwhile, peel, rag, seeds and other solids are transported for the feed mill for processing. Figure 5 shows the typical process steps for juice clarification. Two finishers are commonly aligned sequentially. The standard squeezer-type extractor includes a pre-finishing tube in the extractor, and the pulpy juice flows directly to the primary finisher. This first finisher is set less tightly to allow a higher flow capacity, while the secondary finisher is set for finer separation. Finishers are used to refine the pulpy juice by removing remaining pulp, seeds, and other solid materials. Additional processes to clarify orange juice are based on applying centrifugal force and turbofilters. Both processes are integral to juice production and make the final NFC orange juice suitable for quality standards and subsequent industrial processes.

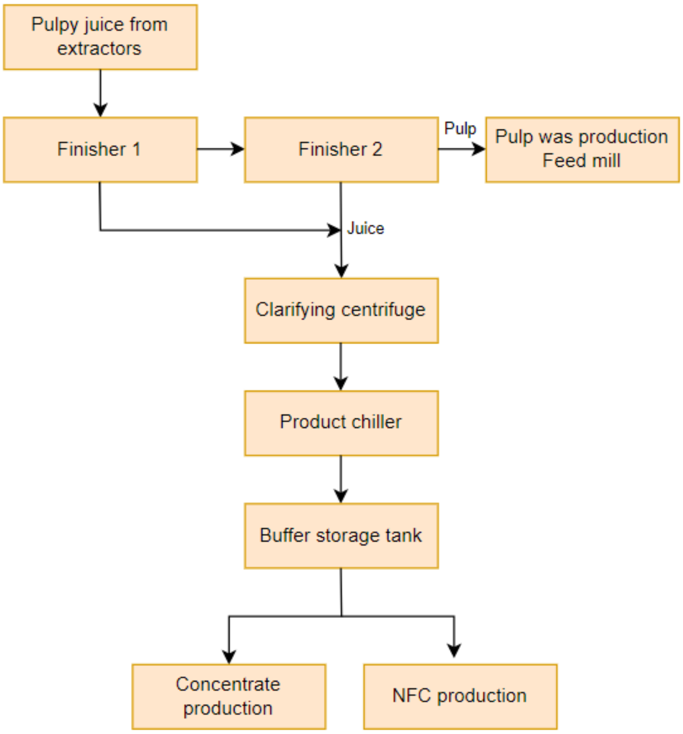

Figure 5. Flow chart of the clarification process applied to produce pulp-free orange juice (or concentrated orange juice).

Following clarification, it's common practice to blend the juice with that from other batches to harmonize its physicochemical, sensory, and nutritional quality prior to additional processing. For NFC orange juice, cooling clarified juice to 4ºC is crucial to minimize potential microbiological activity before being passed into the buffer/blending tanks.

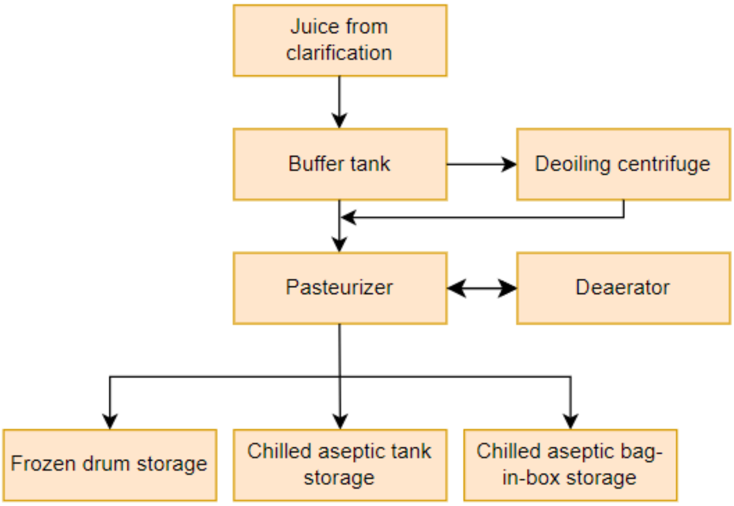

Figure 6. Flow chart of the final industrial steps for the NFC orange juice production and storage.

Once the juice is clarified and blended, it is ready to face the last industrial steps of NFC orange juice production (Figure 6). Despite fine juice extraction and clarification processes, oil content in the juice may exceed acceptable limits, influenced by fruit variety and the efficiency of the extractor. Regulatory standards often dictate these limits. For example, the Association of the Industry of Juice and Nectars from the Fruits and Vegetables of the European Union (AIJN) specifies a maximum oil content of 0.03% (AIJN, n.d.). In terms of sensory impact, acceptable levels of oil in ready-for-consumption orange juice range from 0.015 to 0.03% (Tetra Pak, n.d.). Therefore, it is important to control oil content of orange juice. There are different ways to apply oil reduction. However, deoiling with centrifuges is the most common method because it allows juice yield from the extractors to be maintained at a high level and there is no heating of the juice. The deoiled is stored in buffer tanks for a short time before being pasteurized. Additional blending with other juice batches may be carried out to even out any variations in quality.

NFC orange juice destined for bulk chilled storage demands for rigorous hygiene practices. For this reason, a primary pasteurization is applied with two purposes: to inactivate quality-degrading enzymes and to make the juice microbiologically stable. However, juice going to pasteurization is normally saturated with dissolved oxygen and some air bubbles, which poses a risk of insufficient heat treatment. Additionally, oxygen could destroy a significant amount of vitamin C (and other antioxidants), so reducing its presence is desirable. Thereby, it is recommended to include deaeration as part of the pasteurization process, which is usually carried out by passing the product through a vacuum chamber. Free air bubbles in the juice expand and tend to escape quite easily from the juice. However, dissolved oxygen is more difficult to remove.

Once pasteurized, NFC orange juice must be stored. The three major options for long-term storage are: frozen storage, aseptic storage in tanks and aseptic storage in bag-in-box bulk containers. At Refresco Iberia, all NFC orange juice produced is stored in chilled aseptic tanks until it is demanded to be filled in the final packaging. It is very important to consider that for pulp-added orange juice, it is necessary to add pulp again to the juice. Pulp is usually used as add-back in juice and juice drinks. That is why producers aim to recover pulp from NFC orange juice manufacturing (Figure 4).

Fruit juice preservation methods and packaging

After juice extraction, clarification, blending, deoiling, deaeration and storage in chilled aseptic tanks, it needs to be reprocessed to be packed and supplied to the market to be available for consumers. Processing orange juice to extend its shelf-life is essential to ensure their safety for consumers. It is also a crucial aspect of juice production as it dictates its quality (Agcam *et al.*, 2018). Traditionally, pasteurization has been the most widespread preservation method for extending the shelf life of fruit juices because its efficiency in the reduction of microbial load and enzymatic activity (Perez-Cacho & Rouseff, 2008). Nevertheless, thermal processing leads to the degradation of fruit juice quality in terms of nutritional, functional, physicochemical, and sensory properties (Jiménez-Sánchez *et al*., 2017), especially when a first pasteurization has already been applied. Nowadays, obtaining minimal-processed fruit juices is of interest to better retain the natural health-related compounds content, often heat labile constituents, and sensory-appealing properties of unprocessed fruit juices (Jiménez-Sánchez *et al*., 2017). This is particularly interesting if it is somehow communicated to consumers on the labelling, as an added value that differentiates the product. Non-thermal technologies such as high-pressure processing and pulsed electric fields, among other, have been successfully applied to fruit juices (Jiménez-Sánchez *et al*., 2017; Song *et al*., 2022; Roobab *et al*., 2022). On the other hand, current limitations of these emerging technologies are their high costs, as they require significant investment in equipment, and operational issues related to fully control process variables (Nonglait *et al*., 2022). Therefore, pasteurization is still the most common fruit juice preservation method because its effectiveness in microbiologic and quality-degrading enzymes inactivation, equipment and operational costs that make it accessible for large-scale production, and because its familiarity that ensures safety and consistency in juice quality across the industry (Perez-Cacho & Rouseff, 2008).

Once pasteurized, orange juice is ready to be filled and packed. A wide variety of packaging options could be used for fruit juices. The most common materials for fruit juices are multilayer packages for liquids (made mainly by paperboard, polyethylene, and aluminium foil) and polyethylene terephthalate bottles. Glass, aluminium cans, or plastic pouches are not frequently used, even though it is industrially viable. The final packaging format will depend on the filling technology (aseptic or non-aseptic) and on different criteria such as technological (barrier properties) or commercial (final perception of the product). At Refresco Iberia, all fruit juices are filled with aseptic technology and packed in multilayer packages for liquids and polyethylene terephthalate bottles. Once packed, NFC orange juice is ready to be supplied to the market, being Spain and Portugal the main destinations in the case of Refresco Iberia.

1.4. THE MAIN CHALLENGE FOR THE FRUIT JUICE INDUSTRY

Sharp drop in volume year after year

The fruit juice industry is facing challenging situations. From adapting to changing consumers preferences towards healthier products with less added sugar and more natural ingredients to environmental considerations that push to take action to use sustainable materials and industrial processes. However, the main challenge for the juice industry in Spain is related to the large decrease in terms of volume that has suffered in the last decade (Figure 7).

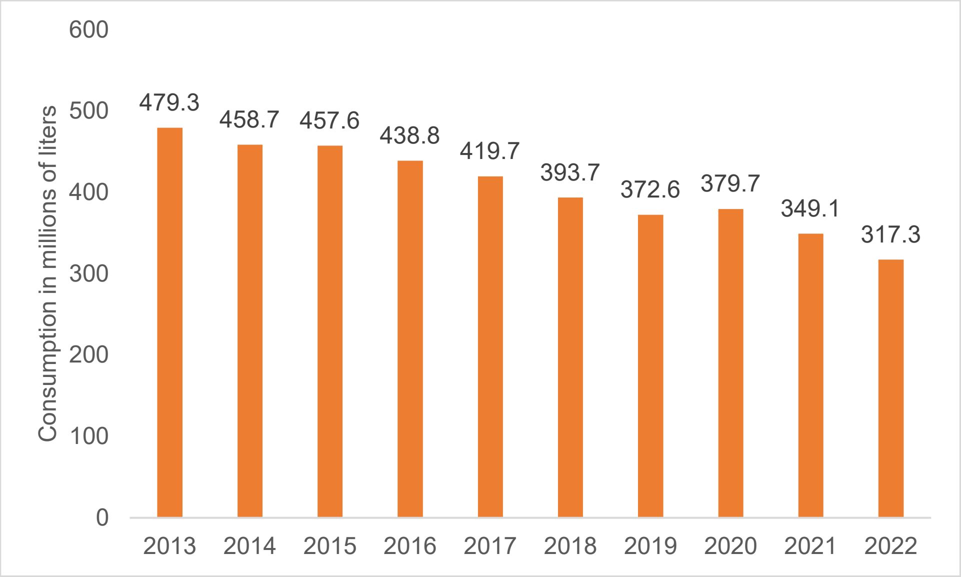

Figure 7. Annual evolution of the volume of nectars and fruit juices consumed in Spanish homes from 2013 to 2022. Source: Statista, n.d.b.

From 2013 to 2022, the whole juice industry has experienced a 33.8% drop in volume, which practically means that each year they produce less juice than the previous one. The moderately positive expectations of growth for the next years in terms of value for the Spanish juice industry (1.79% CAGR 2023-2027) (Statista, n.d.b) are based on different factors such as price increase due to economic inflation, the recovery of hospitality and out-of-home consumption, highly affected by the recent COVID-19 pandemic, and, especially, because it is expected to stimulate the market by adding value through innovation.

The fruit juice market healthy transformation

The fruit juice sector in Spain is a category that has reached a mature status within the market, possibly due to the lack of innovation and new products developed in recent years. For this reason, current efforts are focused on adding value to a mature category with the aim to improve the whole juice industry situation. For example, juices fortified with health-related substances are demonstrating greater market resilience. Other notable subcategories are refrigerated juices, organic juices, hybrid beverages with vegetables, species, cereals, teas, etc. In general, the development of the so-called ¨premium¨ varieties and the advancement of refrigerated products and smoothies stands out, with a growing assortment of organic and vegetable products towards a healthy approach.

Innovation is imperative in the competitive landscape of a global economic force such as the food industry, to meet changing consumer needs. Authors stress the necessity of developing healthier, sustainable food products, driven by accumulated knowledge from intensive research in food science, nutrition, and clinical settings. In this context, one key and highly promising path for the juice industry is its health-focused transformation, driven by consumers seeking alternatives to soft drinks. Manufacturers are strategically differentiation their products, moving beyond mere taste and general health benefits. The shift in consumer preferences towards natural fruit juices presents substantial growth opportunities. Moreover, nowadays consumers are better informed and, as economies grow and disposable incomes increase, they are more willing to spend on added-value beverage options (IMARC, 2023). This results in an increase in demand for healthy, high-quality, and quick sources of nutrition products (GlobeNewswire, 2022b). Thus, the functional food trend emerges as a promising way to enhance human diet's nutritional quality, especially considering increased life expectancy and healthcare costs (Castillo, 2019). One prominent trend is the rise of beverages containing functional ingredients such as prebiotics, which are microbiota-management ingredients that aim to improve human health.

1.5. PREBIOTICS AS FUNCTIONAL INGREDIENTS

Prebiotics

Prebiotics encompass a group of substances whose selective fermentation in the gastrointestinal tract allows specific changes, both in the composition and/or activity in the microbiota that confer benefits upon host well-being and health (Roberfroid, 2007). According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), prebiotics are defined as ¨substrates that are selectively utilized by host microorganisms conferring a health benefit¨ (Salminen *et al*., 2021). The guidance principle of this definition is that a limited number of microbial groups and their metabolites from utilizing prebiotics substrates are linked to a beneficial health effect. Gibson *et al*. (2004) outlined the criteria for classifying a food ingredient as a prebiotic:

- Resistance to gastric acidity, mammalian enzyme hydrolysis, and gastrointestinal absorption.
- Fermentation by intestinal microbiota.
- Selective stimulation of the growth and/or activity of intestinal bacteria that contribute to health and well-being.

Particularly, inulin-type fructans, including inulin, FOS (fructooligosaccharides) and oligofructose, and GOS (galactooligosaccharides) and lactulose, have been extensively studied for their health-promoting prebiotic effects (Gibson et al., 2004; Corzo *et al*., 2015; Vandeputte et al., 2017). The use of such fibers by gut microbiota leads to an increase in microbial population such as Lactobacilli and Bifidobacteria (Manning & Gibson, 2004). This is considered beneficial as the increased presence of these bacteria can result in the suppression of harmful pathogens, thereby reducing the risk of gastrointestinal infections and improving the gut barrier function (Slavin, 2013). In addition to selectively stimulating beneficial bacteria, the health effect of prebiotics is strongly related to the production of short chain fatty acids (SCFAs) like acetate, propionate, and butyrate, which are end products of the metabolism of prebiotics and play key roles in human health (Zhang *et al*., 2023; Bevilacqua *et al*., 2024). Fermentation and SCFAs production also inhibit the growth of pathogens by reducing luminal and fecal pH. Low pH reduces peptide degradation and the resultant formation of toxic compounds such as ammonia, amines, and phenolic compounds, and decreases the activity of undesirable bacterial enzymes (Walker *et al*., 2005; Yamamura *et al*., 2023).

SCFAs play a dual role in epithelial cellular metabolism. On the one hand, SCFAs are a primary energy source for intestinal epithelial cells, even when competing substrates such as glucose and glutamine are available (Zhang *et al*., 2023). They also regulate the functions of intestinal epithelial cells, including proliferation, differentiation, and modulation of subpopulations like enteroendocrine cells. This impacts gut motility and strengthens gut barrier functions (Martin-Gallausiaux *et al*., 2021). Particularly, butyrate has significant intestinal and immune-modulatory functions, protecting against colon cancer since it inhibits the proliferation of cancerous cells. This is known as the butyrate paradox or Warburg effect, and it is due to a metabolic shift in cancerous cells that preferentially use glucose as their energy source, instead of butyrate (Salvi & Cowles, 2021). Consequently, when cancer cells are exposed to butyrate, they do not use it as a primary energy source but rather accumulate it to levels that can inhibit cell proliferation by inducing cell cycle arrest and promoting apoptosis in cancer cells. Therefore, in this context, butyrate acts more as an inhibitor of growth rather than as a fuel.

On the other hand, SCFAs regulate endocrine functions in the gut, playing a vital role in secreting hormones that regulate food intake, insulin secretion, and other gut functions (Martin-Gallausiaux *et al*., 2021). They acutely stimulate enteroendocrine cells to release hormones like glucagon-like peptide-1 and peptide YY (Lu *et al*., 2018). These effects involve activation of receptors like GPR43 and GPR41 (G protein-coupled receptors expressed in human adipocytes, colon epithelial cells and peripheral blood mononuclear cells) and modulate long-term hormonal production, influencing gut hormone gene expression and secretion (Zhang *et al.*, 2023).

In addition to their effects on the gut, prebiotic also have systemic influences. Since SCFAs are water-soluble, they are absorbed from the gastrointestinal tract into the blood stream. They can modulate the immune system, potentially leading to reduce inflammation throughout the body (Kim *et al.*, 2014; McLoughlin *et al.*, 2017). This immunomodulation is particularly beneficial in chronic diseases that have an inflammatory component. Furthermore, the impact of prebiotics on lipid metabolism and insulin resistance suggests potential benefits in managing cardiovascular diseases and type 2 diabetes (Kim *et al.*, 2018; Oniszczuk *et al.*, 2021).

The concept of "cross-feeding" elaborates on the interdependent relationships between different gut microbes. It can be understood as the metabolic exchange between microorganisms. This process ensures the diversity and stability of the gut microbiome, which is vital for efficient nutrient absorption and overall health (Culp & Goodman, 2023). For example, the lactate and acetate produced by fructan-utilizing bacteria become substrates for other bacteria that produce butyrate, which is linked to various health benefits (Saa *et al.*, 2022; Culp & Goodman, 2023).

Additionally, recent studies suggest that prebiotics can contribute to mental health by multiple mechanisms, but largely through attenuating the inflammatory response and serotonin availability (Bistas *et al.*, 2023). For example, prebiotics have been shown to prevent lipid peroxidation, thus offering protection from oxidative stress, and associated inflammatory reactions (Romo-Araiza & Ibarra, 2020). This connection between gut health and mental wellbeing is part of the emerging field of the gut-brain axis (Stengel *et al.*, 2021). Most studies on this topic converge on the need for more research, with larger sample sizes over longer periods of time. Therefore, the actions of prebiotics extend beyond the gastrointestinal tract, influencing systemic health, mental wellbeing, and immune responses. This underscores the importance of prebiotics in dietary strategies aimed at promoting overall health and preventing various disorders.

In addition to their direct health-related effects, prebiotics can be used with technological purposes to create nutritionally improved foods. From the food science viewpoint, prebiotics could be added to food products also with other technological applications other than its health-promoting effects. For example, since inulin (long chain fructans) creates a smooth, creamy gel structure, it can be used as fat replacer, providing a spreadable texture, smooth fatty mouthfeel, and glossy appearance or as a viscosity modifier, as it possesses thickening and gelation properties (Hughes *et al*., 2022; Mudannayake *et al*., 2022). Conversely, shorter chain fructans like FOS and oligofructose increase its solubility and sweetness (30-50% compared to sugar sweetness) and does not substantially increase the viscosity. Because their sugar-like properties, FOS and oligofructose could be used as sugar replacers (Hughes *et al*., 2022; Mudannayake *et al*., 2022).

In addition to inulin-type fructans and GOS, and because of the great potential of prebiotics, research has been focused on evaluating new potential prebiotic substances (Guarino *et al*., 2020). Among them all, resistant maltodextrin (RMD) has gained much attention in recent years because its potential prebiotic effect in clinical trials (Włodarczyk & Śliżewska, 2021).

1.6. RESISTANT MALTODEXTRIN AND ITS PROVEN HEALTH EFFECTS

Resistant maltodextrin

RMD is a fiber produced by the heat treatment of corn starch that resists digestion in the small intestine, reaching the colon and getting fermented, resulting in enhanced SCFA production (Lockyer & Nugent, 2017). Figure 8 shows the glycosidic linkages and molecular structure of RMD, which comprises a small ratio of saccharides with a degree of polymerization (DP) 1-9 and many polysaccharides with a DP 10 or more. RMD contains a random distribution of 1-2 and 1-3 linkages, which are formed during the dextrinization process, and 1-4 and 1-6 linkages naturally found in starch (Hashizume & Okuma, 2009). In terms of food technology and food processing, RMD is a very user-friendly fiber because of its high-water solubility, low viscosity, and its high stability to heat and acid conditions. RMD can be added to a wide range of food products, but because its properties, it seems to specially fit well the fruit juices manufacturing, processing, and storage conditions.

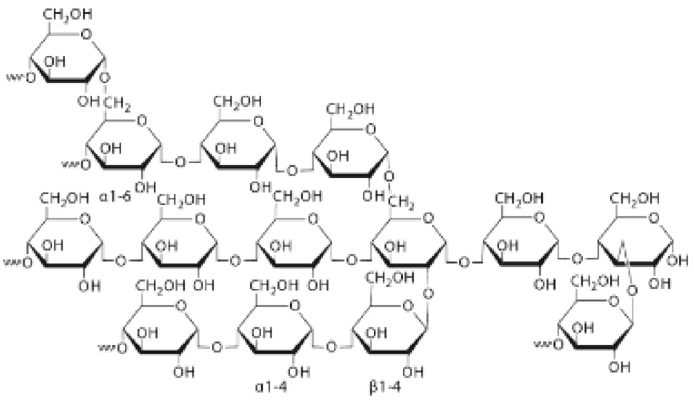

Figure 8. Molecular representation of RMD showing glycosidic linkages.

Health evidence of RMD

Weight loss and gut microbiota diversity

The interest in RMD as a prebiotic fiber stem from its potential to improve gut microbiota composition, enhance gastrointestinal health, and possibly aid in controlling blood glucose and cholesterol levels. A meta-analysis of randomized controlled trials sought to determine the efficacy of RMD as a dietary intervention for weight management. The results indicated a positive impact of RMD on reducing body weight and body mass index, suggesting its potential utility in weight loss strategies (Mukai *et al*. 2017). Another study, a randomized placebo-controlled double-blinded dietary intervention involving 116 overweight or obese participants, investigated the effects of RMD on weight loss during a 12-week energy-restricted diet. The findings revealed no significant differences in weight loss between groups. However, the RMD supplemented led to a greater reduction in systolic and diastolic blood pressure compared to placebo, therefore being beneficial for cardiovascular health. RMD also induced changes in gut microbiota by improving its diversity, leading to higher abundances of *Parabacteroides* and *Bifidobacteria* (Hess *et al*., 2020).

Colonic transit time and bowel movements

In addition, another randomized, placebo-controlled, double-blind study investigated the effects of RMD on colonic transit time (CTT) and other defecation characteristics. The study involved 66 healthy adults who underwent a 7-day run-in period followed by a 21-day intervention period. The total CTT significantly decreased in the RMD group compared to the placebo group, and notably the stool volume increased by 56% in the RMD group compared to the baseline, while it remained unchanged in the placebo group. Stool consistency also improved in the RMD group (Abellán *et al*., 2016). Reducing CTT is important for several reasons. First, because it makes bowel movements more regular and comfortable, which is particularly beneficial for individuals with chronic constipation or irregular bowel habits. Also, by ensuring regular bowel movements, RMD reduces the time toxins and carcinogens stay in contact with the colon lining, potentially lowering the risk of gastrointestinal disorders. Moreover, studies suggest a link between improved gut motility and metabolic health (Müller *et al*., 2018; Fayfman *et al*., 2019). Regular bowel movements can positively influence blood glucose and lipid levels, contributing to better management of conditions like metabolic syndrome. Finally, constipation and irregular bowel movements can significantly affect one's quality of life and mental health, so improving CTT with RMD could lead to increased comfort and psychological well-being, and to reduce risk of colonic diseases such as diverticulosis.

Blood glucose and lipid levels

Furthermore, a meta-analysis of randomized controlled trials explored the effects of RMD on blood glucose levels and the glycemic response to carbohydrate-rich foods. As a result, RMD significantly attenuated the rise of blood glucose levels following carbohydrate intake, being the evidence stronger for RMD in drinks than in solid foods (Livesey & Tagami, 2009). This indicates its potential benefit in managing blood sugar levels. Another study, conducting both animal (rats) and human (13 healthy adults) experiments, demonstrated that RMD can significantly suppress postprandial increases of blood triacylglycerol, remnant-like particle cholesterol (RLD-cholesterol) and insulin levels, which are known to promote arteriosclerosis (Kishimoto *et al*., 2007). This suggests that RMD might be beneficial to modulate lipid absorption and metabolism following high-fat meals, which is of interest to maintain a good cardiovascular health. Accordingly, another study demonstrated that RMD can significantly increase the excretion of lipids in feces in both rats and humans (10 healthy adults), indicating a reduction in lipid absorption in the body, meaning that less fat is available for storage or conversion into blood lipids like triglycerides (Kishimoto *et al*., 2009).

However, current studies, although enlightening and hopeful, often involve small sample sizes and short-term interventions. The long-term effects of RMD intake on various population groups, including those with pre-existing health conditions, remain largely unexplored. There is also a need to understand the potential interactions of RMD with different dietary patterns and other lifestyle factors. Also, for integrating RMD consumption more substantially into dietary recommendations, it is needed to ensure the robustness of scientific evidence through comprehensive long-term research. This will not only reinforce current understanding but also ensure safe and effective application of RMD in improving public health outcomes.

1.7. WORK JUSTIFICATION

The scope of this work needs to be explored from two related but different perspectives. Firstly, from the consumer’s viewpoint. Until now, the scientific interest of prebiotic fibers has been focused on its human clinical health effects, which is understandable because its positive implications. On the other hand, other studies have focused on the stability of the prebiotic fiber itself during processing and storage, as well as their health functional effects. However, there was a significant gap in knowledge regarding how prebiotics influence the intrinsic properties of the foodstuff they are added to, which is of great relevance for technological feasibility, industrial purposes, and social acceptance.

Due to the scarcity in the scientific literature on this topic, it is preferably to study prebiotic addition in a matrix that is easy to analyze, being the effects clearly shown. Since orange juice is the most consumed fruit juice worldwide, it emerges as a simple and appropriate matrix to use. Moreover, analyzing the impact of a prebiotic fiber in the orange juice is important because it usually undergoes industrial processes, such as heat treatments, aimed to extend its shelf life but also negatively affecting its health-related compounds by degrading them. Therefore, adding new functional ingredients to orange juice could potentially interact with its desirable compounds, displaying a positive effect by better retaining or protecting them from degradation.

Furthermore, adding prebiotic fibers to conventional foods is not yet commonplace in the Spanish retail market, so consumers are not familiar with them. For this reason, a better understanding of how prebiotics influences the sensory properties of foodstuff will help to design nutritionally improved, tailor-made foods and beverages that meet the requirements of exigent and better-informed consumers.

Besides, inulin, FOS and GOS are the most studied substances because their prebiotic effects. Nevertheless, RMD is a relatively newer area of study in the prebiotic field that represents an opportunity for innovative research and its emerging applications in food science and nutrition. Also, because of its chemical characteristics, naming its high-water solubility and resistance to heat and acid conditions, RMD emerges as an ideal prebiotic fiber to be added to pasteurized orange juice.

Secondly, from the commercial viewpoint, the fruit juice industry in Spain is facing a complex and very competitive business landscape. With the market reaching a mature status, many companies are trying to add value to the juice category. The business objective of this is to improve the sales and revenue of a declining beverage category. By innovating, the aim is to improve the entire market prospects. For a multinational and highly competitive company as Refresco, it is necessary to address this context with a strategy that allows it to increase its market share. Therefore, this project represents a way of introducing the company into lines of research for the added-value new-product development strategy.

Additionally, the research aligns with the broader objective of enhancing human diets by introducing novel, nutritious, and safe food products. The study of RMD in pasteurized orange juice serves as a model for understanding how new food components can be effectively incorporated into everyday foodstuff.

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CHAPTER 2. RESEARCH OBJECTIVES AND THESIS OUTLINE

2.1. RESEARCH OBJECTIVES

In last years, RMD has gained much attention in clinical settings because its positive health implications. However, little is known on how RMD impacts the food in which it is added. For this reason, the primary objective of this thesis is:

- To enlighten the effects of RMD addition on the relevant characteristics of standard pasteurized orange juice, with and without pulp.

To do so, it was necessary to address different works based on the multiple characteristics of orange juice. Consequently, the secondary research objectives of this thesis are as follows:

- To elucidate the feasibility of adding RMD at different concentrations to orange juice, with and without pulp, before pasteurization process.
- To analyze the impact of RMD addition at different concentrations, and orange pulp presence, on the physicochemical properties of pasteurized orange juice.
- To determine if adding RMD before orange juice pasteurization has a protective effect on its bioactive compounds and its antioxidant capacity.
- To quantify the impact of orange pulp presence in the bioactive compounds and the antioxidant capacity of pasteurized orange juice.
- To study how adding RMD before orange juice pasteurization affects the bioaccesibility of its bioactive compounds.
- To explore the impact of RMD addition to pasteurized orange juice, with and without pulp, on its original sensory attributes.
- To assess the quantification of the aroma volatile compounds of pasteurized orange juice, with and without pulp, and the impact of RMD on such flavor-related compounds.
- To elucidate the effect of adding RMD in the bioactive compounds and the antioxidant capacity of pasteurized orange juice, with and without pulp, over storage time.

2.2. THESIS OUTLINE

Before pointing out the thesis outline, it is important to clarify that all works were performed using pasteurized orange juice with pulp (2.5%) and without it, based on different consumer preferences and on the high impact of pulp in the characteristics of orange juice. Likewise, same RMD concentrations were applied in all studies: 0% (standard samples), 2.5%, 5% and 7.5%. These specific concentrations were determined based on what was previously done in clinical studies to achieve positive health outcomes. This way, a wide spectrum of possibilities, from low to high RMD concentration, can be evaluated, analyzing the tolerance of RMD addition and its multiple effects.

The previous parts of this works represent the argumentative justification and provide insights to acknowledge and frame the thesis within the scientific and business field. **Chapter 1** is key to introduce the Spanish fruit juice market and the importance and complexity of orange juice for this industry. It also helps to better understand the status of prebiotics and the research for new substances with prebiotic activity, where RMD is gaining much attention because its reported health effects up to date. **Chapter 2** presents the objectives of the research as they were set out at the beginning of the thesis, according to the experimental design. Here, the thesis outline is also shown according to all different works that have been performed to elucidate the multiple research objectives.

Firstly, it was needed to address the feasibility of adding RMD to pasteurized orange juice, and what are the outcomes in terms of physicochemical properties compared to regular pasteurized orange juice. Previous studies on prebiotics have largely focused on the stability of the fiber itself during processing and storage, as well as their functional effects. However, there was a significant gap in knowledge regarding in how the fiber influences the intrinsic properties of the foodstuff they are added to. That encouraged the study performed in **Chapter 3**, where key parameters for fruit juice such as ºBrix, pH, acidity, particle size distribution, density, turbidity, rheology, and color were measured.

Secondly, orange juice is also well known as a great source of bioactive compounds with antioxidant properties that are desirable for human health. However, the application of common heat treatments such as pasteurization leads to a reduction of these health-related compounds. Therefore, the aim in **Chapter 4** was to explore whether adding RMD before heat treatment positively affects the preservation of intrinsic bioactive compounds of pasteurized orange juice, and their bioaccesibility. To do so, ascorbic acid, vitamin C, total phenols, total carotenoids, and the antioxidant capacity were analyzed. After that, *in vitro* digestibility was assessed to evaluate the effectiveness of RMD in the protection and release of the bioactive compounds after simulated pre-absorptive events in the gastrointestinal tract.

Thirdly, a critical aspect of introducing functional ingredients into foods is to figure out how they impact the organoleptic properties of the conventional foodstuff they are added to. This is particularly relevant for a market in which prebiotic addition to day-to-day foods is not common, so consumers are not used to it. This motivated to perform a sensory analysis of pasteurized orange juices with RMD in **Chapter 5**, focusing on parameters such as color, aroma, sweetness acidity, bitterness, mouthfeel, off-flavor, and overall rating. Additionally, important sensory-related physicochemical parameters such as ºBrix, pH, acidity, color, particle size distribution, and density were analyzed along with the aroma volatile compounds (alcohols, aldehydes, terpenes and terpenoids, ketones and acids), as they directly influence the flavor of the finished product. Moreover, once again, it is well-known that pasteurization negatively affects the aroma volatile compounds by degrading them, causing a loss of the original aroma. Therefore, it was interesting to address whether RMD could be used to positively counteract their loss through the evaporation process.

The last work of this thesis is exposed in **Chapter 6**, where the stability over time (until 170 days, roughly 6 months of storage) of the bioactive compounds of pasteurized orange juice with RMD, naming ascorbic acid, vitamin C, total phenols, total carotenoids, and the antioxidant capacity, was studied. This was important as it provided valuable insights on the effects found in **Chapter 4**, to better know if they were limited to the time of juice processing or if they extend throughout the storage life of juices. Assessing the effects of RMD on processed food over time is appropriate to determine whether it helps in preserving health-related compounds during the shelf life of that foodstuff.

The final conclusions of the whole thesis are synthesized in **Chapter 7** based on the 4 published articles discussed in the previous chapters.

CHAPTER 3. IMPACT OF RESISTANT MALTODEXTRIN ADDITION ON THE PHYSICOCHEMICAL PROPERTIES IN PASTEURIZED ORANGE JUICE

This chapter has been published as: Arilla, E., Igual, M., Martínez-Monzó, J., Codoñer-Franch, P., & García-Segovia, P. (2020). Impact of resistant maltodextrin addition on the physicochemical properties in pasteurized orange juice. *Foods*, *9*(12), 1832. doi: 10.3390/foods9121832

ABSTRACT

Resistant maltodextrin (RMD) is a water-soluble fiber that can be fermented in the colon and exert prebiotic effects. Therefore, its addition to food and beverage products could be beneficial from both technological and nutritional viewpoints. However, to date, most studies have focused on the stability of the prebiotic fiber rather than its impact in the original food matrices. Therefore, this work aimed to evaluate the addition of RMD on the physicochemical properties of pasteurized orange juice (with and without pulp). °Brix, pH, acidity, particle size distribution, density, turbidity, rheology, and color were measured in orange juices with increasing RMD concentrations (2.5, 5, and 7.5%). Control samples without RMD were also prepared. RMD added soluble solids to the orange juice, affecting the °Brix, density, turbidity, and rheology. Slight color differences were observed, and lower citric acid content was achieved because of orange juice replacement with RMD. Differences in particle size distribution were exclusively because of pulp content. Further studies are needed to elucidate if potential consumers will appreciate such physicochemical changes in organoleptic terms.

INTRODUCTION

Adding new food components to formulate novel nutritious and safe food products has become a method to improve the quality of human diets. One major food component that has tremendous interest from scientists, companies, and consumers is prebiotics fibers. Prebiotics are defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health” (Gibson *et al*., 2004). The most studied and accepted prebiotic are non-digestible carbohydrates that include inulin, fructo-oligosaccharides, galacto-oligosaccharides, lactulose, and human milk oligosaccharides (Corzo *et al*., 2015). However, other food components, such as resistant maltodextrin (RMD), could exert functional effects too and, therefore, attract considerable interest. RMD is a water-soluble and fermentable fiber produced by the heat treatment of corn starch, indigestible in the small intestine but fermentable in the colon, resulting in enhanced short-chain fatty acid production (Lockyer & Nugent, 2017). Figure 1 shows the glycosidic linkages and molecular structure of RMD, which comprises a small ratio of saccharides with a degree of polymerization (DP) 1–9 and many polysaccharides with a DP 10 or more. RMD contains a random distribution of 1–2 and 1–3 linkages, which are formed during the dextrinization process, and 1–4 and 1–6 linkages naturally found in starch (Hashizume & Okuma, 2009). In terms of food technology and food processing, RMD is a very user-friendly fiber because of its low viscosity and its high stability to heat and acid conditions. In addition, it is tasteless and flavorless, so it could be easily added to a wide range of food products.

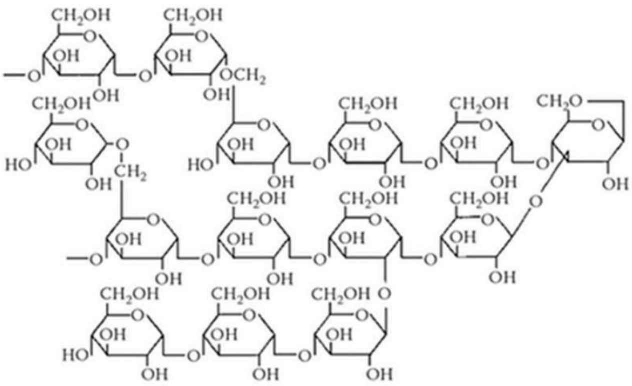


Figure 1. RMD chemical structure model.

In past studies, RMD has shown its potential prebiotic effect. In a double blind, randomized controlled crossover study, the daily intake of 25 g of RMD for 3 weeks (followed by a 2-week washout) increased fecal bifidobacteria counts and stool wet weight (Burns *et al*., 2018), suggesting health benefits. However, using RMD in food products is not limited to its potential prebiotic effect. For instance, in another double blind, randomized controlled crossover study, RMD demonstrated short-term decreased hunger and increased satiety hormones when ingested with a meal (Ye *et al*., 2015). In addition, according to a systematic review of randomized placebo-controlled trials, the functional effect of RMD seems to be more effective in liquid foods rather than solid foods (Livesey & Tagami, 2009), so its use in beverages could be beneficial. These named studies suggest that RMD could have an adequate functional effect in a liquid matrix.

These functional effects can be linked to the maintenance of intestinal homeostasis because the gastrointestinal microflora plays a key role in the overall health status, affecting important human bodily functions such as metabolic, trophic, and protective (Guarner & Malagelada, 2003). Several factors have been shown to influence gastrointestinal microflora composition, being the environmental factors associated with diet and lifestyle the most predominant ones to shape it (Rothschild *et al*., 2018).

To provide a better diet, the food and beverage industry works to develop novel products that meet consumers' requirements and population wellbeing. Therefore, the beverage industry, and especially the juice industry, is probably one of the most dynamic and innovative sectors. In accordance with the growing consumer inclination toward healthier food products (Saba *et al*., 2019), the beverage industry is increasing the number of healthier ingredients in their juices, for example, by developing functional beverages through adding prebiotics (Priyadarshini & Priyadarshini, 2018).

In terms of flavor, orange juice is the most demanded (Priyadarshini & Priyadarshini, 2018), as it is the most consumed fruit juice worldwide. Aspects like the fruit origin, fruit cultivar, maturity, juice processing conditions, packaging, and storage conditions affect the physicochemical properties of the juice. Moreover, orange juice, one of the most representative citrus juices, contains many nutritive and biologically active micronutrients, besides its natural sugar content. The most significant micronutrients for orange juice are potassium, copper, folate, vitamin C, flavonoids (mostly hesperidin), and dietary fibers (Ivanova *et al*., 2017). From the viewpoint of juice processing, one of the primary considerations is the technology applied to assure the microbial stabilization. Although alternative processing treatments have been developed (for example, high-pressure processing technologies or pulsed electric fields), thermal pasteurization is still the most cost-effective method to reduce microbial populations and enzyme activity (Perez-Cacho & Rouseff, 2008).

Because of their technological characteristics, food components perceived as prebiotics have shown not only an upgrade in the nutritious quality of the food product but also an improvement in the quality regarding sensory properties, texture, and physicochemical properties (De Paulo Farias *et al*., 2019). Thus, based on previous evidence, adding RDM could be beneficial from nutritional and technological viewpoints, to give a novel potentially prebiotic pasteurized orange juice. However, it is necessary to specifically evaluate how each functional food component affects the matrix in which it is added. In addition, most of the studies that have been previously completed in this field focused on the stability of the prebiotic fiber in fruit juice processing and storage conditions or in the functionality of the finished beverages (Renuka *et al*., 2009; Yousaf *et al*., 2010; Ghavidel *et al*., 2014; Davim *et al*., 2015). These are undoubtedly of great importance, but few studies show how the prebiotic fibers added affect the physicochemical properties of the finished beverage. In addition, most of the studies that have been done were focused on the effect of prebiotic addition on stability, storage conditions, or functionality of the prebiotic fiber in the finished beverages (Renuka *et al*., 2009; Yousaf *et al*., 2010; Ghavidel *et al*., 2014; Davim *et al*., 2015). Only a few studies show how the addition of prebiotic fibers impact the physicochemical properties of the finished beverage. Therefore, this study aimed to evaluate the addition of RMD on the physicochemical properties of pasteurized orange juice (with and without pulp). How the RMD affects °Brix, pH, acidity, particle size distribution, density, turbidity, rheology, and color were elucidated to help develop novel functional products that could be accepted by a consumer.

MATERIALS AND METHODS

Raw materials

This study was conducted with freshly squeezed orange juice supplied by Refresco Iberia S.A.U. (Valencia, Spain). All oranges were from Spanish origin. RMD (Fibersol-2) added to the juice was purchased from ADM/Matsutani, LLC (Decatur, IL, USA). Frozen pasteurized orange pulp was provided by a local fruit processing company (Zumos Valencianos del Mediterráneo, Valencia, Spain).

Sample preparation and pasteurization

A total of eight samples of orange juice were prepared. Four were orange juice with pulp (OJP) and the other four were orange juice without pulp (OJWP). Fresh orange juice was directly collected from the industrial squeezed lines. Orange pulp (2.5%) was added to the OJP samples, and increasing RMD concentrations (2.5, 5, and 7.5%) were added into both OJP and OJWP samples. Both orange pulp and RMD concentrations were applied in weight/weight percentage. Pulp content was homogenized using a stirrer (LH Overhead Stirrer, VELP Scientifica, Usmate, Italy), by applying 200 rpm for 5 min. Increasing RMD concentrations were mixed and stirred (200 rpm, 15 min) into both OJP and OJWP samples. Thus, for a finished beverage portion of 200 g, either 5, 10, or 15 g of RMD would be ingested, enough to display functional effects according to other studies (Livesey & Tagami, 2009; Ye *et al*., 2015; Burns *et al*., 2018). Control samples without RMD addition (OJP0 and OJWP0) were also prepared, and they complied with the European Fruit Juice Association orange juice guidelines (AIJN, n.d.), so no adulteration or deviation occurred during the juice extraction. Finally, all samples were pasteurized (Fruchtsaftdispenser, Mabo Steuerungselemente GmbH, Eppingen, Germany) at 85 °C for 10 s, and were hot filled into 250 mL polyethylene terephthalate (PET) bottles. All bottles were immersed in a cold-water bath (<10 °C) for 30 min to cool down their temperature after the heat treatment.

Physicochemical determinations

ºBrix, Acidity and pH

Measurement of total soluble solids (°Brix) was conducted using refractometry (Abbemat 200, Anton Paar, Graz, Austria). Acidity, expressed as grams of citric acid per 100 mL of orange juice (gCA/100 mL), was determined using a DL53 acid titrator (Mettler Toledo, Greifensee, Switzerland). Determination of pH was made using a Basic 20 pH meter (Crison, Alella, Spain). All determinations were performed in triplicate in accordance with AOAC guidelines (Latimer, 2012).

Particle size distribution

Juice particle size distribution was determined by applying the laser diffraction method and Mie theory, following the ISO13320 regulation (ISO, 2020), by using a particle size analyzer (Malvern Instruments Ltd., Mastersizer 2000, Malvern, UK) equipped with a wet sample dispersion unit (Malvern Instruments Ltd., Hydro 2000 MU, Malvern, UK). Laser diffraction reports the volume of material of a given size, since the light energy reported by the detector system is proportional to the volume of material present. The Mie theory requires the information on both the sample and the dispersant optical property. For orange juice, the particle refraction and absorption were 1.52 and 0.1, respectively, and the water refraction index was 1.33. The sample was dispersed in distilled water and pumped through the optical cell under moderate stirring (1800 rpm) at 20 °C. The volume (%) against particle size (in µm) was obtained and the size distribution was characterized by the volume mean diameter (D[4,3]). The standard percentile d(0.1) or size of particle below which 10% of the sample lies and d(0.9) or size of particle below which 90% of the sample lies were also considered for juice characterization.

Rheological measurements

Juice flow behavior was analyzed using a controlled shear stress rheometer coupled to a thermostatic bath (Thermo Electron Co., Haake RheoStress 1, Waltham, MA, USA) with coaxial cylinders (Z34 DIN) using sensor system set at 20 °C following the Igual *et al*. (2014) methodology. A relax time of 900 s was selected for the sample before running the test. Shear rate, (γ; s^{-1}), was increased from 0 to 150 s^{-1} in 20 step (fixed duration for each step 30 s) and shear stress σ (Pa), was recorded.

Density

Density was determined by using a pycnometer (50 mL) and distilled water at 25 °C as a reference.

Turbidity

Orange juice was centrifuged at 3000 rpm for 10 min. The turbidity of the upper layer solution was determined using a spectrophotometer UV-VIS (Thermo Scientific, Helios Zeta UV-Vis, Loughborough, LE, UK) at 600 nm, as described by Chandler and Robertson [23]. Sample transmittance (T) was obtained in relation to distilled water, and the turbidity (Tb) was calculated using Equation (1) (Matsui *et al*., 2008):

$$\boldsymbol{Tb}\,(\%) = \mathbf{100} - \boldsymbol{T} \tag{1}$$

Color measurement

Color values were obtained from the reflection spectrum. Samples color was measured using a colorimeter (CM-700d, Konica Minolta, Tokyo, Japan) with a standard illuminant D65 and a visual angle of 10°. Results were obtained in terms of L* (brightness: L* = 0 (black), L* = 100 (white)), a* (−a* = greenness, +a* = redness), and b* (−b* = blueness, +b* = yellowness), according to the CIELab system (CIE, 2018). Chroma, C*ab (saturation) and hue angle, h*ab, were also calculated, using equations 2 and 3, respectively. The color difference was calculated regarding the control samples in each case, in all OJP and OJWP samples, to evaluate the RMD addition effect.

$$\boldsymbol{C^*_{ab}} = \left(\left(\boldsymbol{a}^{*2} + \boldsymbol{b}^{*2}\right)\right)^{\mathbf{1/2}} \tag{2}$$

$$\boldsymbol{h^*_{ab}} = \mathbf{arctan}\left(\frac{\boldsymbol{b}^*}{\boldsymbol{a}^*}\right) \tag{3}$$

Statistical analysis

Analysis of variance (ANOVA) was applied with a confidence level of 95% ($p < 0.05$), to evaluate the differences among samples. Furthermore, a correlation analysis among studied properties of juices and RMD concentration was made, with a 95% significance level. Statgraphics (Centurion XVII Software, version 17.2.04, Statgraphics Technologies, Inc. The Plains, VA, USA) was used.

RESULTS AND DISCUSSION

°Brix, acidity, and pH were evaluated as the basic control parameters, as is the general protocol in the juice industry (Table 1). Increasing concentrations of RMD implied a significant increase in total soluble solids in both OJP and OJWP samples ($p < 0.05$). This makes sense since, as explained, RMD is a water-soluble fiber. OJWP samples showed slightly higher values of °Brix ($p < 0.05$), mainly because a small percentage of orange juice was replaced in OJP samples by orange pulp, which is an insoluble fiber. Ghavidel *et al*. (2014) also reported than an increase in fiber content (fructo-oligosaccharides, FOS) produced an increase in the total soluble solids in an orange juice-based sugar-added beverage. In addition, the substitution of sugar by other prebiotic fibers, namely oligofructose and inulin, did not change the °Brix range of papaya nectar (Braga & Conti-Silva, 2015). Because of this soluble solid addition to the matrix and its light sweetness taste, such fibers have been proposed as sugar replacers, among other food technology applications (Wang, 2009; Priya, 2020). This could be beneficial in acidic food products, such as orange juice, to help balance its sensory profile without adding sugar but adding functional ingredients.

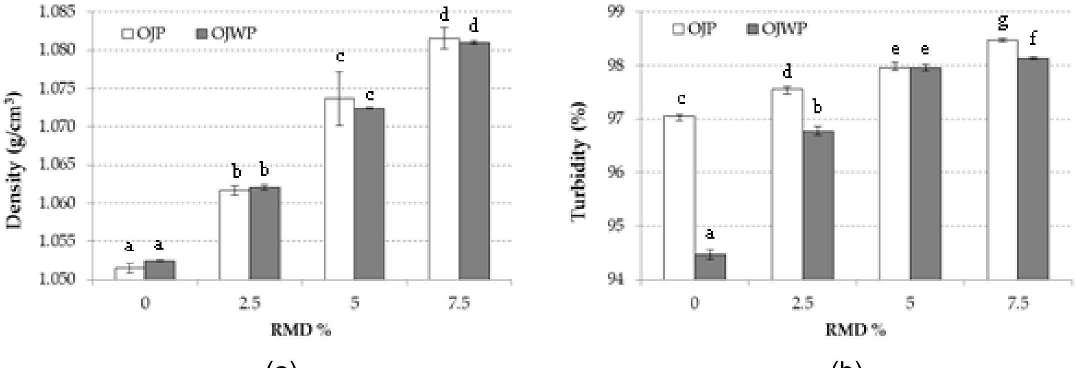
Table 1. Mean values (and standard deviations) of °Brix, pH, and acidity of pasteurized orange juice.

Sample	°Brix	Acidity (gCA/100 mL)	pH
OJP0	11.36 ± 0.04^{a}	0.773 ± 0.003^{h}	3.36 ± 0.04^{a}
OJP2.5	13.58 ± 0.03^{c}	0.756 ± 0.003^{g}	3.46 ± 0.08^{b}
OJP5	15.83 ± 0.08^{e}	0.733 ± 0.002^{f}	3.50 ± 0.07^{bc}
OJP7.5	17.98 ± 0.04^{g}	0.7133 ± 0.0006^{e}	3.44 ± 0.04^{b}
OJWP0	11.52 ± 0.05^{b}	0.686 ± 0.003^{d}	3.55 ± 0.03^{cd}
OJWP2.5	13.75 ± 0.03^{d}	0.671 ± 0.002^{c}	3.59 ± 0.03^{d}
OJWP5	15.96 ± 0.04^{f}	0.656 ± 0.002^{b}	3.54 ± 0.03^{cd}
OJWP7.5	18.18 ± 0.08^{h}	0.6380 ± 0.0005^{a}	3.57 ± 0.03^{cd}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp.

However, higher RMD concentrations significantly decreased acidity values in both OJP and OJWP samples ($p < 0.05$). This is because RMD addition helped to reduce the quantity of raw orange juice, and therefore citric acid. Moreover, OJP samples had significantly higher acidity values than OJWP ($p < 0.05$). In terms of pH, the differences were small but significant ($p < 0.05$), as OJP samples presented lower pH values than OJWP. Therefore, orange pulp addition showed an impact on the acidity and pH values ($p < 0.05$), whereas RMD addition decreased citric acid content by replacing orange juice content ($p < 0.05$). The acidity of the orange pulp could be higher than the acidity from the orange juice, thus leading to an increase in acidity and a decrease in pH in OJP samples. RMD addition had less impact on the pH.

°Brix, pH, and acidity values of the control samples are hard to compare with those in other studies, since the oranges used for the juice extraction were all from Spanish origin. Citric acid values reported from Mexican orange juices were lower (Farnworth *et al.*, 2001). However, °Brix and pH were almost the same as those reported from Cortés *et al*. (2008), who used the Navel cultivar from Spain. This enhances the importance of the raw material origin and the complexity to properly compare the physicochemical properties in fruit-derived products. Juice density is also an important quality control parameter in the juice industry (Kimball, 2012). Figure 2a shows that density values were not affected by pulp addition ($p > 0.05$) and that they increased as RMD concentration increased ($p < 0.05$). This could be explained because RMD was dissolved completely in the orange juice. Moreover, it is widely known that, in fruit juices, the soluble solids quantity affects density values, while insoluble solids, such as cloud and pulp, contribute little to density measurements (Kimball, 2012). The relationship between density and soluble solids has been extensively studied and regression models have been developed, like the one obtained by Ramos and Ibarz (1998).

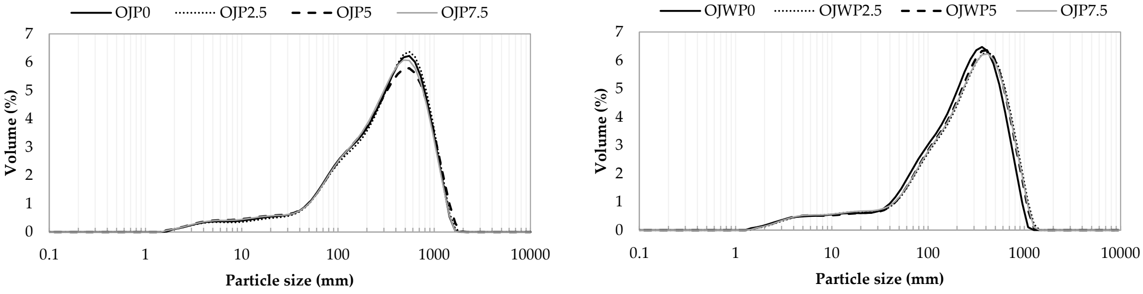


(a) (b)

Figure 2. (a) Mean values (and standard deviations) of density of pasteurized orange juice; (b) Mean values (and standard deviations) of turbidity of pasteurized orange juice. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

Turbidity is represented in Figure 2b. Turbidity provides a measure of the concentration of disperse particles in a solution by measuring its light-scattering properties (Vaillant *et al*., 2008). Therefore, as RMD was completely dissolved, higher RMD concentrations led to higher turbidity values ($p < 0.05$). However, in contrast to the density measurements, pulp content played a role in the turbidity values ($p < 0.05$), clouding OJP samples. This can be checked by comparing turbidity values of the control samples (OJP0 and OJWP0). However, the clouding effect because of pulp content was limited as turbidity difference between OJP and OJWP samples decreased as RMD concentration raised. For example, no turbidity difference was found in 5% RMD-added samples.

Volume particle size distribution is represented for the OJP samples (Figure 3a) and OJWP samples (Figure 3b). Both OJP and OJWP samples showed a similar trend, as it can be observed in Figure 3. Table 2 compiles the mean values (and standard deviations) of volume mean diameter D[4,3] and the standard percentiles d(0.1), d(0.5), and d(0.9). Particles size of OJP samples presented significantly greater volume mean diameter than OJWP samples ($p < 0.05$). RMD addition did not have an impact in the volume mean diameter ($p > 0.05$), as it was dissolved in the orange juice. Therefore, the difference between OJP and OJWP samples regarding volume mean diameter were exclusively because of pulp content ($p < 0.05$). This phenomenon is demonstrated by comparing the standard percentiles. As the number of analyzed particles grows, the particle size distribution becomes homogenized; this is seen in Table 2 by comparing d(0.1) values to d(0.9) values. In the first percentile d(0.1), greater differences in particle size distributions were found between all orange juice samples ($p < 0.05$). However, by increasing the number of particles analyzed (d(0.9)), the same relationship is obtained as with the volume mean diameter. Thus, OJP obtained a greater quantity of particles of larger size ($p < 0.05$).



(a) (b)

Figure 3. (a) Volume particle size distributions (representative curves) of pasteurized orange juice with pulp; (b) Volume particle size distributions (representative curves) of pasteurized orange juice without pulp. OJP, orange juice with pulp; OJWP, orange juice without pulp.

The particle size distribution obtained for these orange juice samples differed from the one performed by Stinco *et al.* (2012), who, on average, reported larger particle size for the industrially squeezed orange juices (both fresh and pasteurized). Achieving smaller particles could be beneficial as the food matrix is one of the key factors related to the release of bioactive compounds, such as carotenoids (Stinco *et al*., 2012).

Table 2. Mean values (and standard deviations) of volume mean diameter (µm) D[4,3], standard percentiles (µm) d(0.1), d(0.5), and d(0.9) of pasteurized orange juice.

Sample	D[4,3]	d(0.1)	d(0.5)	d(0.9)
OJP0	368 ± 27^{b}	44 ± 4^{d}	305 ± 25^{cd}	791 ± 55^{b}
OJP2.5	382 ± 33^{b}	49 ± 6^{e}	321 ± 30^{d}	814 ± 71^{b}
OJP5	374 ± 39^{b}	38 ± 5^{c}	299 ± 30^{b}	825 ± 92^{b}
OJP7.5	361 ± 27^{b}	40 ± 4^{c}	296 ± 22^{b}	783 ± 61^{b}
OJWP0	300 ± 14^{a}	33 ± 3^{b}	253 ± 12^{a}	637 ± 33^{a}
OJWP2.5	297 ± 20^{a}	28 ± 3^{a}	248 ± 16^{a}	638 ± 44^{a}
OJWP5	288 ± 12^{a}	30 ± 2^{ab}	241 ± 10^{a}	615 ± 28^{a}
OJWP7.5	289 ± 15^{a}	27 ± 3^{a}	240 ± 13^{a}	623 ± 32^{a}

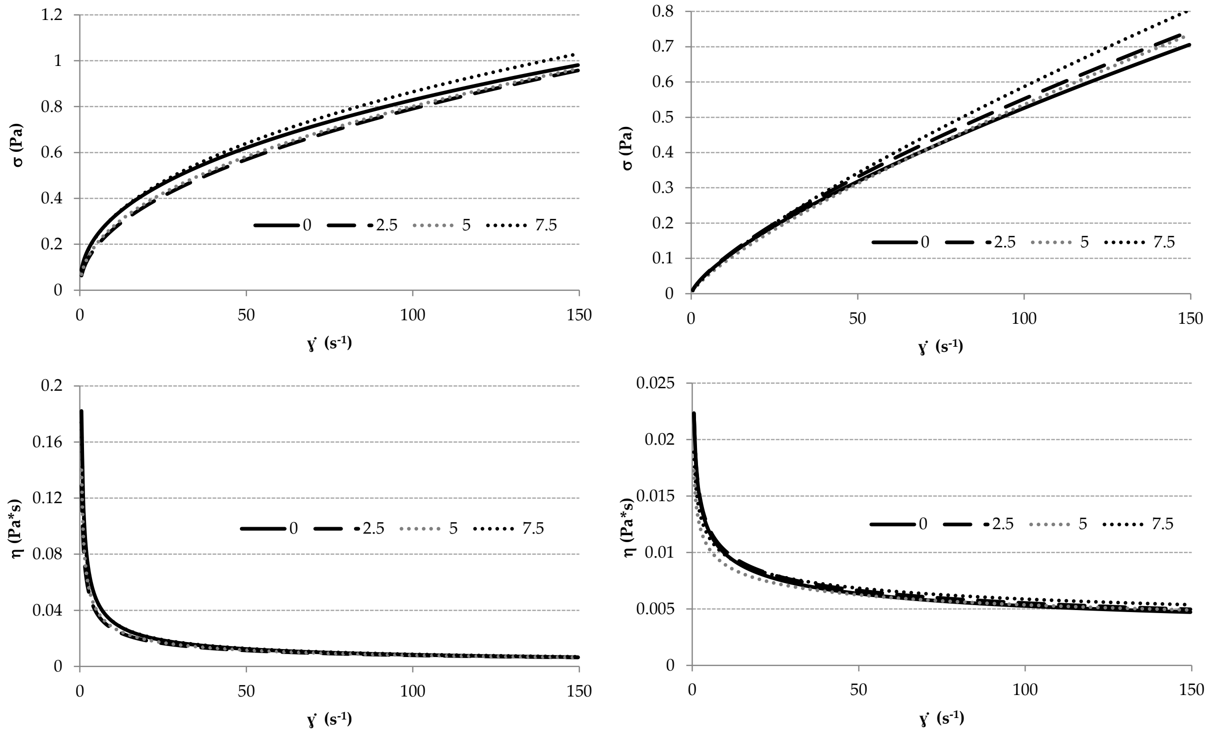
The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp.

Regarding rheology, all orange juices showed a non-Newtonian, non-time dependent, pseudoplastic behavior as observed in Figure 4. The obtained flow curves were well-fitted ($R^2 \geq 0.99$) to the Ostwald de Waele model (Equation (4)), where k is the consistency index (Pa·sn) and n is the flow index (Table 3). This mathematical relationship is useful because of its simplicity (Steffe, 1996).

$$\boldsymbol{\sigma} = \boldsymbol{k}\,\dot{\gamma}^{\,\boldsymbol{n}} \tag{4}$$

OJP samples showed significantly higher k values than OJWP samples ($p < 0.05$), meaning that pulp addition could slightly increase the viscosity of the orange juice. This can be observed in Figure 4 comparing viscosity profiles of OJP and OJWP. Rega *et al*. (2004) also demonstrated that viscosity increased as pulp content increased. This could be explained because suspended solids increase the apparent viscosity of the fruit juice (Hernandez *et al*., 1995), probably because of the pectin content of orange pulp (Schalow *et al*., 2018). RMD addition did not produce a significant change in k of OJWP samples ($p > 0.05$).

Moreover, RMD addition did not produce a clear trend in the k of both OJP and OJWP samples. Despite soluble solids supposedly increasing viscosity (Hernandez *et al*., 1995), in this study, RMD addition did not show differences in the viscosity profiles. However, OJWP samples marked significantly higher n values than OJP samples ($p < 0.05$), thus, its flow behavior was slightly more Newtonian than OPJ samples. This suggests that OJP exhibits a more pseudoplastic behavior than OJWP (Figure 4). RMD addition had also a significant effect on the n behavior in OJWP, as n values in samples with 5 and 7.5% RMD increased ($p < 0.05$). However, orange pulp addition seems to have a stronger impact on the rheology of orange juice samples.



(a) (b)

Figure 4. (a) Flow behavior (σ-γ) and viscosity profiles (η-γ) of pasteurized orange juice with pulp (OJP); (b) Flow behavior (σ-γ) and viscosity profiles (η-γ) of pasteurized orange juice without pulp (OJWP).

Table 3. Mean values (and standard deviations) of consistency index (k), flow index (n) of pasteurized orange juice.

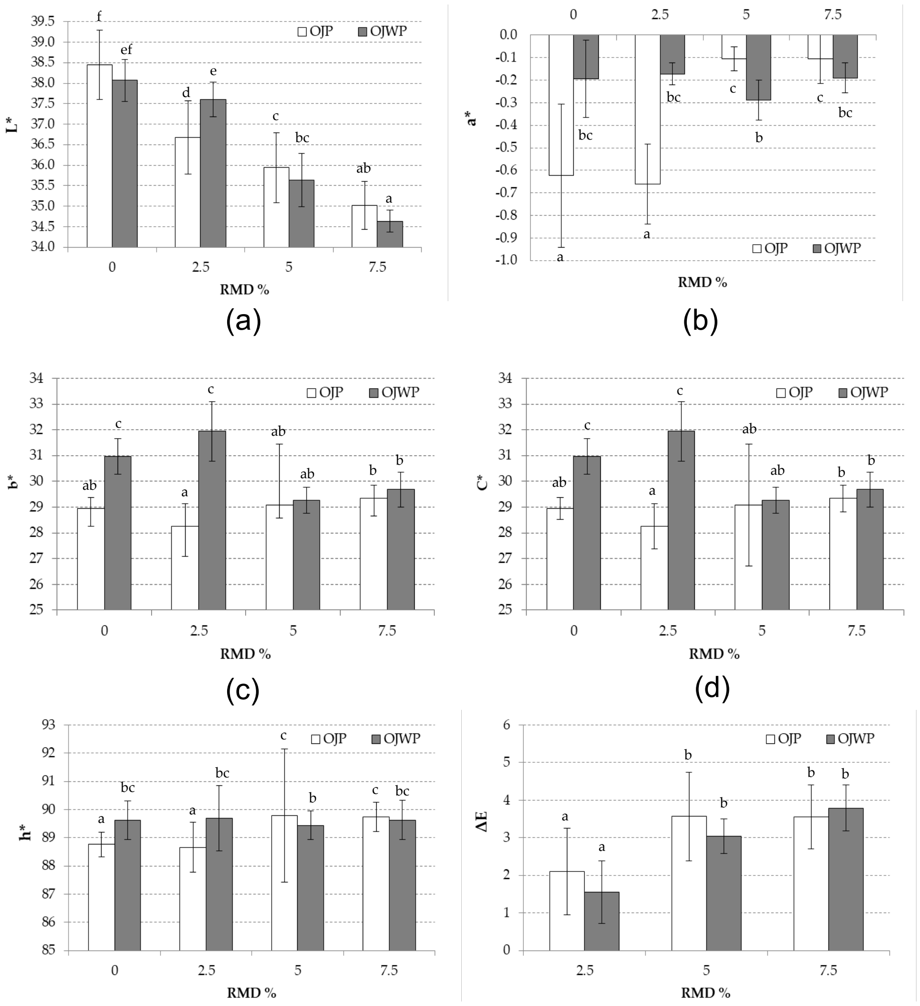
Sample	*k* (Pa*s^{n})	*n*
OJP0	0.121 ± 0.009^{c}	0.4276 ± 0.0102^{a}
OJP2.5	0.0891 ± 0.0009^{b}	0.47 ± 0.02^{c}
OJP5	0.096 ± 0.005^{b}	0.461 ± 0.012^{bc}
OJP7.5	0.117 ± 0.015^{c}	0.43 ± 0.02^{ab}
OJWP0	0.0184 ± 0.0002^{a}	0.728 ± 0.002^{d}
OJWP2.5	0.0186 ± 0.0002^{a}	0.73675 ± 0.00106^{d}
OJWP5	0.01477 ± 0.00009^{a}	0.7800 ± 0.0012^{e}
OJWP7.5	0.01618 ± 0.00006^{a}	0.7799 ± 0.0013^{e}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp.

A statistical correlation was conducted to explain the relationship between the studied parameters in the orange juice samples. There were significant correlations among particle size parameters and rheological values according to Pearson coefficients ($p < 0.05$). As was observed in other citric fruit juices (Igual *et al.*, 2014), k showed a positive correlation with D[4,3] (0.9238), d(0.1) (0.8512), d(0.5) (0.9068), and d(0.9) (0.9263); n showed a negative correlation with D[4,3] (−0.9609), d(0.1) (−0.8932), d(0.5) (−0.9467), and d(0.9) (−0.9609). Therefore, the higher the particle size in juices, the higher the consistency index in juices but the lower the flow index. Moreover, k and n were correlated with pH and acidity showing −0.7875 and 0.8766, and 0.7628 and −0.9168 Pearson coefficients, respectively.

Finally, color is one of the most important parameters for consumers, as it is related to quality perception (Cortés *et al.*, 2008); color changes are shown in Figure 5. It was shown that higher RMD concentrations significantly decreased L* values ($p < 0.05$) (Figure 5a), thus that all orange juices turned slightly darker, as RMD concentration was raised. This could be explained as higher RMD concentrations helped to reduce orange juice content. Orange pulp showed no significant effect on the L* values ($p > 0.05$). L* presented significant correlations with density (0.9493) and turbidity (0.7467) ($p < 0.05$). Igual *et al.* (2014) also found a high correlation between L* and turbidity in grapefruit juices. In addition, OJP0 and OJP2.5 had the lowest a* values ($p < 0.05$) (Figure 5b), which implies that they were greener than the rest. This could probably be explained because RMD addition led to a protective effect on the carotenoids content (Arilla *et al.*, 2020), therefore leading to a protective effect in the reddish tones during the pasteurization process. For the yellow-blue content (b*, Figure 5c), RMD addition showed no significant effect ($p > 0.05$), while orange pulp content had a significant effect ($p < 0.05$) on b* values. Thus, in the OJWP samples, an increasing addition of RMD seemed to lower the yellowish tones, as OJWP5 and OJWP7.5 had lower b* values. However, few differences exist in the OJP because of RMD addition. This suggests that orange pulp content could have a protective effect on the natural yellow/orange tones of orange juice. C* values (Figure 5d) resulting from Equation (2) were almost the same as b*, as a* values were all close to 0. Regarding h*, RMD addition on OJWP samples did not play a significant role ($p > 0.05$). However, h* values (Figure 5e) were significantly increased ($p < 0.05$) as RMD concentration was higher in OJWP samples.

RMD addition in OJP and OJWP samples had almost the same result in terms of total color difference (ΔE) regarding control samples (Figure 5f), thus, that pulp content did not interfere in color differences ($p > 0.05$). Total color differences between juices with RMD 2.5% and the control samples were lower than three units. Therefore, they are not perceptible to human eye, which only distinguishes color differences if ΔE is larger than three (Bodart *et al.*, 2008). Increasing RMD addition implied small but significant ($p < 0.05$) color differences, but they were limited because samples with 5% and 7.5% RMD had almost no color difference between them.



(a)

(b)

(c)

(d)

(e)

(f)

Figure 5. Mean values (and standard deviations) of color coordinates L* (a), a* (b), b* (c), C* (d), and h* (e) and total color differences (ΔE, (f)) of pasteurized orange juice. Letters indicate homogeneous groups established by the ANOVA (*p* < 0.05) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

CONCLUSIONS

In this study, physicochemical differences were found due to adding RMD to orange juice with and without pulp. Samples with 7.5% added RMD presented a greater impact on the physicochemical properties in both OJP and OJWP samples. With RMD, a soluble-water fiber, its addition to orange juice increased the total soluble solids content, which raised Brix, density, and turbidity values, the last more evident in OJWP samples. Citric acid content was lowered because of the orange juice replacement by RMD, and small but significant changes were observed in terms of pH. Differences in particle size distribution were exclusively because of pulp content. Orange pulp content, and not RMD addition, appears to have an impact on the orange juice rheology. Slight color differences were found; however, only higher RMD concentration would be perceptible by the human eye.

This study demonstrates that RMD addition in a wide range of concentrations is feasible from a food technology viewpoint. However, the optimal dose of RMD will depend on the functional effect to be achieved.

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CHAPTER 4. EFFECT OF ADDING RESISTANT MALTODEXTRIN TO PASTEURIZED ORANGE JUICE ON BIOACTIVE COMPOUNDS AND THEIR BIOACCESIBILITY

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ABSTRACT

Resistant maltodextrin (RMD) is a water-soluble and fermentable functional fiber. RMD is a satiating prebiotic, reducer of glucose and triglycerides in the blood, and promoter of good gut health, and its addition to food is increasingly frequent. Therefore, it is necessary to study its potential effects on intrinsic bioactive compounds of food and their bioaccessibility. The aim of this study was to evaluate the effect of adding RMD on the bioactive compounds of pasteurized orange juice with and without pulp, and the bioaccessibility of such compounds. RMD was added at different concentrations: 0 (control sample), 2.5%, 5%, and 7.5%. Ascorbic acid (AA) and vitamin C were analyzed using HPLC, whereas total phenols, total carotenoids (TC), and antioxidant capacity were measured using spectrophotometry. After that, sample *in vitro* digestibility was assessed using the standardized static *in vitro* digestion method. The control orange juice with pulp presented significantly higher values of bioactive compounds and antioxidant capacity than the control orange juice without pulp ($p < 0.05$). RMD addition before the juice pasteurization process significantly protected all bioactive compounds, namely total phenols, TC, AA, and vitamin C, as well as the antioxidant capacity (AC) ($p < 0.05$). Moreover, this bioactive compound protective effect was higher when higher RMD concentrations were added. However, RMD addition improved phenols and vitamin C bioaccessibility but decreased TC and AA bioaccessibility. Therefore, the AC value of samples after gastrointestinal digestion was slightly decreased by RMD addition. Moreover, orange pulp presence decreased total phenols and TC bioaccessibility but increased AA and vitamin C bioaccessibility.

INTRODUCTION

Fruit consumption has historically been associated with a healthy diet, as they provide key nutrients, especially bioactive compounds such as vitamins, minerals, dietary fiber, and phytochemicals. They have also been linked to a reduced risk of developing chronic diseases such as cardiovascular disease, cancer, diabetes, or aged-related functional decline (Liu, 2013). Furthermore, in the last decades, consumers have developed a greater awareness of how food affects their health and wellbeing. This, along with the consumers' need to save time, is causing the fruit juice sector to experience a positive and dynamic growth. Thus, fruit juices are becoming part of the so-called new-age beverages (Allegra *et al*., 2019).

With this pathway to a better diet, the juice industry has focused on product differentiation and development of juices that go beyond basic nutrition and good tasting (Day *et al*., 2009; Priyadarshini & Priyadarshini, 2018). This new paradigm expanded the limits of an already mature market, introducing novel functional ingredients that are founded on the premise that, beyond its nutritional contribution, helps to promote optimal health conditions and reduce the risk of diseases through positively modulating host gut microbiota (Butel, 2014; Granato *et al*., 2020). One of these novel functional ingredients are prebiotics, which are defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health" (Gibson *et al*., 2004). Prebiotics can improve the survival, growth, metabolism, and beneficial health activities of probiotics in the digestive system (Butel, 2014). Many oligosaccharides and polysaccharides–including dietary fiber–have been studied because of their potential prebiotic activity, such as inulin, fructo-oligosaccharides, lactulose, isomalto-oligosaccharides, lactosucrose, xylo-oligosaccharides, gluco-oligosaccharides, and human milk oligosaccharides, among others (Gibson *et al*., 2004; Corzo *et al*., 2015). Resistant maltodextrin (RMD) is a dietary fiber that has attracted a lot of interest in recent times. RMD is a water-soluble fiber produced from the heat treatment of corn starch, indigestible in the small intestine but possibly fermented in the colon, resulting in enhanced short-chain fatty acid production (Lockyer & Nugent, 2017). It has been proven to exert a satiating effect (Ye *et al*., 2015), to reduce post-meal glucose (Livesey & Tagami, 2009) and triglycerides (Kishimoto *et al*., 2007) levels in blood, and to promote good gut health (Baer *et al*., 2014).

Fruit juices have been suggested as an ideal medium for such functional ingredients because of their natural content of beneficial nutrients, and because they are generally well-accepted by all age groups in terms of organoleptic properties (Tuorila & Cardello, 2002; Fonteles & Rodrigues, 2018). Among all fruit juices, the most valued is orange juice, which was the most consumed fruit juice worldwide in 2018, representing 43.8% of the fruit juice market (Neves *et al*., 2020). Moreover, orange juice provides an important dietary source of bioactive compounds, such as phenolic compounds, carotenoids, ascorbic acid (AA), and vitamin C (Sánchez-Moreno *et al*., 2003), that contribute to its antioxidant properties. Since bioactive compounds are associated with better diet quality and an increase of positive health outcomes (Lima *et al*., 2019), it is important to optimize their stabilization in the food matrix, especially in those food products subjected to industrial processes.

Pasteurization is the most widely used preservation method for fruit juices, as it is the most cost-effective method (Perez-Cacho & Rouseff, 2008). However, although thermal processing gives microbial safety, it causes irreversible losses of bioactive compounds and antioxidant properties (Agcam *et al*., 2014; Lu *et al*., 2018), thus reducing their beneficial health effects. Therefore, it is important to evaluate whether adding a prebiotic fiber, such as RMD, positively or negatively affects the preservation of intrinsic bioactive compounds in pasteurized orange juice. Furthermore, it is important to explore the food processing field of RMD addition to elucidate its effects on the intrinsic bioactive compounds of orange juice. It is also necessary to analyze whether incorporating functional ingredients plays a role in the bioaccessibility of health-promoting compounds in the gastrointestinal tract, since bioaccessibility represents compounds that may be absorbed in the gut (Saura-Calixto *et al*., 2007). Therefore, *in vitro* models provide information on the pre-absorption phase that can be expressed quantitatively based on the fraction of bioactive compounds released from the food matrix during ingestion (Dima *et al*., 2020). This analyses if the desired functionality is achieved through improving the protection and release of such compounds from the food matrix.

Most studies have mainly focused on the effect of prebiotic addition on stability, storage conditions, or functionality of the prebiotic fiber in the finished beverage (Yousaf *et al*., 2010; Davim *et al*., 2015; Ghavidel *et al*., 2014; Renuka *et al*., 2009). Therefore, it is necessary to evaluate the addition of a potential prebiotic fiber such as RMD and its impact on the food matrix. Furthermore, the effect of RMD addition to the physicochemical properties of pasteurized orange juice was studied (Arilla *et al*., 2020).

This study aimed to evaluate the effect of RMD addition before pasteurization treatment on the bioactive compounds (total phenols, total carotenoids (TC), AA, and vitamin C) and the antioxidant capacity (AC) of orange juice. Moreover, an *in vitro* digestion simulation was performed to analyze their bioaccessibility.

MATERIALS AND METHODS

Raw materials

This study was conducted with freshly squeezed orange juice supplied by Refresco Iberia S.A.U. (Valencia, Spain). All oranges were from Spanish origin. RMD (Fibersol-2) added to the juice was purchased from ADM/Matsutani, LLC (Decatur, IL, USA). Frozen pasteurized orange pulp was provided by a local fruit processing company (Zumos Valencianos del Mediterráneo, Valencia, Spain).

Sample preparation and pasteurization

Eight orange juice samples were prepared to conduct this study. Four were with orange pulp-added (orange juice with pulp, OJP), and four were without orange pulp (orange juice without pulp, OJWP). To minimize differences between samples, one single batch of orange juice was selected and separated into two tanks. Orange pulp was added to one tank and the other was left pulp-free. Pulp content was homogenized using a stirrer (LH Overhead Stirrer, VELP Scientifica, Usmate, Italy), by applying 200 rpm for 5 min. Then, each tank was transferred to 4 new tanks (8 in total) to add increasing RMD concentrations (2.5%, 5%, and 7.5%). This way, for a finished drink portion of 200 g, 5, 10, and 15 g of RMD would be ingested, enough to display its prebiotic effect. Control samples without RMD were also prepared. All orange juices were pasteurized (Fruchtsaftdispenser, Mabo Steuerungselement GmbH, Eppingen, Germany) at 85 °C for 10 s and hot-filled in 250 mL polyethylene terephthalate (PET) bottles.

Analytical determinations

ºBrix, Acidity and pH

Measurement of total soluble solids (TSS) by refractometry (Abbemat 200, Anton Paar, Austria), acidity as grams of citric acid per 100 mL (g AC/100 mL) (DL53 acid titrator, Mettler Toledo, Switzerland), and pH (Basic 20 pH meter, Crison, Spain) were performed as basic quality control parameters for the orange juices.

Total phenols (TP)

Determining TP was based on the Folin–Ciocalteu method. The extraction procedure consisted of homogenizing 35 g of the orange juice for 1 min with 50 mL of methanol. The homogenate was centrifuged (10,000 rpm, 10 min, 4 °C) to obtain the supernatant.

Then, 15 mL of distilled water and 1.25 mL of Folin–Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) were added to 250 µL of the supernatant. The samples were mixed and allowed to stand for 8 min in darkness before 3.75 mL of 7.5% sodium carbonate aqueous solution was added. Water was added to adjust the final volume to 25 mL. Samples were allowed to stand for 2 h at room temperature before measurement. Absorbance was measured at 765 mm in a UV-visible spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA). The total phenolic content was expressed as mg of gallic acid equivalents (GAE) (Sigma-Aldrich, Steinheim, Germany) per 100 mL or g of orange juice (Igual *et al*., 2016). Samples were analyzed in triplicate before and after *in vitro* digestion.

Total carotenoids (TC)

The TC in the samples before and after *in vitro* digestion were extracted with a solvent hexane/acetone/ethanol mixture following the method of Olives Barba *et al*. (2006) in triplicate. Sample absorbance was measured at 446 nm in a UV-visible spectrophotometer (Thermo Electron Corporation). The TC content was expressed as mg of β-carotene (Fluka-Biochemika) per 100 mL or g of orange juice.

Ascorbic acid (AA) and vitamin C

AA and vitamin C (ascorbic acid + dehydroascorbic acid) were determined using a HPLC-UV detector (Jasco equipment, Italy) in triplicate. The method proposed by Xu *et al*. (2008) was used to determine the ascorbic acid with some modifications made by Igual *et al*. (2016). To determine the ascorbic acid, 1 g of the sample was extracted with 9 mL 0.1% oxalic acid for 3 min and immediately filtered (0.45 µm) before injection. The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as the reductant reagent. A 0.5 mL aliquot sample was taken to react with 2 mL of a 20 g/L dithiothreitol solution for 2 h at room temperature and in darkness. Afterwards, the same procedure as that used for the ascorbic acid method was performed. The HPLC method and instrumentation was Ultrabase-C18, 5 µm (4.6 × 250 mm) column (Scharlab, Barcelona, Spain); mobile phase 0.1% oxalic acid, volume injection 20 µL, flow rate 1 mL/min, detection at 243 nm and at 25 °C. An AA standard solution (Sigma-Aldrich, Steinheim, Germany) was prepared.

Antioxidant capacity (AC)

AC was assessed using the free radical scavenging activity of the samples evaluated with the stable radical 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) following the methodology of Igual *et al*. (2019) in triplicate. Samples were mixed with methanol. The homogenate was centrifuged (10,000 rpm, 10 min, 4 °C) to obtain the supernatant. A total of 0.1 mL of supernatant was added to 3.9 mL of DPPH (0.030 g/L, Sigma-Aldrich, Steinheim, Germany) in methanol. A UV-visible spectrophotometer (Thermo Electron Corporation) was used at the absorbance of 515 nm. The results were expressed as milligram Trolox equivalents (TE) per 100 mL or g.

In Vitro Digestion

Sample *in vitro* digestibility (IVD) (%) was assessed using the standardized static *in vitro* digestion method suitable for food (COST INFOGEST network) proposed by Minekus *et al*. (2014). Four steps were followed: the oral phase, mixing the sample and simulate salivary fluid (SSF) (1:1) with amylase at pH 7 for 2 min; the gastric phase, mixing the oral bolus and simulate gastric fluid (SGF) (1:1) with pepsin at pH 3 for 2 h; the intestinal phase, mixing the gastric chyme and simulate intestinal fluid (SIF) (1:1) with enzymes at pH 7 for 2 h; centrifuging at 4500 rpm for 30 min and then a filtration, filtering through a 1 µm glass-fiber membrane (Uribe-Wandurraga *et al*., 2020).

The *in vitro* digestibility was calculated as the difference between the initial mass and the undigested mass (after correcting for the blank assay), divided by the initial mass, and multiplied by 100, according to Batista *et al*. (2017). Analyses were repeated in triplicate. Samples obtained at the end of *in vitro* digestion were collected according to Minekus *et al*. (2014). These were freeze-dried with a protease inhibitor. With crude protein values calculated, the bioaccessibility was determined using Equation (1), proposed by Khouzam *et al*. (2011).

$$\mathbf{Bioaccessibility} = \left(\frac{\mathrm{A}}{\mathrm{B}}\right) \times \mathbf{100} \qquad (1)$$

where, A is the concentration of the bioactive compounds in the bio-accessible fraction after *in vitro* digestion (filtrate after filtration); B is the concentration of the bioactive compounds in the sample before digestion. Possible bioactive compounds present in tap water and the reagents were also analyzed and corrected in the final bio-accessible fraction.

Statistical Analysis

Analysis of variance (ANOVA) was applied with a confidence level of 95% ($p < 0.05$), to evaluate the differences between the samples. Furthermore, a correlation analysis among studied bioactive compounds and antioxidant capacity of juices, with a 95% significance level was conducted. Statgraphics Centurion XVII Software, version 17.2.04 (Statgraphics Technologies, Inc., The Plains, VA, USA) was used.

RESULTS AND DISCUSSION

Effect of RMD on TSS, Acidity, pH, and Bioactive Compounds of Pasteurized Orange Juice

TSS, acidity, and pH (Table 1) were evaluated as basic quality control parameters, as they are related to the stability of bioactive compounds in plant-derived products (Sánchez-Moreno *et al.*, 2003). Control samples without RMD (OJP0 and OJWP0) agreed with values obtained by other authors (Sánchez-Moreno *et al.*, 2003; Wibowo et al., 2015; Mennah-Govela & Bornhorst, 2017). In orange juice, soluble solids are sugars, mainly fructose, sucrose, and glucose, that, with the citric acid content, determine the characteristic balance of sweetness and sourness that makes orange juice appealing to consumers (Wibowo et al., 2015). Increasing RMD concentrations led to increasing TSS values ($p < 0.05$). RMD is a water-soluble fiber and, therefore, dissolves in aqueous matrices, such as orange juice. Other studies have also reported that prebiotic fiber addition led to an increase in TSS in fruit-based beverages (Pimentel *et al.*, 2015; Igual *et al.*, 2019). Therefore, prebiotics go beyond their functional properties contributing to sweetness, texture, and mouthfeel and have been proposed as sugar replacers (Singla & Chakkaravarthi, 2017; Priya, 2020). Nevertheless, OJP samples had slightly lower soluble solids content because they contained orange pulp, which is an insoluble fiber. Contrary to the TSS, citric acid content significantly decreased ($p < 0.05$) with higher RMD concentrations in both OJP and OJWP samples, because its addition implied the replacement of raw orange juice in the finished beverage.

Table 1. Mean values (and standard deviations) of TSS, pH, and acidity of pasteurized orange juice.

Sample	TSS	Acidity (g CA/100 mL)	pH
OJP0	11.38 ± 0.03^{a}	0.773 ± 0.04^{h}	3.683 ± 0.006^{a}
OJP2.5	13.58 ± 0.02^{c}	0.747 ± 0.002^{g}	3.677 ± 0.006^{a}
OJP5	15.75 ± 0.04^{e}	0.725 ± 0.002^{f}	3.69 ± 0.02^{a}
OJP7.5	17.99 ± 0.04^{g}	0.711 ± 0.002^{e}	3.71 ± 0.02^{a}
OJWP0	11.47 ± 0.08^{b}	0.691 ± 0.002^{d}	3.80 ± 0.03^{b}
OJWP2.5	13.72 ± 0.03^{d}	0.670 ± 0.003^{c}	3.90 ± 0.04^{b}
OJWP5	15.9 ± 0.05^{f}	0.6530 ± 0.0005^{b}	3.827 ± 0.006^{b}
OJWP7.5	18.09 ± 0.02^{h}	0.636 ± 0.002^{a}	3.823 ± 0.006^{c}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp.

Moreover, orange pulp addition seemed to play a role in the citric acid content, because OJWP had significantly lower acidity values ($p < 0.05$) than OJP samples. This suggests that orange pulp contains higher citric acid content than orange juice; therefore, its addition increased citric acid values. It also played a significant role ($p < 0.05$) in terms of pH. This similar behavior for TSS, acidity, and pH were reported in our previous paper on the same topic (Arilla *et al.*, 2020).

Oxidative stress is related to several detrimental effects on human health (Pisoschi & Pop, 2015; Pizzino *et al.*, 2017). To manage this condition through diet, maintaining the maximum amount of antioxidant compounds from the food matrix is key, as antioxidants may prevent or delay oxidative cell damage (Apak *et al.*, 2018). Citrus fruits, and especially orange juice, have been reported to provide an important dietary source of bioactive compounds, including TP, TC, AA, and vitamin C, which contribute to its AC (Sánchez-Moreno *et al.*, 2003; Sádecká *et al.*, 2014). Table 2 compiles the mean values (with standard deviations) of these compounds and AC of control OJP0 and OJWP0 samples. OJP0 showed higher ($p < 0.05$) TP content than OJWP0, meaning that pulp addition to orange juice may increase TP content in the finished beverage. This may be because orange pulp contains hesperidin, which is the main phenolic compound in oranges for health-promoting activities (Iglesias-Carres *et al.*, 2019). Sádecká *et al.* (2014) also reported that pulp-added pasteurized orange juice showed higher TP content (hesperidin) compared to pasteurized OJWP. Moreover, De Ancos *et al.* (2017) found that orange pulp had a 1.6 times higher TP concentration than orange juice. In addition, OJP0 also obtained significantly higher ($p < 0.05$) TC content than OJWP0. Other studies on citrus products also demonstrated the importance of pulp in terms of TC content. For example, Rodrigo *et al.* (2015) found an increase close to 40% in the TC content in orange pulp compared to freshly prepared orange juice. Following the same trend as with TP and TC content, OJP0 exhibited higher ($p < 0.05$) AA and vitamin C contents than OJWP0. This could be explained by the fact that citrus by-products, including orange pulp, have been suggested as a good source of AA in terms of quantity (Al-Juhaimi, 2014). Thus, OJP0 had higher AC than OJWP0 ($p < 0.05$), exclusively because of orange pulp presence. Therefore, orange pulp addition to orange juice could be beneficial, leading to an increased AC.

Table 2. Mean values (and standard deviations) of total phenols (TP), total carotenoids (TC), ascorbic acid (AA), and vitamin C content of pasteurized orange juice with and without RMD addition, OJP0 and OJWP0, respectively (control samples).

Compounds	OJP0	OJPW0
TP (mg$_{GAE}$/100 mL)	99.8 ± 1.2^{a}	88.9 ± 0.6^{b}
TC (mg$_{\beta\text{-carotene}}$/100 mL)	7.13 ± 0.02^{a}	6.97 ± 0.02^{b}
AA (mg$_{AA}$/100 mL)	$5.81 \pm 0.13_{a}$	5.76 ± 0.07^{a}
Vitamin C (mg$_{\text{Vitamin C}}$/100 mL)	6.80 ± 0.04^{a}	6.66 ± 0.02^{b}
AC (mg$_{TE}$/100 mL)	106.1 ± 0.5^{a}	102.3 ± 0.3^{b}

The same letter in superscript within the row indicates homogeneous groups established by ANOVA ($p < 0.05$).

Some differences were found between the bioactive compounds registered in both OJP0 and OJWP0 samples compared to other orange and citrus juice-based studies. Agcam *et al.* (2014) showed lower TP content in orange juices treated using pulsed electric fields and conventional pasteurization. Moreover, Velázquez Estrada *et al.* (2013) and Stinco *et al.* (2020) reported lower TC content in fresh, high-pressure homogenized and pasteurized orange juices. Similarly, Sánchez-Moreno *et al.* (2003) also had a lower TC content in commercial orange juices stored at room, cold, and frozen temperatures. In contrast, Aschoff *et a*l. (2015) found higher AA and vitamin C contents in fresh and pasteurized orange juices. Bioactive compounds content in fruit-based juices is influenced by many aspects, such as the post-harvest, cultivar, or processing system. Therefore, it may be difficult to find similar ranges of bioactive compounds, as many aspects influence its quantification.

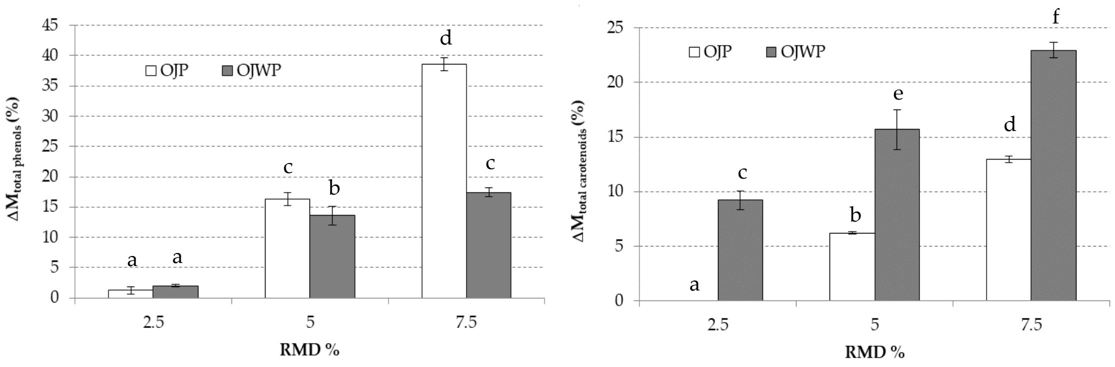
To evaluate the effect of RMD addition on the intrinsic bioactive compounds of pasteurized orange juice, the variation of each component (ΔMi) in all RMD-added samples with increasing RMD concentrations (2.5%, 5%, and 7.5%), referred to as OJP0 and OJWP0, respectively, was calculated according to Equation (2):

$$\Delta \boldsymbol{M}_i^{\boldsymbol{RMD}\%} = \frac{(\boldsymbol{M}_i^{\boldsymbol{RMD}\%} - \boldsymbol{M}_i^{\boldsymbol{Control}})}{\boldsymbol{M}_i^{\boldsymbol{Control}}} \times \mathbf{100} \qquad (2)$$

where, Mi: mass of compound i in the sample obtained from 100 g of pasteurized orange juice control (OJP and OJWP) and superscripts, RMD%: percentage of RMD of the sample (2.5, 5, and 7.5) and control (OJP or OJWP).

According to this equation, the greater the positive variation of each pasteurized orange juice RMD-added sample compared to its control sample, the greater the protective effect RMD displays on the intrinsic bioactive compounds of orange juice, as higher amounts of each bioactive compound would be found.

Figure 1a shows that higher concentrations of RMD added before orange juice pasteurization led to a higher ($p < 0.05$) protective effect on TP content in both OJP and OJWP samples. Besides this, OJP samples showed significantly ($p < 0.05$) higher TP variation values than OJWP samples, following the same trend as in control samples (Table 2). The difference between OJP and OJWP samples in terms of TP variations was higher, as higher concentrations of RMD were applied. Therefore, according to several studies, the protective effect that RMD displays on TP might help to preserve, or even improve, the health-promoting and prebiotic-like effect that phenolic compounds exert (Dueñas *et al*., 2015; Espín *et al*., 2017; Lima *et al*., 2019; Iglesias-Carres *et al*., 2019).

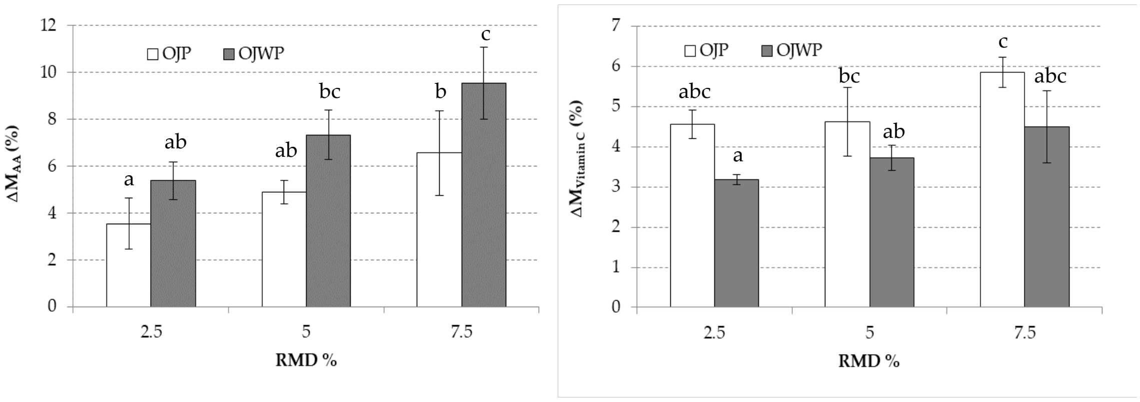


(a) (b)

Figure 1. (a) Mean values and standard deviation of total phenols variation of pasteurized orange juice (OJP and OJWP) with 2.5, 5, and 7.5 RMD%. (b) Mean values and standard deviation of total carotenoids variation of pasteurized orange juice (OJP and OJWP) with 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

The assessment of carotenoids in citrus products is difficult because of their complex carotenoid profile and because of the inherent acidity of these products (Meléndez-Martínez *et al*., 2008; Valente *et al*., 2014). TC were measured to compare the effect of RMD addition in OJP and OJWP samples. As it was observed in Table 2, OJP0 had significant higher TC content than OJWP0 ($p < 0.05$), probably because the TC content is higher in the pulp than in the orange juice. Figure 1b shows that both OJP and OJWP presented higher positive variations of TC content as RMD concentration increased significantly ($p < 0.05$), an effect greater in OJWP samples.

Vitamin C comprises two biologically active forms, AA and DHAA (Valente *et al*., 2014). Content variations of AA and vitamin C because of RMD addition in OJP and OJWP samples are represented in Figure 2. Higher RMD concentrations led to significantly increased AA and vitamin C content ($p < 0.05$), more noticeable in OJWP and OJP samples, respectively. Therefore, orange pulp seems to interact with RMD to increase vitamin C protection from oxidative enzymes (i.e., AA oxidase and peroxidase) when the food matrix is disrupted by heat treatment (Rodríguez-Roque *et al*., 2013). Loss of vitamin C, because of heat treatment, is undesirable because of its many health-related implications. In fact, the role that vitamin C might play for the prevention and treatment of COVID-19 is being investigated (Carr & Rowe, 2020). RMD addition to orange juice has been shown to exert a protective effect on vitamin C, which is of prime interest nowadays. In accordance with these results, Alves Filho *et al*. (2018) pointed out that adding prebiotic fibers, such as inulin and gluco-oligosaccharides, exhibited a protective effect on the vitamin C of heat-treated acerola juice. Finally, despite the observation that RMD addition to orange juice displayed a protective effect on AA and vitamin C content, this effect was more noticeable in TP and TC, as the positive variations registered in those compounds were greater.



(a) (b)

Figure 2. (a) Mean values and standard deviation of ascorbic acid variation of pasteurized orange juice (OJP and OJWP) with 2.5, 5, and 7.5 RMD%; (b) Mean values and standard deviation of vitamin C variation of pasteurized orange juice (OJP and OJWP) with 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA (p < 0.05) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

Figure 3 shows the AC variation in each RMD-added OJP and OJWP sample. As with all bioactive compounds, the higher the RMD concentration in the pasteurized orange juice, the significantly greater the protective effect on the AC (p < 0.05). This was seen in the pulp-free samples, where OJWP7.5 achieved the highest (p < 0.05) AC variation. Therefore, adding prebiotic fibers, such as RMD, could play a key role in preserving intrinsic health-promoting compounds from thermal degradation. Fonteles *et al*. (2021) also reported that inulin addition to acerola juice displayed a protective effect on preserving bioactive compounds (vitamin C and TP) after thermal processing, therefore leading to higher AC.

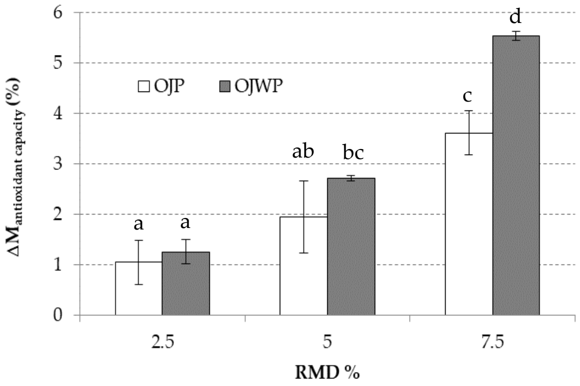


Figure 3. Mean values and standard deviation of antioxidant capacity (AC) variation of pasteurized orange juice (OJP and OJWP) with 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA (p < 0.05) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

To explain the relationships in the different compounds quantified in this study with the AC and the relationships among them, correlation statistical analyses were performed. The studied bioactive compounds showed a positive Pearson's correlation coefficient with AC. Vitamin C and TP played a key role in the AC of orange juices, showing 0.8916 ($p < 0.05$) and 0.8647 ($p < 0.05$), respectively. This behavior has been observed by other authors in citric products (Sánchez-Moreno *et al*., 2003; Xu *et al*., 2008; Igual *et al*., 2016). In fruit juices, it is widely accepted that AC is mainly related to AA and TP content (Sádecká *et al*., 2014). Likewise, Igual *et al*. (2016) found a significant correlation (0.8313, $p < 0.05$) between the AA and TC content in other citric-based products, probably because of the stabilizing effect of AA on carotenoids (Choi *et al*., 2002).

Effect of RMD on In Vitro Digestibility and Bioactive Compounds Bioaccessibility of Pasteurized Orange Juice

Figure 4 shows the IVD% of all OJP and OJWP samples. All orange juice samples obtained a high IVD% because orange juice has an aqueous matrix with monosaccharides (glucose and fructose) and disaccharides (sucrose) (Wibowo *et al*., 2015), which are easy to digest. Although RMD addition to orange juice slightly changed its digestibility, especially when higher doses of RMD were applied, no clear trend was observed. Thus, OJWP5 had the significantly lowest IVD% ($p < 0.05$), whereas OJWP7.5 had a significantly higher digestibility ($p < 0.05$). Moreover, despite not exerting a significant effect ($p > 0.05$), orange pulp slightly reduced the digestibility of the orange juice, because OJP samples presented lower IVD% than OJWP. This could be explained by the fact that orange pulp is an insoluble fiber that typically contains large amounts of cell wall polysaccharides (Schalow *et al*., 2018), which could pose difficult digestibility compared to the pulp-free orange juices.

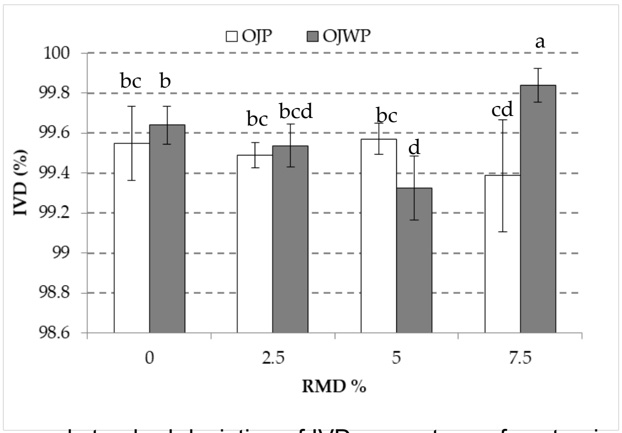
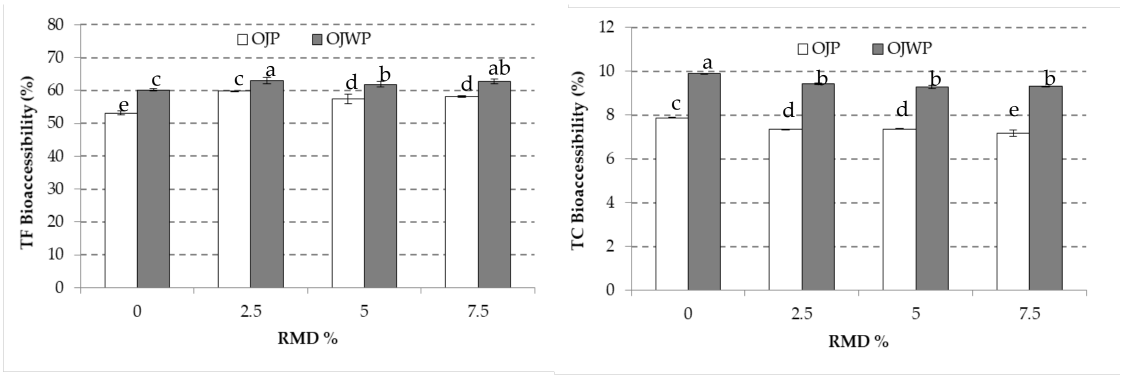


Figure 4. Mean values and standard deviation of IVD percentage of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp.

In vitro digestion is also useful for the estimation of pre-absorptive events, such as bioaccessibility of nutrients from a food matrix (Thakur *et al*., 2020). Figure 5a shows the TP bioaccessibility of all OJP and OJWP samples. TP bioaccessibility of OJWP samples was slightly higher ($p < 0.05$) than OJP samples, suggesting that orange pulp, despite adding hesperidin to orange juice (Iglesias-Carres *et al*., 2019), decreases phenols release from food matrices. However, RMD in the orange juice improved ($p < 0.05$) TP bioaccessibility, although higher RMD concentrations did not have a significant effect ($p > 0.05$). Likewise, Fonteles *et al*. (2021) reported that inulin addition to acerola juice increased TP bioaccessibility. Furthermore, Moser *et al*. (2020) also concluded that adding orange pomace and commercial pulverized citrus pulp fiber to orange juice enhances flavonoids bioaccessibility. In contrast, other studies on fruit juices have reported that the interaction between phenols and dietary fibers in the *in vitro* digestion process might reduce its solubility and availability (Rodríguez-Roque *et al*., 2013; De Ancos *et al*., 2017). The effect of dietary fibers on TP bioaccessibility was more related to the solubilization capacity of the fiber in the food matrix, as orange pulp (insoluble) reduced TP bioaccessibility, but RMD (soluble) presence improved it. In this regard, Schallow *et al*. (2018), who studied the water-binding and gelling properties of orange by-products, suggested that at a low TSS content, more water might be bound by the insoluble pulp fiber, promoting the aggregation of adjacent pectin chains, and helping to form larger and more resistant rupture junction zones. Therefore, orange pulp presence could impair TP release from an orange juice matrix. Furthermore, the TP bioaccessibility of all samples was higher than those reported in other blended fruit juice-based (orange, kiwi, and pineapple) (Rodríguez-Roque *et al*., 2013) and other orange fruit and juice-based studies (Moser *et al*., 2020). Besides this, the pasteurization process has been reported to improve TP bioaccessibility in fruit juices (Gil-Izquierdo *et al*., 2001; He *et al*., 2016).

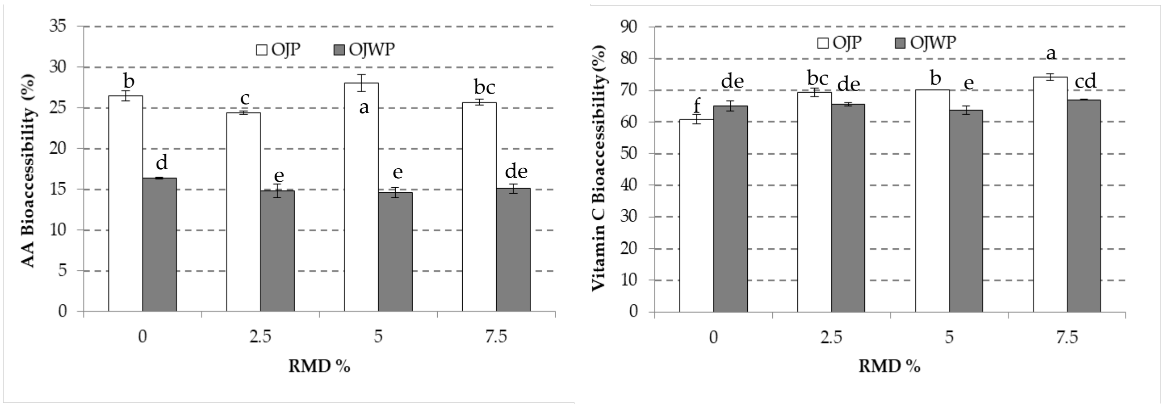
The TC bioaccessibility obtained for all samples (Figure 5b) were in the same range as those reported in other fruit juice-based studies (Rodríguez-Roque *et al*., 2013; Stinco *et al*., 2020). As with TP, orange pulp addition to orange juice also decreased TC bioaccessibility significantly ($p < 0.05$). These results agree with those reported in both *in vitro* (Rodríguez-Roque *et al*., 2013) and *ex vivo* (Hoffmann *et al*., 1999) studies that suggested that the bioaccessibility and absorption rate of carotenoids is negatively affected by fiber presence. In fact, intrinsic citrus pectin has been suggested to have a strong inhibitory effect on β-carotene absorption (Yonekura & Nagao, 2007; Aschoff *et al*., 2015). This might also explain why RMD addition to orange juice moderately decreased TC bioaccessibility compared to control samples but significantly ($p < 0.05$), although higher concentrations did not have a stronger effect ($p > 0.05$).



(a) (b)

Figure 5. (a) Mean values and standard deviation of total phenols (TP) bioaccessibility of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD%. (b) Mean values and standard deviation of total carotenoids (TC) bioaccessibility of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

Figure 6 shows the mean values and standard deviations of AA and vitamin C bioaccessibility of all OJP and OJWP samples. OJP samples obtained a greater ($p < 0.05$) AA bioaccessibility than OJWP. This indicates that orange pulp might play a protective role in the AA oxidation to DHAA during the *in vitro* digestion process. RMD addition to orange juice slightly decreased AA bioaccessibility in OJWP samples, although a higher RMD concentration did not have a stronger effect ($p > 0.05$).



(a) (b)

Figure 6. (a) Mean values and standard deviation of ascorbic acid (AA) bioaccessibility of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD%. (b) Mean values and standard deviation of vitamin C (VC) bioaccessibility of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD%. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

Regarding vitamin C bioaccessibility, except for the control samples where OJWP0 obtained a significantly higher vitamin C bioaccessibility ($p < 0.05$) than OJP0, the rest of RMD-added OJP samples showed significantly higher vitamin C bioaccessibility ($p <$

0.05) than OJWP samples. This suggests that RMD incorporation to orange juice might interact with orange pulp to increase DHAA protection during *in vitro* digestion. RMD significantly improved vitamin C bioaccessibility in OJP samples ($p < 0.05$), especially when higher RMD concentrations were applied, but did not have a significant effect in OJWP samples ($p > 0.05$). Similarly, inulin has been reported to improve vitamin C bioaccessibility in acerola juice (Fonteles *et al*., 2021). All OJP and OJWP showed comparable vitamin C bioaccessibility to those reported by De Ancos *et al*. (2017), who studied the influence of orange cultivars and mandarins post-harvest on vitamin C during *in vitro* digestion. Instead, other studies on fruit-based beverages reported lower vitamin C bioaccessibility (Rodríguez-Roque *et al*., 2013; Rodríguez-Roque *et al*., 2015; Uğur *et al*., 2020).

Figure 7 shows the mean values and standard deviations of AC of all OJP and OJWP samples after gastrointestinal digestion. The AC ranged from 19.0 ± 1.2 to 21.0 ± 0.7. The OJWP control gave the significantly highest ($p < 0.05$) AC. The OJP control presented significantly lower AC values ($p < 0.05$), probably due to the pulp effect on bioactive bioaccessibility. RMD addition slightly decreased the AC, more noticeably as higher RMD concentrations were added. Consequently, both 7.5% RMD-added OJP and OJWP samples gave the lowest significant AC ($p < 0.05$).

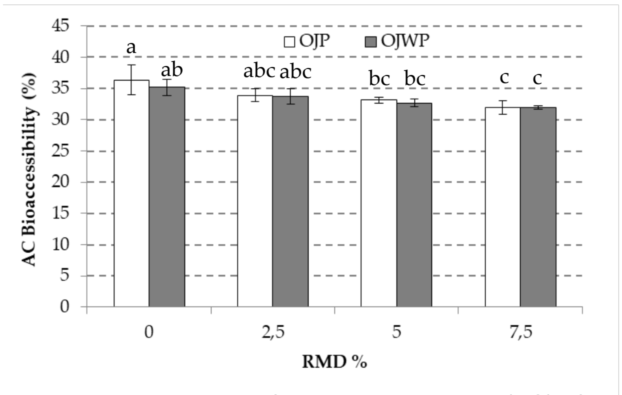


Figure 7. Mean values and standard deviation of antioxidant capacity (AC) of pasteurized orange juice (OJP and OJWP) with 0, 2.5, 5, and 7.5 RMD% after gastrointestinal digestion. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each parameter analyzed. OJP, orange juice with pulp; OJWP, orange juice without pulp.

However, even though RMD addition to orange juice displayed a slight effect by diminishing all bioactive compounds' bioaccessibility, the total amount of bioactive compounds that remained available to be absorbed in the human gut were higher than in control samples. This is because RMD-added samples before *in vitro* digestion showed a significantly higher bioactive compounds content, in absolute terms, than the control samples.

CONCLUSIONS

RMD addition before the pasteurization juice process protected all bioactive compounds, namely TP, TC, AA, and vitamin C, as well as the AC. Moreover, this protective effect for the bioactive compounds of orange juice was higher when higher RMD concentrations were applied.

Orange pasteurized juice with pulp presented significantly higher values of all bioactive compounds than orange pasteurized juice without pulp; therefore, orange pulp could play a key role regarding the antioxidant properties of pasteurized orange juice.

Concerning bioaccessibility, RMD addition improved TP and vitamin C bioaccessibility but decreased TC and AA bioaccessibility. Subsequently, the AC value of samples after gastrointestinal digestion was slightly decreased by RMD addition. Besides, orange pulp presence decreased TP and TC bioaccessibility but increased AA and vitamin C bioaccessibility.

This study shows that RMD could have interesting applications in the food technology field, leading to health-related benefits. Besides the prebiotic component of RMD, it also displays other important activity to protect health-promoting compounds from degradation because of heat treatment, which is the most common means to preserve fruit juices. However, it would be interesting to know the evolution and stability of the bioactive compounds studied during storage. For this reason, the authors are developing an experiment, and the results obtained now are favorable.

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CHAPTER 5. SENSORY EVALUATION, PHYSICOCHEMICAL PROPERTIES AND AROMATIC PROFILE OF PASTEURIZED ORANGE JUICE WITH RESISTANT MALTODEXTRIN

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ABSTRACT

The beneficial health effects of prebiotics have been demonstrated in numerous research papers. However, their incorporation into daily food remains unfamiliar to consumers. This work evaluates the effects of the addition of resistant maltodextrin (RMD) on the sensory attributes of pasteurized orange juice, together with the physicochemical properties and the aromatic profile. RMD addition increased the sweetness and decreased the acidity and bitterness, resulting in a higher overall panelists' rating of orange juice. It also proportionally increased °Brix together with density and decreased acidity. Color changes were registered with higher RMD concentrations. Orange pulp presence affected the volume particle size distribution analysis, while RMD addition did not have any effect. The aroma volatile compounds were also analyzed. Pulp-added samples showed a higher quantity of alcohol and aldehydes, whereas pulp-free samples registered higher terpene and terpenoid values. Ketones and acids were also quantified. RMD had a moderate impact on volatile compound quantifications, with the orange pulp presence playing a much more decisive role. A correspondence analysis was also performed to relate instrumental and sensory determinations for all samples. This work proves that the addition of RMD to orange juice is technologically feasible while also achieving a good response at the sensory level.

INTRODUCTION

Consumers' consciousness towards a healthy diet in recent years has increased the demand for high-value food products, such as functional ingredients (Karelakis *et al.*, 2020), aiming to improve the nutritional properties of conventional food and beverages. Within the functional ingredients, prebiotics emerge as a route of great interest to meet consumers' needs due to their contribution to human health. Prebiotics are a group of dietary fibers whose selective fermentation results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health (Gibson *et al.*, 2010). Substances such as inulin, fructo-oligosaccharides, gluco-oligosaccharides, lactulose, isomalto-oligosaccharides, or lactosucrose have been widely studied because of their prebiotic activity and their effects on human health (Gibson *et al.*, 2004; Corzo *et al.*, 2015). In addition, resistant maltodextrin (RMD), which is a corn-based non-digestible fiber (Lockyer & Nugent, 2017), is currently gaining popularity in clinical settings (Kishimoto *et al.*, 2007; Livesey & Tagami, 2009; Baer *et al.*, 2014; Ye *et al.*, 2015).

The functional foodstuff trend represents an innovation focus for the industry to develop new food products that go beyond basic nutrition and good taste (Day *et al.*, 2009; Priyadarshini & Priyadarshini, 2018; Singla & Chakkaravarthi, 2017). As such, it entails technological challenges depending on the food matrix, processing, packaging, shelf life, and other aspects. For this reason, it is convenient to investigate the application of these substances to easily manageable matrices, such as beverages. Accordingly, orange juice is a suitable vehicle for this kind of functional ingredient because it already contains functional compounds (Fonteles & Rodrigues, 2018) and because it is the most preferred fruit juice flavor among consumers (Priyadarshini & Priyadarshini, 2018; Neves *et al.*, 2020). In addition, prebiotics added to liquid matrices could be more effective in terms of health impact because they are usually easier to digest than solid foods. The application of a pasteurization process to commercial orange juice is widespread. However, it is well known that thermal treatments cause a complex series of chemical reactions which directly affect the aroma volatile compounds, either losing the original aroma or developing foreign odors to fresh orange juice (Ruiz Perez-Cacho & Rouseff, 2008). Since RMD is a starch-based ingredient, it could be used to retain and protect volatile compounds, therefore positively counteracting their loss through the evaporation process (Jørgensen *et al.*, 2012). Packaging technologies and materials also have a role in the flavor retention of orange juice, reducing the intensity of aroma compounds (Berlinet *et al.*, 2007). For instance, van Willige *et al.* (2003) highlighted that limonene, myrcene, and decanal amounts could be reduced during orange juice storage through the absorption of the PET packaging. Therefore, protecting or improving the original sensory attributes of heat-treated orange juice is an interesting challenge that needs to be further exploited.

To achieve social acceptance of prebiotic-added foods, it is of great relevance to elucidate the impact of such compounds on food products. The potential health benefits of prebiotics have been described in clinical trials (Pujari & Banerjee, 2021). However, the incorporation of functional ingredients into day-to-day foods is not yet commonplace (Baker *et al*., 2022). Therefore, this work aimed to evaluate the sensory attributes of pasteurized orange juice with RMD, in addition to the physicochemical properties and the aromatic profile. Analyzing how functional ingredients influence the sensory properties of conventional foods is essential to improving consumer predisposition towards healthier eating habits.

MATERIALS AND METHODS

Raw materials

This study was conducted with freshly industrially squeezed orange juice supplied by Refresco Iberia S.A.U. (Valencia, Spain). All oranges used were of Spanish origin from different cultivars, such as Navelina, Salustiana, Navel, Navel Late, Lane Late, Navel Powell, and Valencia. Oranges were grown in conventional planting conditions. The orange juice extraction followed the standard manufacturing procedure: reception of oranges from suppliers, surface cleaning of the orange peel, classification according to fruit diameter, juice extraction in industrial juicers (Citrus Juice Extractor Model 593, JBT, Chicago, IL, USA), and sieving to separate the residual pulp from orange juice. Orange juices from different cultivars were mixed to ensure a homogeneous quality, as is commonly performed in the juice industry. RMD (Fibersol-2) added to the juice was purchased from ADM/Matsutani, LLC (Decatur, IL, USA). Frozen pasteurized orange pulp was provided by a local fruit processing company (Zumos Valencianos del Mediterráneo, Valencia, Spain).

Sample preparation and pasteurization

Eight samples of orange juice were prepared. Four were orange juice with pulp (OJP), and the other four were orange juice without pulp (OJWP). Fresh orange juice was directly collected from the industrially squeezed lines. Orange pulp (2.5%) was added to the OJP samples. Pulp content was homogenized using a stirrer (LH Overhead Stirrer, VELP Scientifica, Italy) by applying 200 rpm for 5 min. Increasing RMD concentrations (2.5, 5, and 7.5%) were mixed into OJP and OJWP samples. Thus, for a final beverage portion of 200 mL, 5, 10, or 15 g of RMD, respectively, would be ingested, enough to display functional effects according to other studies (Kishimoto *et al*., 2007; Livesey & Tagami, 2009; Baer *et al*., 2014; Ye *et al*., 2015). Control samples without RMD addition (OJP0 and OJWP0) were also prepared, and they complied with the European Fruit Juice Association orange juice guidelines (AIJN, n.d.), so no adulteration or deviation occurred during the juice extraction. To properly dissolve RMD in the fresh orange juice, the same stirrer was used at 200 rpm for 15 min. Finally, all samples were pasteurized (Fruchtsaftdispenser, Mabo Steuerungselemente GmbH, Germany) at 85 °C for 10 s, and were hot filled into 250 mL polyethylene terephthalate (PET) bottles. After the heat treatment, all bottles were immersed in a cold-water bath (<10 °C) for 30 min to cool down.

Sensory analysis

An expert panel of 15 members, 6 men and 9 women, performed a sensory analysis of the orange juice samples. All members of the panel, aged between 28 and 55 years old, volunteered from the Quality Control department of Refresco Iberia S.A.U., an orange juice manufacturer and bottling company. Therefore, they had professional experience testing orange juice. They evaluated the color, aroma, sweetness, acidity, bitterness, mouthfeel (softness, thickness), off-flavor, and overall rating as the attributes of the orange juice samples using a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) (ISO, 2003).

During the test session, panelists worked isolated in individual booths. All samples were presented in a random order to the panelists at 20 °C under normal lighting conditions in 50 mL cups, and a 3-digit random number was placed on them, identifying each sample. Water at room temperature was given to the panelists to clean the palate before trying the next sample. A testing sheet for each sample identified with the 3-digit random number was given to each panelist to record the results of the sensory evaluation. During the session, the panelists evaluated all 8 samples (with and without orange pulp addition and with and without RMD addition).

All participants gave their informed consent before engagement in this study. For data confidentiality purposes, each member of the panel was assigned a random three-digit code. Data were treated anonymously and following the European General Data Protection Regulation (Regulation E.C. (2016). No 679/2016 of the European Parliament and of the Council of 27 April 2016).

Physicochemical determinations

ºBrix, pH, acidity and density

Total soluble solids (°Brix) were measured with refractometry (Abbemat 200, Anton Paar, Austria). pH determination was conducted using a Basic 20 pH meter (Crison, Barcelona, Spain). Acidity, expressed as grams of citric acid per 100 mL (gCA/100 mL), was determined using a DL53 acid titrator (Mettler Toledo, Greifensee, Switzerland). Density was obtained using a densimeter (DMA 5000, Anton Paar, Austria). All determinations were performed in triplicate, following AOAC guidelines (Latimer, 2012).

Particle size

Particle size distribution of juices was determined with the laser diffraction method and Mie theory (ISO, 2020), using a particle size analyzer (Malvern Instruments Ltd., Mastersizer 2000, UK) equipped with a wet sample dispersion unit (Malvern Instruments Ltd., Hydro 2000 MU, UK). Laser diffraction reports the volume of material of a given size, since the light energy reported by the detector system is proportional to the volume of material present. The Mie theory requires information on the sample and the dispersant optical properties. For orange juice, the particle refraction and absorption were 1.52 and 0.1, respectively, and the water refraction index was 1.33. The sample was dispersed in distilled water and pumped through the optical cell under moderate stirring (1800 rpm) at 20 °C. The volume (%) against particle size (in μm) was obtained, and the size distribution was characterized by the volume mean diameter (D[4,3]). The standard percentile d(0.1), or particle size below which 10% of the sample lies, and d(0.9), or particle size below which 90% of the sample lies, were also considered for juice characterization.

Color measurement

The sample color was measured using a colorimeter (Konica Minolta CM-700 d/600 d series, Tokyo, Japan) with a standard illuminant D65 and a visual angle of 10°. Results were obtained in terms of L* (brightness: L* = 0 (black), L* = 100 (white)), a* (−a* = CIELab system (CIE, 2018). Total color differences (ΔE) were calculated by comparing each sample with RMD with its corresponding control (OJWP0 or OJP0).

Analysis of aroma volatile compounds with ITEX/GS-MS

The extraction and analysis of volatile compounds were performed according to Igual *et al*. (2021) using the in-tube extraction technique (ITEX) followed by their separation and identification with gas chromatography–mass spectrometry (GC-MS), using a GC-MS

QP-2010 model (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a Combi-PAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland) and a capillary column (ZB-5 ms, 30 m × 0.25 mm i.d. × 0.25 µm, Phenomenex, Torrance, CA, USA). For the volatile extraction step, a hermetically sealed headspace vial containing 0.5 g of sample was incubated at 60 °C under continuous agitation for 10 min. After the incubation, from the headspace phase, the volatile compounds were adsorbed (aided by the headspace syringe) repeatedly (15 strokes) into a porous polymer fiber microtrap (ITEX-2TRAPTXTA, Tenax TA 80/100 mesh, ea). The extraction of the volatile compounds and their thermal desorption and injection into the GC-MS injector were performed automatically using the Combi-PAL AOC-5000 autosampler. The following parameters were used for the column oven: from 38 °C, the temperature rose to 110 °C and then to 250 °C at 4 °C/min and 20 °C/min, respectively, and the final temperature was held for 5 min. The identification of the samples' volatiles was based on their mass spectra using the software's NIST27 and NIST147 mass spectra libraries and was verified and compared with retention indices drawn from databases (The Pherobase, n.d.; Flavornet and Human Odor Space, n.d.). Results are expressed as a relative percentage of the total peak area.

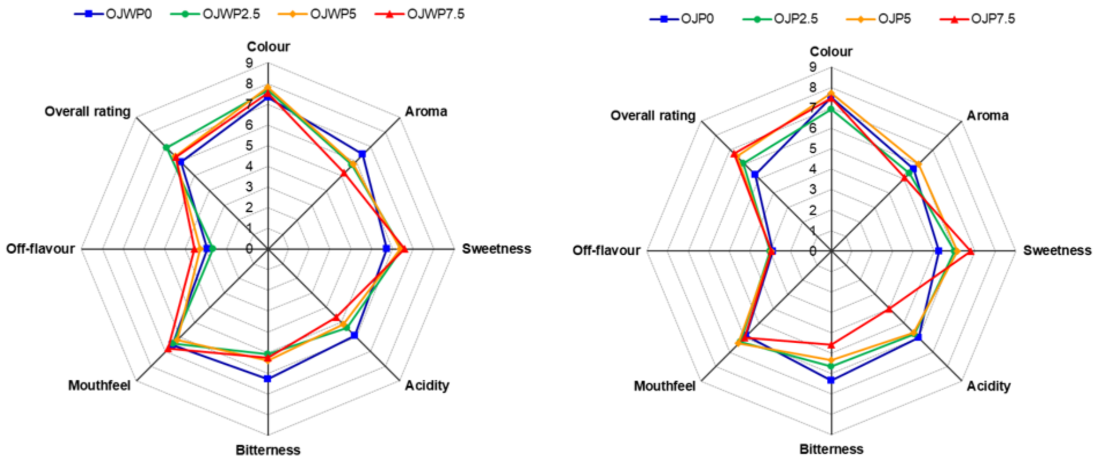
Statistical analysis

Analysis of variance (ANOVA) was applied with a confidence level of 95% ($p < 0.05$) to evaluate the differences among samples. Furthermore, a correspondence analysis (CA) among sensory attributes of juices was conducted with a 95% of significance level. A multiple factor analysis (MFA) was also carried out with the mean values of all data (instrumental and sensorial) to explore the relationship between them. Each sample is represented by 8 points, grouping sensory and instrumental variables studied in the samples. The consensus representation that considers the 8 variables simultaneously is also represented for each juice. From the sensory analysis, sweetness, acidity, and bitterness were grouped into sensory taste variables. On the other hand, off-flavors and flavor appeal were grouped into a variable named sensory flavor. Color and mouthfeel in the sensory test have been kept individually as sensory color and sensory texture, respectively. The variables obtained from the instrumental measurements have been grouped as follows: L*, a*, and b* as the instrumental color; °Brix and titratable acidity as the instrumental flavor; density and particle size parameter D[4,3] as the instrumental texture; and the different groups of analytically determined aromatic compounds as the instrumental aroma. Statgraphics Centurion XVII software, version 17.2.04 (Statgraphics Technologies, Inc., The Plains, VA, USA), and XLSTAT statistical software version 2021 were used (Lumivero, 2023).

RESULTS AND DISCUSSION

Sensory evaluation

Figure 1 shows the mean value scores of each sample without orange pulp (a) and with orange pulp (b) for each evaluated sensory attribute. In general terms, the most noticeable changes occurred in the sensory parameters related to smell and taste, such as aroma, sweetness, acidity, and bitterness, which affected the overall rating of the samples in both OJWP (a) and OJP (b) samples, respectively. OJWP0 was marked as having the most appealing aroma of all samples, with a mean value of 6.47 ± 2.17. OJWP0 also scored the highest value for acidity, with a mean value of 5.93 ± 1.44, and bitterness, with a mean value of 6.27 ± 1.62, and was ranked the lowest in terms of sweetness, with a mean value of 5.73 ± 1.91. OJP0 had similar scores to OJWP0, which led to the lowest overall rating scores for both standard samples, with mean values of 5.27 ± 1.39) and 5.93 ± 1.53, respectively. This was expected since the standard samples consist only of pasteurized orange juice (OJWP0) and pasteurized orange juice with pulp (OJP0), without any addition of RMD.



(a) (b)

Figure 1. Mean values scores of the different sensory attributes evaluated in pasteurized orange juice with or without pulp and 0–7.5 % of RMD addition. (a) OJWP, orange juice without pulp; (b) OJP, orange juice with pulp. Concentric octagonal isolines show the axis tick marks.

RMD addition to OJWP and OJP samples improved almost all the sensory attributes, except for the aroma, which obtained slightly lower scores. For example, samples with a 7.5% RMD concentration were scored mean values of 5.07 ± 2.19 for the pulp-added orange juice and 5.20 ± 1.90 for the pulp-free orange juice. This could be due to RMD addition, which led to a reduction in the orange juice content in the final sample, and therefore, an aromatic loss could be expected. Moreover, RMD-added OJP samples did not show any additional off-flavor compared to the standard OJP0 sample. In contrast, RMD-added OJWP samples presented more off-flavors, especially at high RMD concentrations, where the 7.5% sample had a mean value of 3.53 ± 2.10, in comparison to the OJWP0 with a mean value of 2.93 ± 1.79. Rega *et al*. (2024) stated that orange pulp strongly influences flavor release in orange juice and increases the fresh orange juice character. Thus, orange pulp seems to play a role in masking possible off-flavors derived from RMD addition to orange juice. In addition, the sweet/acid ratio has been identified as a basic precept when judging the sensory quality of many fruit-based products (Jayasena & Cameron, 2008). RMD incorporation increased the sweetness scores and decreased the acidity and bitterness scores, with this effect being higher at higher RMD concentrations and clearer in the OJP samples. Thus, OPJ7.5 had mean values of 6.80 ± 1.42, 4.00 ± 1.60, and 4.60 ± 1.24 for sweetness, acidity, and bitterness, respectively. This resulted in a higher overall rating of the RMD-added samples. This can be clearly observed in both OJP7.5 and OJWP7.5, which scored mean values of 6.73 ± 1.87 and 6.27 ± 1.58, respectively. This contrasts with the study by Luckow and Delahunty (2004), who found that consumers prefer the sensory characteristics of conventional orange juices to their functional (probiotic and prebiotic) counterparts. This could be an indicator that RMD is more suitable for developing sensory-attracting functional foods in comparison with other functional ingredients.

The Tukey's HSD (Honestly Significant Difference) method applied to the sum of ranks was used to perform a multiple comparison among the samples. The calculated Tukey's HSD value, according to assay conditions, was 40.7. When the difference between the sums of rank of each pair of samples, for each attribute, was greater than 40.7, significant differences between paired samples were assumed. Significant differences were not observed among the eight samples studied ($p > 0.05$). Therefore, the expert judges could not appreciate the differences among the samples for any attribute that showed a significant effect.

A correspondence analysis was carried out to relate the samples by means of the different juices with all the attributes evaluated and the assessors' preferences. From this analysis, two factors were obtained that explained 90% of the variability in the results (Figure 2). The first factor (F1) explained 76.08% of the variability, and the second (F2) explained 13.79%. Figure 2 shows the projection in the plane of the juices and attributes derived from the correspondence analysis. Samples are mainly ordered from left to right in the graph, from the lowest to the highest RMD concentration on F1. OJP0 was identified as more bitter, whilst OJWO was related to acidity and flavor intensity. The OJWP5 sample was favorably evaluated for its color and mouthfeel, and juices with higher concentrations of RMD were identified as the sweetest. Furthermore, sweetness is the sensory attribute that has the most weight on overall liking.

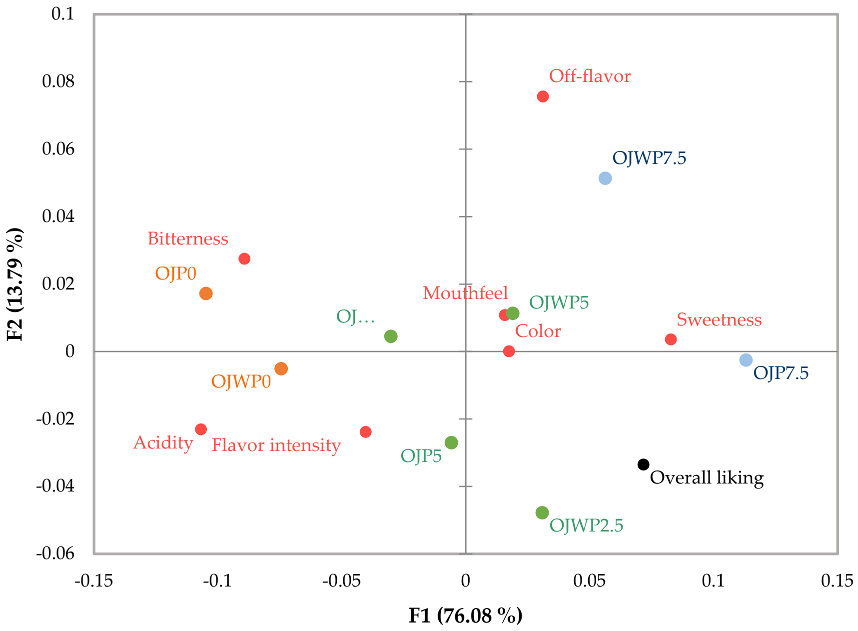


Figure 2. Correspondence analysis. Representation of sensory attributes and samples tested in the normalized plane defined by the two factors, explaining the variability in the results of the sensory analysis. OJWP, orange juice without pulp; OJP, orange juice with pulp; 0–7.5 % of RMD addition. Colors orange, green, and blue indicate different groups of clusters in CA.

Physicochemical properties

The °Brix content of orange juice comes from its sugar content, mainly fructose, sucrose, and glucose. Also, acidity is a result of its citric acid content. The °Brix/acid ratio plays a major role in the sensory properties of orange juice. For this reason, it is commonly used as a juice quality and fruit maturity indicator (Kimball, 2012). They are also measured to ensure non-deviation during the juice extraction process, in accordance with the AIJN (n.d.). OJWP0 had almost the same ($p > 0.05$) °Brix as OJP0 (Table 1). RMD addition proportionally increased ($p < 0.05$) the °Brix values of orange juice in both OJWP and OJP samples. This phenomenon was expected as RMD displays good dissolving properties in water (Lockyer & Nugent, 2017). Contrariwise, orange pulp, an insoluble fiber, did not have a significant ($p > 0.05$) impact on °Brix. Unlike with °Brix, adding higher concentrations of RMD to orange juice proportionally decreased ($p < 0.05$) its acidity. This could be explained since RMD addition reduced the quantity of raw orange juice, and therefore citric acid. Similar results were obtained in previous work on RMD addition to orange juice (Arilla *et al.*, 2020; Arilla *et al.*, 2021; Arilla *et al.*, 2022). Other studies on prebiotic-added fruit-based beverages also showed the same behavior (Ghavidel *et al.*, 2014; Braga & Conti-Silva, 2015; Pimentel *et al.*, 2015). Thus, adding RMD to orange juice helped to achieve a favorable sweet/acid balance, as RMD-added samples obtained higher overall rating scores (Figure 1).

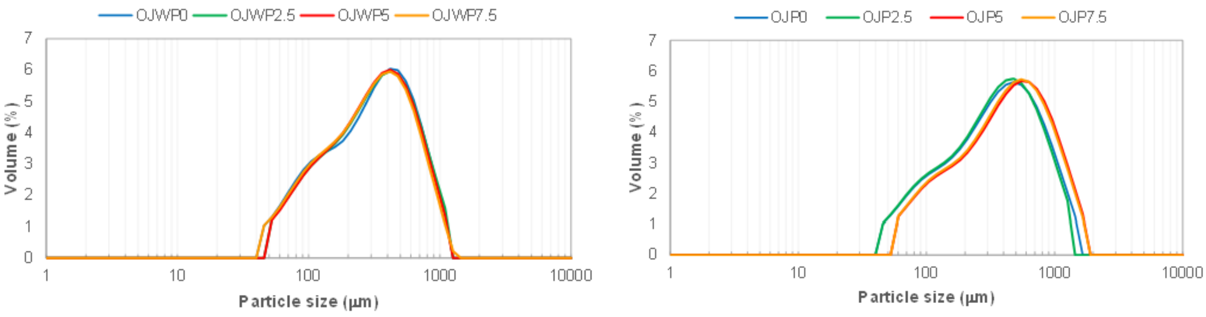
Table 1. Mean values (and standard deviations) of °Brix, pH, acidity (gCA/100 mL), and color coordinates (L*, a*, and b*) of pasteurized orange juice and total color differences (ΔE).

Sample	°Brix	pH	Acidity	L*	a*	b*	ΔE
OJWP0	12.3 ± 0.2^{d}	3.67 ± 0.02^{b}	0.920 ± 0.002^{a}	40.68 ± 0.13^{a}	-1.54 ± 0.13^{d}	29.6 ± 0.3^{a}	-
OJWP2.5	14.4 ± 0.2^{c}	3.67 ± 0.02^{b}	0.882 ± 0.002^{c}	37.89 ± 0.16^{d}	-1.29 ± 0.07^{bc}	28.70 ± 0.07^{c}	2.95 ± 0.13^{e}
OJWP5	16.5 ± 0.2^{b}	3.67 ± 0.02^{b}	0.861 ± 0.002^{e}	36.80 ± 0.12^{e}	-1.48 ± 0.09^{d}	27.4 ± 0.4^{d}	4.5 ± 0.2^{c}
OJWP7.5	18.7 ± 0.2^{a}	3.67 ± 0.02^{b}	0.838 ± 0.002^{g}	35.12 ± 0.02^{g}	-1.51 ± 0.02^{d}	26.43 ± 0.12^{e}	6.41 ± 0.05^{a}
OJP0	12.2 ± 0.2^{d}	3.70 ± 0.02^{a}	0.891 ± 0.002^{b}	40.13 ± 0.10^{b}	-1.35 ± 0.12^{c}	29.4 ± 0.4^{ab}	-
OJP2.5	14.4 ± 0.2^{c}	3.68 ± 0.02^{b}	0.872 ± 0.002^{d}	38.84 ± 0.13^{c}	-1.240 ± 0.002^{ab}	28.76 ± 0.15^{bc}	1.46 ± 0.07^{f}
OJP5	16.5 ± 0.2^{b}	3.71 ± 0.02^{a}	0.849 ± 0.002^{f}	37.9 ± 0.3^{d}	-1.350 ± 0.014^{c}	26.5 ± 1.2^{e}	3.7 ± 0.5^{d}
OJP7.5	18.8 ± 0.2^{a}	3.68 ± 0.02^{b}	0.832 ± 0.002^{h}	35.69 ± 0.13^{f}	-1.16 ± 0.05^{a}	26.5 ± 0.5^{e}	5.3 ± 0.2^{b}

The same letter in superscript within the column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJWP, orange juice without pulp; OJP, orange juice with pulp; 0–7.5 % of RMD addition; L*, brightness; a*, greenness–redness tones; b*, blueness–yellowness tones; □E, total color differences.

RMD incorporation into orange juice did not affect ($p > 0.05$) the pH, which ranged between 3.67 and 3.71 in all samples. The potential technological applications of prebiotics not only for a nutritious upgrade but also for sensory and physicochemical improvements in conventional food have been discussed (De Paulo Farias *et al*., 2019). The color of orange juice, which is mainly due to carotenoid pigments, plays an important role as a quality indicator and is key to consumer acceptance (Wibowo *et al*., 2015a). RMD addition at higher concentrations significantly decreased L* values ($p < 0.05$) (Table 1). Therefore, all orange juices turned slightly darker when the RMD concentration was raised. This is probably because adding RMD reduced the orange juice content in the finished samples. Orange pulp showed a small but significant ($p < 0.05$) effect on the L* values, as all OJP RMD-added samples showed higher L* values than the free-pulp samples at the same RMD concentrations. In addition, OJWP samples obtained, in general, lower a* values than OJP samples, meaning that OJP samples were slightly redder than OJWP samples. This could be an indicator that pulp incorporation into orange juice could add carotenoids to orange juice. Accordingly, in a past study on orange juice bioactive compounds, pulp-added orange juice presented higher carotenoid content than pulp-free orange juice (Arilla *et al*., 2021). The b* value was barely affected by orange pulp presence. Additionally, RMD incorporation into orange juice decreased ($p < 0.05$) the L* and b* values, meaning that samples lost brightness and yellowness, especially at higher RMD concentrations. The a* values were also affected by RMD but not in a significant way. Higher RMD concentrations caused ($p < 0.05$) color changes in both OJWP and OJP samples, mainly in pulp-free samples. According to Bodart *et al*. (2008), if the ΔE is larger than 3 units, color changes may start to be perceptible by the human eye. Instrumentally, it was proven that the addition of pulp helped to better retain the original color of the orange juice in the RMD-added samples, although panelists did not find evident color differences among all samples during sensory evaluation (Figure 1).

The analysis of the volume particle size distribution for all OJWP (Figure 3a) and OJP (Figure 3b) samples indicated that both followed a similar trend. However, pulp-free samples had less data dispersion. Pulp-added samples, on the contrary, seemed to show more data dispersion. This is likely due to the addition of orange pulp rather than the addition of RMD, since pulp is an insoluble solid of variable size, while RMD has good water-soluble properties and, therefore, should barely affect the volume particle size distribution. Table 2 summarizes the mean values (and standard deviations) of volume mean diameter D[4,3] and the standard percentiles d(0.1), d(0.5), and d(0.9). The particle size of OJP samples presented significantly greater volume mean diameter ($p < 0.05$) than OJWP samples exclusively because of orange pulp addition. Also, RMD addition to orange juice hardly had an impact ($p > 0.05$) on the volume mean diameter mainly because of its water-soluble properties.



(a) (b)

Figure 3. Volume particle size distributions (representative curves) of pasteurized orange juice with or without pulp and 0–7.5 % of resistant maltodextrin. (a) OJWP, orange juice without pulp; (b) OJP, orange juice with pulp.

Juice density is also an important quality control parameter in the juice industry (Luckow & Delahunty, 2004). The OJWP0 and OJP0 samples presented the same ($p > 0.05$) density values. However, adding higher concentrations of RMD to orange juice proportionally increased the density values ($p < 0.05$). In addition, the RMD-added OJP samples presented slightly higher ($p < 0.05$) density values compared to the OJWP samples at the same RMD concentrations. The effect of RMD on orange juice density was expected as it completely dissolved in water. Moreover, density values and °Brix (soluble solids) have traditionally presented a clear relationship in matrices that practically only contain soluble solids, such as fruit juices. As a result, regression models have been proposed (Ibarz & Miguelsanz, 1989; Ramos & Ibarz, 1998). On the contrary, insoluble solids such as cloud and pulp contribute little to the density measurement (Luckow & Delahunty, 2004). Accordingly, the °Brix (Table 1) and density values (Table 2) of both OJP and OJWP RMD-added samples proportionally followed the same trend in this study.

Table 2. Mean values (and standard deviations) of volume mean diameter (µm) D[4,3], standard percentiles (µm) d(0.1), d(0.5), and d(0.9), and density (g/cm3) of pasteurized orange juice.

Sample	D[4,3]	d(0.1)	d(0.5)	d(0.9)	Density
OJWP0	302 ± 13^{cd}	25.6 ± 1.6^{c}	245 ± 10^{cd}	665 ± 30^{c}	1.0487 ± 0.0002^{g}
OJWP2.5	307 ± 4^{c}	26.1 ± 1.2^{c}	247 ± 5^{c}	677 ± 8^{c}	1.0575 ± 0.0003^{f}
OJWP5	299 ± 14^{cd}	23 ± 2^{e}	242 ± 11^{cd}	657 ± 31^{c}	1.0659 ± 0.0009^{d}
OJWP7.5	286 ± 12^{d}	19.9 ± 1.2^{f}	230 ± 9^{d}	632 ± 28^{c}	1.0757 ± 0.0004^{b}
OJP0	427 ± 26^{ab}	43 ± 3^{ab}	332 ± 18^{ab}	963 ± 66^{a}	1.0487 ± 0.0005^{g}
OJP2.5	410 ± 37^{b}	41 ± 4^{b}	324 ± 29^{b}	916 ± 87^{b}	1.0579 ± 0.0002^{e}
OJP5	433 ± 20^{a}	41 ± 3^{ab}	339 ± 18^{a}	974 ± 47^{a}	1.0670 ± 0.0003^{c}
OJP7.5	427 ± 25^{ab}	44 ± 4^{a}	335 ± 20^{ab}	957 ± 62^{ab}	1.0762 ± 0.0007^{a}

The same letter in superscript within the column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJWP, orange juice without pulp; OJP, orange juice with pulp; 0–7.5 % of RMD addition; D[4,3], volume mean diameter; d(0.1), standard percentile which encompasses the particles whose size is below 10% of the sample; d(0.5), standard percentile which encompasses the particles whose size is below 50% of the sample; d(0.9), standard percentile which encompasses the particles whose size is below 90% of the sample.

Aroma volatile compounds

The taste of food is mainly based on the flavors and aromas of ingredients (Seifullah, 2019). Flavor has a direct influence on consumer satisfaction, directly influencing consumption. In the present study, the aroma volatile compounds were divided into five groups: alcohols, aldehydes, terpenes and terpenoids, ketones, and acids. Table 3 shows the analysis of volatile compounds of these groups for all OJWP and OJP samples. The OJP samples reported higher ($p < 0.05$) alcohol content than the OJWP samples, mainly because the content of 1-terpinen-4-ol increased ($p < 0.05$) in pulp-added samples. 1-terpinen-4-ol has been considered by some authors as an index of degradation of the aroma in the orange juice or as an indicator of the age of orange juice (Jordan *et al*., 2003). OJP0 showed the highest ($p < 0.05$) amount of 1-terpinen-4-ol. RMD addition slightly decreased ($p < 0.05$) 1-terpinen-4-ol content in all OJWP and OJP samples, with this effect being clearer in OJWP samples. Keeping 1-terpinen-4-ol at the lowest possible levels is desirable, as it has been reported as one of the predominant compounds contributing to storage off-flavor development in citrus juices (Wibowo *et al*., 2015). Moreover, 1-terpinen-4-ol comes from the acid-catalyzed hydration of limonene and linanool (Wibowo *et al*., 2015), which could be enhanced when the °Brix/acid ratio is low, in acidic conditions. Therefore, a decrease in 1-terpinen-4-ol could be expected since adding RMD to orange juice increased °Brix and decreased citric acid content (Table 1).

Aldehydes are secondary metabolites formed during the ripening and maturation period of orange fruit (Kelebek & Selli, 2011). The main representant within the aldehydes was octanal, followed by decanal and nonanal. Ruiz Perez-Cacho and Rouseff (2008) reported that these compounds were present at 45 (octanal), 22 (nonanal), and 10 (decanal) times greater concentrations in mechanically squeezed orange juice compared to hand-squeezed because industrial juice extraction practices introduce relatively high levels of peel oil. Brat *et al*. (2003) also quantified greater amounts of octanal, nonanal, and decanal in orange pulp than in orange juice. This explains why pulp-added samples showed higher ($p < 0.05$) amounts of octanal, nonanal, and decanal, and subsequently higher ($p < 0.05$) total values of aldehydes, than OJPW samples. Furthermore, RMD addition to OJWP samples decreased ($p < 0.05$) total aldehyde content. Interestingly, it displayed the opposite effect in pulp-added orange juice, with OJP5 showing the highest ($p < 0.05$) value of total aldehydes among all samples. Therefore, RMD addition to pulp-added orange juice seems to retain higher aldehyde concentrations, especially of octanal, which along with nonanal and decanal has been related to a more intense orange-like/green note in the overall aroma profile of juice from Valencia variety oranges (Sellami *et al*., 2018).

The terpenes and terpenoids group reached the largest amount of all five volatile compound groups. Limonene and β-myrcene were the main compounds in this group, with higher ($p < 0.05$) quantification in the OJWP samples than in the OJP samples in both cases. The presence of limonene and β-myrcene at these values seems to be related to orange juice extraction, as industrial processes apply higher manufacturing pressures compared to manual hand-squeezing processes, therefore leading to higher orange peel oil extraction (Averbeck & Schieberle, 2009; Neves *et al*., 2020). Pulp incorporation implied a reduction in orange juice in OJP samples. As a result, pulp-free samples obtained higher ($p < 0.05$) total values of terpenes and terpenoids, ranging between 84.38% and 86.74%, whilst pulp-added samples registered values in the range of 81.07% to 82.19%. Although limonene concentration could be influenced by several factors, such as orange maturity stage, origin, variety, way of harvesting, and juice processing conditions (Bylaite & Meyer, 2006), it has been also found to be one of the predominant volatile compounds in other orange juice studies (Wibowo *et al*., 2015). Despite its high concentration, the contribution of limonene to orange aroma is considered the most conflicted of any of the volatiles in orange juice. In fact, Perez-Cacho and Rouseff (2008) stated that limonene does not display a key impact in orange juice, although it is a necessary component of any orange juice odor model. In other study, they also hypothesized that limonene could work as a “lifting agent” for other volatiles, in a similar way as ethanol does in wine (Ruiz Perez-Cacho & Rouseff, 2008).

In addition to limonene and β-myrcene, α-pinene was the next highest quantified volatile compound in the terpenes and terpenoids group, especially in the pulp-added samples. Brat *et al*. (2003) reported a higher amount of α-pinene in orange pulp than in orange juice. Therefore, this seems to be exclusively due to orange pulp incorporation. RMD addition slightly increased ($p > 0.05$) the total values of terpenes and terpenoids in pulp-free samples. However, higher RMD concentration did not cause a higher impact. Moreover, it did not have an effect ($p > 0.05$) on pulp-added samples.

From the ketones group, no difference ($p > 0.05$) was observed between OJP0 and OJWP0. RMD hardly increased ($p > 0.05$) total ketone quantification in pulp-free samples. However, in pulp-added samples, RMD incorporation did had a significant effect by increasing ($p < 0.05$) total ketone values. Ketones have been related to the aromatic quality of orange juice (Saifullah *et al*., 2019). Moreover, from the acids group, the biggest amount was identified in OJWP0 and OJP0 in an increasing manner ($p < 0.05$), with butanoic acid-ethyl ester and acetic acid-octyl ester being the mainly identified acids. This could be correlated with the fact that, during sensory analysis, panelists identified control samples with higher acidity. Adding higher RMD concentrations reduced ($p < 0.05$) total acid values, probably because the addition of RMD implied a reduction in orange juice in the final samples.

Consumers expect high-quality juices, with sensory properties like those found in unprocessed fresh juices. However, pasteurization is known to affect aroma release in a negative way (Perez-Cacho & Rouseff, 2008). This seems to be related to interactions such as the polymerization of proteins and pectin during heat treatment (Schalow *et al*., 2018). Orange juice can be considered as a multiphase system of an aqueous phase and a water-insoluble phase comprising both cloud and pulp, which contains large amounts of cell wall polysaccharides and can be a source of pectin (Sellami *et al*., 2018). Therefore, pulp particles could enhance such chemical reactions during the orange juice pasteurization process.

Table 3. Mean values (and standard deviations) of aroma volatile compounds quantification (relative % from total peaks area) of pasteurized orange juice.

Alcohols	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
1-Octanol	0.09 ± 0.03^{bc}	0.11 ± 0.03^{bc}	0.07 ± 0.03^{c}	0.07 ± 0.01^{c}	0.21 ± 0.02^{a}	0.22 ± 0.02^{a}	0.18 ± 0.01^{a}	0.11 ± 0.02^{b}
3-methyl-1-Butanol,	0.07 ± 0.02^{b}	0.06 ± 0.02^{bc}	0.06 ± 0.03^{bc}	0.07 ± 0.02^{b}	0.16 ± 0.02^{a}	0.02 ± 0.01^{d}	n.d.	0.03 ± 0.02^{cd}
2-methyl-1-Butanol	n.d.	0.04 ± 0.02^{b}	n.d.	n.d.	n.d.	n.d.	0.35 ± 0.02^{a}	n.d.
1-Terpinen-4-ol	0.69 ± 0.02^{c}	0.60 ± 0.02^{d}	0.57 ± 0.02^{d}	0.49 ± 0.02^{e}	1.03 ± 0.02^{a}	0.88 ± 0.02^{b}	0.87 ± 0.02^{b}	0.94 ± 0.02^{b}
cis-p-Mentha-2,8-dien-1-ol		0.13 ± 0.02^{c}	0.16 ± 0.03^{bc}	0.15 ± 0.02^{bc}	0.17 ± 0.02^{b}	0.14 ± 0.02^{bc}	0.28 ± 0.02^{a}	0.25 ± 0.02^{a}
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-//cis-Carveol	0.34 ± 0.03^{bc}	0.24 ± 0.02^{d}	0.29 ± 0.04^{c}	0.31 ± 0.02^{c}	0.23 ± 0.02^{d}	0.12 ± 0.02^{e}	0.39 ± 0.02^{a}	0.37 ± 0.02^{ab}
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-//trans-Carveol	n.d.	0.08 ± 0.02^{bc}	0.06 ± 0.01^{cd}	0.07 ± 0.02^{bc}	0.03 ± 0.02^{d}	0.03 ± 0.02^{d}	0.11 ± 0.01^{ab}	0.12 ± 0.02^{a}
Total	1.19 ± 0.10^{d}	1.26 ± 0.15^{d}	1.25 ± 0.16^{d}	1.16 ± 0.11^{d}	1.83 ± 0.12^{b}	1.41 ± 0.11^{c}	2.18 ± 0.10^{a}	1.82 ± 0.12^{b}
Aldehydes	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
Dodecanal	0.07 ± 0.02^{bc}	0.05 ± 0.02^{c}	0.06 ± 0.02^{c}	0.05 ± 0.02^{c}	0.12 ± 0.02^{a}	0.10 ± 0.02^{ab}	0.06 ± 0.02^{c}	0.12 ± 0.02^{a}
2-Hexenal, (E)-	0.09 ± 0.02^{c}	0.09 ± 0.02^{c}	0.08 ± 0.03^{cd}	0.09 ± 0.02^{c}	0.20 ± 0.02^{a}	0.05 ± 0.02^{d}	0.14 ± 0.02^{b}	0.16 ± 0.02^{b}
Heptanal	n.d.	0.03 ± 0.02^{a}	0.05 ± 0.02^{a}	0.03 ± 0.02^{a}	0.04 ± 0.02^{a}	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	n.d.
Undecanal	0.04 ± 0.03^{a}	0.05 ± 0.02^{a}	0.04 ± 0.02^{a}	0.06 ± 0.03^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}
Octanal	7.27 ± 0.04^{d}	7.18 ± 0.03^{d}	6.97 ± 0.04^{e}	6.52 ± 0.05^{f}	8.23 ± 0.04^{c}	8.96 ± 0.06^{b}	9.20 ± 0.04^{a}	9.23 ± 0.03^{a}
Nonanal	0.47 ± 0.06^{b}	0.34 ± 0.03^{cd}	0.36 ± 0.02^{cd}	0.43 ± 0.04^{bc}	0.63 ± 0.03^{a}	0.59 ± 0.04^{a}	0.59 ± 0.02^{a}	0.59 ± 0.03^{a}
Decanal	1.64 ± 0.03^{d}	1.36 ± 0.05^{e}	1.43 ± 0.03^{e}	1.62 ± 0.03^{d}	2.83 ± 0.03^{a}	2.28 ± 0.05^{c}	2.43 ± 0.03^{b}	2.30 ± 0.04^{c}
Total	9.58 ± 0.20^{d}	9.10 ± 0.19^{e}	8.99 ± 0.18^{f}	8.8 ± 0.21^{g}	12.13 ± 0.18^{c}	12.13 ± 0.23^{c}	12.56 ± 0.17^{a}	12.48 ± 0.16^{b}
Terpenes and terpenoids	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
Camphene	0.06 ± 0.02^{a}	n.d.	n.d.	n.d.	0.04 ± 0.03^{a}	0.08 ± 0.02^{a}	n.d.	n.d.
β-Pinene	0.49 ± 0.02^{c}	0.12 ± 0.02^{f}	0.25 ± 0.03^{d}	0.18 ± 0.02^{e}	0.62 ± 0.03^{b}	0.08 ± 0.02^{f}	0.62 ± 0.04^{b}	0.69 ± 0.04^{a}
Benzaldehyde	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.02^{a}	0.07 ± 0.03^{a}	0.06 ± 0.02^{a}	0.07 ± 0.02^{a}
β-Myrcene	34.36 ± 0.06^{b}	34.88 ± 0.18^{a}	33.51 ± 0.07^{c}	32.49 ± 0.08^{d}	31.81 ± 0.07^{e}	31.17 ± 0.10^{f}	31.06 ± 0.08^{f}	31.00 ± 0.12^{f}
Limonene	34.18 ± 0.04^{c}	35.08 ± 0.06^{a}	34.87 ± 0.06^{b}	33.87 ± 0.07^{d}	23.02 ± 0.10^{f}	25.02 ± 0.07^{e}	23.16 ± 0.09^{f}	23.08 ± 0.10^{f}

Terpenes and terpenoids	OJWP0	OJWP2.5	OJWP5	OJWP7.5	OPJ0	OPJ2.5	OPJ5	OPJ7.5
β-cis-Ocimene	0.86 ± 0.02^{c}	0.73 ± 0.02^{d}	0.71 ± 0.02^{d}	0.83 ± 0.03^{c}	1.05 ± 0.04^{b}	1.11 ± 0.03^{a}	1.06 ± 0.03^{ab}	1.03 ± 0.03^{b}
α-phellandrene	n.d.	n.d.	0.06 ± 0.02^{ab}	0.03 ± 0.01^{b}	0.05 ± 0.02^{ab}	0.09 ± 0.03^{a}	0.07 ± 0.03^{ab}	n.d.
Γ-terpinene	1.98 ± 0.02^{e}	1.91 ± 0.02^{e}	1.97 ± 0.04^{e}	2.20 ± 0.04^{d}	2.89 ± 0.07^{bc}	3.08 ± 0.05^{a}	2.81 ± 0.06^{c}	2.97 ± 0.05^{ab}
Terpinolene	0.11 ± 0.02^{b}	0.10 ± 0.02^{b}	0.11 ± 0.02^{b}	0.13 ± 0.03^{b}	0.21 ± 0.02^{a}	0.12 ± 0.02^{b}	n.d.	n.d.
α-Pinene	6.90 ± 0.04^{g}	6.37 ± 0.03^{h}	7.24 ± 0.04^{f}	7.82 ± 0.06^{e}	12.11 ± 0.07^{a}	10.27 ± 0.06^{d}	10.66 ± 0.08^{c}	11.36 ± 0.08^{b}
β-Linalool	0.64 ± 0.02^{e}	0.65 ± 0.03^{e}	0.61 ± 0.02^{e}	0.75 ± 0.05^{d}	0.53 ± 0.05^{f}	1.21 ± 0.03^{c}	1.53 ± 0.03^{b}	1.86 ± 0.04^{a}
(+)-4-Carene	1.24 ± 0.02^{b}	1.05 ± 0.03^{b}	1.07 ± 0.04^{b}	1.26 ± 0.03^{b}	1.69 ± 0.06^{a}	1.78 ± 0.05^{a}	1.73 ± 0.04^{a}	1.27 ± 0.03^{b}
Benzene, 2-ethenyl-1,3-dimethyl-	0.22 ± 0.03^{ab}	0.16 ± 0.02^{b}	0.22 ± 0.03^{ab}	0.22 ± 0.03^{ab}	0.27 ± 0.03^{a}	0.26 ± 0.03^{a}	0.23 ± 0.03^{a}	0.21 ± 0.03^{ab}
β-terpineol	0.03 ± 0.03^{e}	0.14 ± 0.03^{d}	0.03 ± 0.02^{e}	0.05 ± 0.03^{e}	0.13 ± 0.02^{d}	0.23 ± 0.02^{c}	0.57 ± 0.03^{b}	0.79 ± 0.03^{a}
α-terpineol	0.23 ± 0.04^{c}	0.19 ± 0.03^{cd}	0.15 ± 0.03^{de}	0.13 ± 0.02^{e}	0.33 ± 0.02^{b}	0.24 ± 0.03^{c}	0.40 ± 0.02^{b}	0.86 ± 0.03a
α-citral	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.02^{a}	0.05 ± 0.03^{b}	n.d.	n.d.
Copaene	0.09 ± 0.02^{cd}	0.09 ± 0.02^{bcd}	0.06 ± 0.03^{d}	0.07 ± 0.02^{d}	0.19 ± 0.03^{a}	0.13 ± 0.03^{abc}	0.17 ± 0.03^{a}	0.15 ± 0.04^{ab}
1,3,8-p-Menthatriene	0.11 ± 0.02^{ab}	0.06 ± 0.03^{b}	0.13 ± 0.02^{a}	0.13 ± 0.02^{a}	0.15 ± 0.03^{a}	0.16 ± 0.03^{a}	0.14 ± 0.03^{a}	0.16 ± 0.02^{a}
Limonene epoxide	0.14 ± 0.03^{a}	0.15 ± 0.03^{a}	0.14 ± 0.03^{a}	0.14 ± 0.03^{a}	0.14 ± 0.03^{a}	0.15 ± 0.04^{a}	0.20 ± 0.03^{a}	0.15 ± 0.02^{a}
Caryophyllene	0.13 ± 0.03^{b}	0.13 ± 0.03^{b}	0.08 ± 0.02^{c}	0.10 ± 0.02^{bc}	0.25 ± 0.03^{a}	0.12 ± 0.03^{bc}	0.22 ± 0.02^{a}	0.20 ± 0.02^{a}
α-Caryophyllene	0.03 ± 0.02^{d}	0.08 ± 0.02^{cd}	0.08 ± 0.02^{cd}	0.08 ± 0.02^{cd}	0.12 ± 0.03^{abc}	0.13 ± 0.03^{ab}	0.17 ± 0.03^{a}	0.11 ± 0.03^{bc}
β-Elemene	n.d.	0.05 ± 0.02^{d}	0.07 ± 0.03^{bcd}	0.06 ± 0.03^{cd}	0.11 ± 0.02^{abc}	0.12 ± 0.03^{ab}	0.13 ± 0.03^{a}	0.10 ± 0.03^{abcd}
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1.8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- //Valencene	1.33 ± 0.03^{f}	2.73 ± 0.08^{d}	2.42 ± 0.05^{e}	2.67 ± 0.05^{d}	4.12 ± 0.08^{b}	4.10 ± 0.06^{b}	4.29 ± 0.07^{a}	3.84 ± 0.02^{c}
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)-// (-)-Carvone	0.90 ± 0.03^{e}	1.00 ± 0.03^{d}	1.41 ± 0.04^{b}	1.80 ± 0.05^{a}	0.85 ± 0.06^{e}	0.29 ± 0.04^{f}	1.40 ± 0.06^{b}	1.21 ± 0.02^{c}
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	0.16 ± 0.03^{e}	0.36 ± 0.03^{c}	1.04 ± 0.03^{b}	1.18 ± 0.04^{a}	0.08 ± 0.02^{f}	0.25 ± 0.03^{d}	0.12 ± 0.03^{ef}	0.17 ± 0.02^{e}
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-//delta.-Cadinene	0.10 ± 0.03^{e}	0.08 ± 0.02^{e}	0.08 ± 0.02^{e}	0.20 ± 0.03^{d}	0.32 ± 0.03^{bc}	0.36 ± 0.03^{ab}	0.41 ± 0.03^{a}	0.29 ± 0.01^{c}
(-)-α-Panasinsen	n.d.	n.d.	n.d.	n.d.	0.21 ± 0.03^{a}	0.24 ± 0.03^{a}	0.23 ± 0.04^{a}	0.20 ± 0.02^{a}
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-//Nerol acetate	0.05 ± 0.03^{b}	n.d.	n.d.	0.05 ± 0.03^{b}	0.06 ± 0.03^{ab}	0.07 ± 0.03^{ab}	0.08 ± 0.02^{ab}	0.11 ± 0.01^{a}
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	0.04 ± 0.03^{d}	0.20 ± 0.03^{c}	0.12 ± 0.03^{c}	0.25 ± 0.03^{bc}	0.21 ± 0.03^{bc}	0.04 ± 0.02^{d}	0.37 ± 0.04^{a}	0.27 ± 0.02^{b}

Terpenes and terpenoids	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
β-Citral	n.d.	n.d.	0.03 ± 0.03^{a}	0.05 ± 0.03^{a}	0.06 ± 0.03^{a}	0.05 ± 0.02^{a}	0.06 ± 0.02^{a}	0.04 ± 0.02^{a}
Total	84.38 ± 0.68^{b}	86.31 ± 0.82^{a}	86.45 ± 0.88^{a}	86.74 ± 0.90^{a}	81.89 ± 1.16^{c}	81.07 ± 1.08^{c}	81.95 ± 1.05^{c}	82.19 ± 0.80^{c}
Ketones	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
Acetophenone	n.d.	0.04 ± 0.02^{c}	0.04 ± 0.02^{c}	0.04 ± 0.02^{c}	0.05 ± 0.02^{c}	0.06 ± 0.03^{c}	0.18 ± 0.02^{b}	0.53 ± 0.03^{a}
Ethanone, 1-(4-methylphenyl)-	0.11 ± 0.03^{bc}	0.11 ± 0.03^{bc}	0.11 ± 0.03^{bc}	0.15 ± 0.04^{b}	0.06 ± 0.03^{c}	0.05 ± 0.04^{c}	0.18 ± 0.04^{b}	0.46 ± 0.05^{a}
Total	0.11 ± 0.03^{c}	0.15 ± 0.06^{c}	0.15 ± 0.05^{c}	0.19 ± 0.07^{c}	0.11 ± 0.05^{c}	0.11 ± 0.07^{c}	0.36 ± 0.06^{b}	0.99 ± 0.08^{a}
Acids	**OJWP0**	**OJWP2.5**	**OJWP5**	**OJWP7.5**	**OPJ0**	**OPJ2.5**	**OPJ5**	**OPJ7.5**
Butanoic acid, methyl ester	0.59 ± 0.08^{b}	0.04 ± 0.01^{d}	0.06 ± 0.02^{d}	0.06 ± 0.02^{d}	1.20 ± 0.02^{a}	0.10 ± 0.02^{cd}	0.07 ± 0.02^{d}	0.15 ± 0.03^{c}
Dodecanoic acid	0.17 ± 0.04^{b}	0.14 ± 0.02^{b}	0.14 ± 0.03^{b}	0.13 ± 0.03^{bc}	0.07 ± 0.02^{c}	0.25 ± 0.03^{a}	0.26 ± 0.04^{a}	0.24 ± 0.02^{ab}
Tetradecanoic acid	0.61 ± 0.08^{a}	0.14 ± 0.04^{d}	0.27 ± 0.01^{c}	0.24 ± 0.04^{c}	0.14 ± 0.04^{d}	0.18 ± 0.02^{cd}	0.38 ± 0.04^{b}	0.24 ± 0.03^{c}
Butanoic acid, ethyl ester	1.89 ± 0.07^{c}	1.35 ± 0.15^{d}	1.37 ± 0.04^{d}	1.41 ± 0.03^{d}	2.33 ± 0.07^{b}	3.48 ± 0.05^{a}	1.95 ± 0.06^{c}	2.19 ± 0.11^{b}
Acetic acid, octyl ester	1.40 ± 0.08^{a}	0.78 ± 0.03^{b}	0.74 ± 0.06^{b}	0.80 ± 0.04^{b}	0.15 ± 0.03^{c}	0.05 ± 0.01^{d}	0.07 ± 0.03^{cd}	0.04 ± 0.01^{d}
Total	4.65 ± 0.36^{a}	2.45 ± 0.25^{d}	2.58 ± 0.16^{cd}	2.63 ± 0.16^{cd}	3.87 ± 0.18^{b}	4.06 ± 0.13^{b}	2.71 ± 0.19^{cd}	2.86 ± 0.20^{c}
n.i.	0.09	0.73	0.58	0.47	0.15	0.99	0.55	0.08

The same letter in superscript within the column indicates homogeneous groups established by ANOVA ($p < 0.05$). n.i., not identified; n.d., not detected; OJWP, orange juice without pulp; OJP, orange juice with pulp; 0–7.5 % of RMD addition.

Instrumental and sensory correlations

MFA was used to study the influences of all the different types of variables and to relate the results from instrumental and sensory determinations for the evaluated juices. The MFA constructs a sensory map in two dimensions. Factors 1 (F1) and 2 (F2) explained 75.55% of the data variability (Figure 4). The sensory acidity detected by panelists corresponds to the instrumentally measured acidity. In turn, the sensory sweetness reported by the panelists is also close to the measured °Brix. The parameter D[4,3] of the particle size determination and the perceived mouthfeel of the juices are related in F2 but in the opposite way. The sensorily evaluated off-flavors are close to the instrumentally quantified terpenes and terpenoids, and the flavor intensity is related to the acids of the determined aroma compounds.

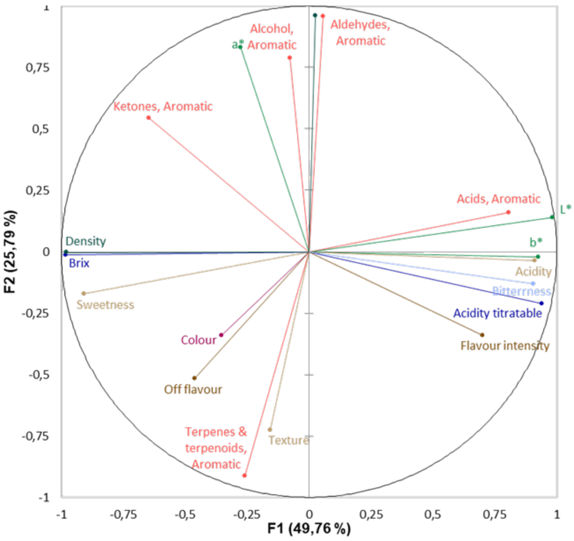


Figure 4. Correlation circle of the response variables.

Figure S1 shows the superimposed MFA representation of the juices. Each sample is represented by eight points grouping sensory and instrumental variables studied in the samples. The consensus representation that considers the eight variables simultaneously is also represented for each juice. From the sensory analysis, sweetness, acidity, and bitterness have been grouped into sensory taste variables. On the other hand, off-flavors and flavor intensity have been grouped into a variable named sensory flavor. Color and mouthfeel in the sensory test have been kept individually as sensory color and sensory texture, respectively. The variables obtained from the instrumental measurements have been grouped as follows: L*, a*, and b* as the instrumental color; °Brix and titratable acidity as the instrumental flavor; density and particle size parameter

D[4,3] as the instrumental texture, and the different groups of analytically determined aromatic compounds as the instrumental aroma.

As shown in Figure 4 and Figure S1, there was a significant difference between juices with and without pulp, according to F2. The juices with pulp are closer to larger particle sizes and aromatic compounds determined in this study. On the other hand, juices without pulp are closer to the sensory attribute of texture in the mouth and to off-flavors. In addition, samples with the highest RMD concentrations are close to the sensory sweetness and °Brix determined, as well as to the density. However, standard samples without RMD addition are close to the sensory evaluation of flavor, acidity, and bitterness and to instrumentally determined L*, a*, titratable acidity, and acid aroma compounds.

The superimposed representation of the samples in the MFA made it possible to evaluate the proximity between the studied variables for all samples. Observing the underlying structure of the instrumental and sensory variables and their proximities, it can be inferred that variables such as sensory color and flavor or instrumental aroma influence the position of the samples in the plane. The most significant differences in the samples are generally produced by the variables mentioned above, and the rest of the variables studied in the MFA present different distances for each sample. Therefore, these differences affect each sample differently. This trend is also observed in the RV coefficients obtained from the MFA (Table 4). The RV coefficient is a multivariate statistic ranging from 0 (full disagreement) to 1 (perfect agreement) [56]. The RV coefficients range from 0.6 to 0.9. Sensory color, flavor, and instrumental aroma showed the lowest RV values against the other variables, while the rest of studied variables showed higher RV coefficients, above all instrumental color. This indicates that sensory color and flavor or instrumental aroma are important variables in the evaluation of the studied juices.

Table 4. RV coefficients obtained from the MFA.

.	**Sensory color**	**Sensory taste**	**Sensory texture**	**Sensory flavor**	**Instrumental color**	**Instrumental taste**	**Instrumental texture**	**Instrumental aroma**	**MFA**
Sensory color	1.000	0.058	0.093	0.025	0.175	0.083	0.099	0.278	0.294
Sensory taste	0.058	1.000	0.733	0.394	0.700	0.645	0.685	0.459	0.808
Sensory texture	0.093	0.733	1.000	0.253	0.732	0.691	0.498	0.346	0.737
Sensory flavor	0.025	0.394	0.253	1.000	0.617	0.527	0.464	0.205	0.605
Instrumental color	0.175	0.700	0.732	0.617	1.000	0.886	0.784	0.489	0.927
Instrumental taste	0.083	0.645	0.691	0.527	0.886	1.000	0.711	0.312	0.834
Instrumental texture	0.099	0.685	0.498	0.464	0.784	0.711	1.000	0.816	0.890
Instrumental aroma	0.278	0.459	0.346	0.205	0.489	0.312	0.816	1.000	0.690
MFA	0.294	0.808	0.737	0.605	0.927	0.834	0.890	0.690	1.000

CONCLUSIONS

Despite the growing background on the beneficial effects of prebiotics on health, their incorporation into day-to-day foods remains unfamiliar to consumers. As an approach, this work provides evidence that the addition of RMD to pasteurized orange juice is technologically feasible while also achieving a good response at the sensory level, even at high RMD concentrations, according to expert panelists. RMD improved almost all sensory attributes, leading to higher overall rating scores than RMD-free control samples. Also, RMD addition to orange juice displayed a very similar impact on the physicochemical properties of orange juice as was previously found by our group. RMD addition had a moderate effect on volatile compound quantification, whereas the presence of orange pulp played a much more decisive role by increasing 1-terpinen-4-ol, octanal, nonanal, decanal, and α-pinene and decreasing limonene and β-myrcene.

Therefore, adding RMD may be of interest to upgrade the organoleptic acceptability of conventional fruit juices, such as orange juice. This can favor the consolidation of prebiotic addition to day-to-day foods in consumers' diet. It would be of interest to perform a sensory analysis of RMD-added orange juice with consumers to tackle this challenge.

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SUPPLEMENTARY MATERIAL

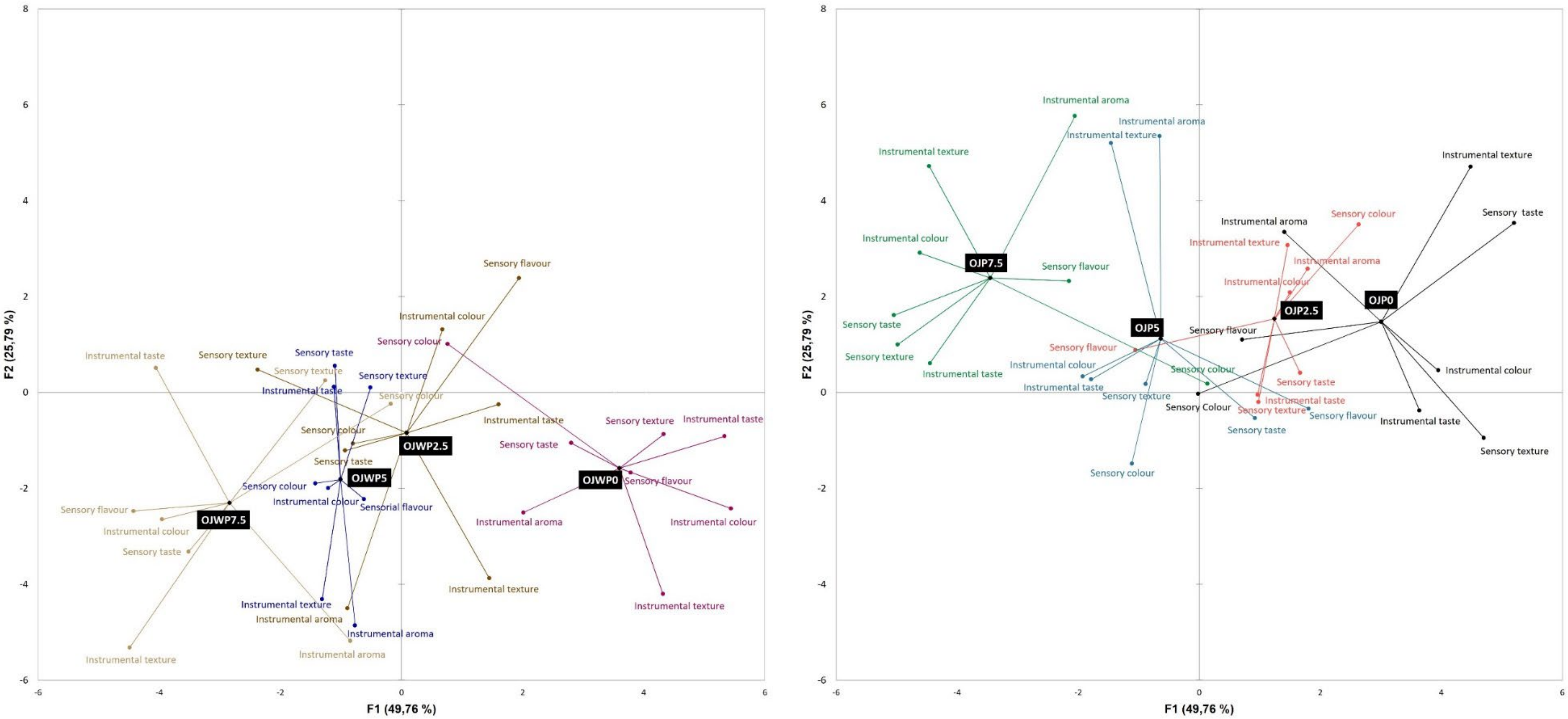

Figure S1. Superimposed MFA representation of the juices. Each sample is represented by eight points grouping sensory and instrumental variables studied in the samples. Each juice representation considers the eight variables simultaneously.

CHAPTER 6. STABILITY OF VITAMIN C, CAROTENOIDS, PHENOLS AND ANTIOXIDANT CAPACITY OF PASTEURIZED ORANGE JUICE WITH RESISTANT MALTODEXTRIN STORAGE

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ABSTRACT

Resistant maltodextrin (RMD) was added at increasing concentrations (0%, 2.5%, 5% and 7.5%) before pasteurization to orange juice to analyze its potential protective effect on the health-related bioactive compounds of pasteurized orange juice throughout its storage time. Samples were characterized in terms of basic physicochemical properties and bioactive compounds at the beginning of the storage. Higher concentrations of RMD proved to better preserve the bioactive compounds of orange juice, thus obtaining a higher antioxidant capacity (AC). Stability of all samples was determined by measuring the same parameters at days 0, 15, 45, 75, 105, 136 and 170 of storage. °Brix and pH were very stable in all samples along storage, while all bioactive compounds had negative variations. However, RMD addition slightly improved ascorbic acid, vitamin C, total phenols, and total carotenoids retention, improving then its AC. This effect was greater in the 5% RMD-added samples. All bioactive compounds showed a positive Pearson's correlation coefficient with AC. Color variations were also measured at days 105 and 170. All samples had a positive variation of all color parameters, being this clearer at day 170. This work enlightens the potential functionality of RMD to better preserve the health-related compounds of pasteurized orange juice.

INTRODUCTION

Prebiotics are a group of non-digestible carbohydrates, mainly dietary fibers, that can selectively influence gut microbiota resulting in desirable effects for human health (Singla & Chakkaravarthi, 2017). To our knowledge, inulin, fructo-oligosaccharides (FOS), isomalto-oligosaccharides, gluco-oligosaccharides, or human milk oligosaccharides, among others, are the most studied substances because of their potential prebiotic activity (Corzo *et al*., 2015). However, the identification of fiber as the potential cause of healthy intestinal function led to the search for substances with prebiotic activity, including resistant maltodextrin (RMD) in recent years (Fuentes-Zaragoza *et al*., 2011).

RMD is a water-soluble fiber produced from the heat treatment of corn starch. It is indigestible in the small intestine but fermentable by the colonic bacteria and, therefore, enhancing the production of short-chain fatty acid (Lockyer & Nugent, 2017). Up to now, RMD has shown to exert a wide variety of positive health effects. For example, the intake of 10 grams of RMD with a meal stimulated the production of satiety hormones, hence decreasing hunger (Ye *et al*., 2015). In a meta-analysis of randomized controlled trials, RMD consumption attenuated the insulin and triacylglycerol response to meal, being this effect stronger for RMD in drinks than in solid foods (Livesey & Tagami, 2009). The suppressive effect on insulin and triacylglycerol levels was also found when consuming a meal with a beverage containing 5 or 10 grams of RMD (Kishimoto *et al*., 2007). Also, in a double-blind placebo-controlled randomized crossover study, a beverage containing 25 or 50 grams of RMD added to water and consumed for 24 days increased fecal bulk (Baer *et al*., 2014). According to these cited articles, liquid matrices seem to facilitate the RMD transport through the gastrointestinal tract, compared to solid foods. In fact, fruit juices have been suggested as an ideal vehicle for prebiotic delivery because their appealing organoleptically properties that makes them well accepted by population of all age groups (Valero-Cases *et al*., 2020). In terms of flavor, citrus juices are popular. Specifically, orange juice is the most consumed fruit juice worldwide, playing an important role at the nutritional and economic level. Orange juice is well known by antioxidant properties thanks to its intrinsic bioactive compounds content, mainly total phenols (TP), total carotenoids (TC), ascorbic acid (AA) and vitamin C. Antioxidants emerge as desirable compounds because their effect against oxidative stress (Adwas *et al*., 2019), as they inhibit reactive oxygen species production and scavenging of free radicals. These reactive oxygen species have been shown to be involved in the development of cancer, cardiovascular and neurodegenerative diseases, in addition to aging (Yang *et al*., 2018). Therefore, preserving the intrinsic bioactive compounds of the orange juice is a challenge worth it because its potential health impact, both at the time of juice processing and along its storage during its shelf life.

Moreover, in plant-derived products there is a relationship between the physicochemical properties and the bioactive compounds (Sánchez-Moreno *et al*., 2003). For instance, the natural orange color of the oranges is mostly due to its carotenoid content, so the chemical transformation of such compounds may imply color changes. Deeply understanding this relationship between the physicochemical properties and the bioactive compounds of orange juice could also be of great interest to develop healthier and higher quality food products. This is especially interesting when applying thermal preservation technologies as pasteurization, which is the most widely technology used as it is the most cost-effective method to reduce microbial populations and enzyme activity (Perez-Cacho & Rousell, 2008). Nevertheless, it is well known that applying thermal processing causes irreversible losses of bioactive compounds and antioxidant properties (Lu *et al*., 2018). In our previous study, RMD addition before the pasteurization juice process showed a protective effect on the bioactive compounds of orange juice, being this effect greater with higher RMD concentrations (Arilla *et al*., 2021). However, the effects of RMD addition on the stability of the bioactive compounds and its antioxidant capacity (AC) along storage needs to be elucidated. Therefore, the aim of the present study is to analyze if the protective effect of RMD addition on orange juice bioactive compounds extends throughout the shelf life of the finished product.

MATERIALS AND METHODS

Raw materials

This study was conducted with freshly squeezed orange juice supplied by Refresco Iberia S.A.U. (Valencia, Spain). All oranges were from Spanish origin. RMD (Fibersol-2) added to the juice was purchased from ADM/Matsutani, LLC (Decatur, IL, USA). Frozen pasteurized orange pulp was provided by a local fruit processing company (Zumos Valencianos del Mediterráneo, Valencia, Spain).

Sample preparation and pasteurization

Eight samples of orange juice were prepared. Four were orange juice with pulp (OJP) and the other four were orange juice without pulp (OJWP). Fresh orange juice was directly collected from the industrial squeezed lines. Orange pulp (2.5%) was added to the OJP samples. Pulp content was homogenized using a stirrer (LH Overhead Stirrer, VELP Scientifica, Italy), by applying 200 rpm for 5 min. Increasing RMD concentrations (2.5, 5, and 7.5%) were mixed into both OJP and OJWP samples. Thus, for a finished beverage portion of 200 mL, 5, 10, or 15 g of RMD would be ingested, enough to display functional effects according to other studies (Livesey & Tagami, 2009; Ye *et al*., 2015). Control samples without RMD addition (OJP0 and OJWP0) were also prepared, and they complied with the European Fruit Juice Association orange juice guidelines (AIJN, n.d.), so no adulteration or deviation occurred during the juice extraction. To properly dissolve RMD in the fresh orange juice, the same stirrer at 200 rpm for 15 min was used. Finally, all samples were pasteurized (Fruchtsaftdispenser, Mabo Steuerungselemente GmbH, Germany) at 85°C for 10 s, and were hot filled into 250 mL polyethylene terephthalate bottles. All bottles were immersed in a cold-water bath (<10°C) for 30 min to cool down their temperature after the heat treatment. Then, samples were stored at 25°C in darkness for 170 days.

Determinations

°Brix, pH, AA, vitamin C, TP, TC and AC were determined at 0, 15, 45, 75, 105, 136 and 170 days of storage.

ºBrix and pH

Measurement of total soluble solids (°Brix) was conducted using refractometry (Abbemat 200, Anton Paar, Austria). Determination of pH was made using a Basic 20 pH meter (Crison, Spain). All determinations were performed in triplicate in accordance with AOAC guidelines (Latimer, 2012).

Ascorbic acid (AA) and vitamin C

AA and vitamin C, which involves AA and dehydroascorbic acid (DHAA), were determined using a HPLC-UV detector (Jasco equipment, Italy) in triplicate. The method proposed by Xu *et al*. (2008) was used to determine the ascorbic acid with some modifications made by Igual *et al*. (2016). To determine the AA, 1 g sample was extracted with 9 mL 0.1% oxalic acid for 3 min and immediately filtered (0.45 µm) before injection. The procedure employed to determine total vitamin C was the reduction of DHAA acid to AA, using DL-dithiothreitol as the reductant reagent. A 0.5 mL aliquot sample was taken to react with 2 mL of a 20 g/L dithiothreitol solution for 2 h at room temperature and in darkness. Afterwards, the same procedure as that used for the AA method was performed. The HPLC method and instrumentation was: Ultrabase-C18, 5 µm (4.6 × 250 mm) column (Scharlab, Barcelona, Spain); mobile phase 0.1% oxalic acid, volume injection 20 µL, flow rate 1 mL/min, detection at 243 nm and at 25 °C. AA standard solution (Sigma-Aldrich, Steinheim, Germany) was prepared.

Total phenols (TP)

Determining TP was based on the Folin-Ciocalteu method. The extraction procedure comprised mixing sample with methanol. The mixture was centrifuged (12,857xg, 10 min, 4 °C) to obtain the supernatant (Igual *et al*., 2016). Absorbance was measured at 765 mm in a UV-3100PC spectrophotometer (VWR, Leuven, Belgium). The total phenolic content was expressed as mg of gallic acid (Sigma-Aldrich, Steinheim, Germany) equivalents per 100 g of orange juice to compare all the samples.

Total carotenoids (TC)

The TC in the samples were extracted with a solvent hexane/acetone/ethanol mixture following the Olives Barba *et al*. (2006) method in triplicate. Sample absorbance was measured at 446 nm in a UV-visible spectrophotometer (Thermo Electron Corporation). The TC content was expressed as mg of β-carotene (Fluka-Biochemika) per 100 g of orange juice to compare all the samples.

Antioxidant Capacity (AC)

AC was assessed using the free radical scavenging activity of the samples evaluated with the stable radical 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) following Igual *et al*. (2019) methodology in triplicate. Samples were mixed with methanol. The homogenate was centrifuged (10.000 rpm, 10 min, 4 °C) to obtain the supernatant. 0.1 mL of supernatant was added to 3.9 mL of DPPH (0.030 g/L, Sigma-Aldrich, Steinheim, Germany) in methanol. A UV-visible spectrophotometer (Thermo Electron Corporation) was used at the absorbance at 515 nm. The results were expressed as milligram Trolox equivalents (TE) per 100 g of orange juice to compare all the samples.

Color measurement

Sample color was measured using a colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with a standard illuminant D65 and a visual angle of 10°. Measurements were realized at 0, 105 and 170 days of storage when visual changes were observed. Results were obtained in terms of L* (brightness: L* = 0 (black), L* = 100 (white)), a* (−a* = greenness, + a* = redness), and b* (−b* = blueness, + b* = yellowness), according to the CIELab system (CIE, 2018). Differences in L*, a* and b* because of storage time were calculated (ΔL*, Δa* and Δb*). The total color difference (ΔE) was calculated with respect to the sample at the beginning of storage to evaluate the storage effect.

Statistical analysis

Analysis of variance (ANOVA) was applied with a confidence level of 95% ($p < 0.05$), to evaluate the differences among samples. Furthermore, a correlation analysis among studied bioactive compounds and antioxidant capacity of juices, with a 95% significance level was conducted. Statgraphics Centurion XVII Software, version 17.2.04 (Statgraphics Technologies, Inc., The Plains, VA, USA) was used.

RESULTS AND DISCUSSION

Orange juice characterization

Table 1 shows the mean values with standard deviations of °Brix, pH and color parameters because they are usually measured in the quality control processes of the citrus fruit industry (Kimball, 2012). Control samples without RMD (OJP0 and OJWP0) obtained comparable physicochemical values than those reported in other orange juice-based studies (Sánchez-Moreno *et al*., 2003; Wibowo *et al*., 2015; Mennah-Govela & Bornhorst, 2017). Orange pulp did not display a significant ($p > 0.05$) effect on the soluble solids fraction. Adding a water-soluble fiber as RMD proportionally increased significantly ($p < 0.05$) °Brix values. The same behavior was also observed in other prebiotic fibers with similar water-dissolving properties (Pimentel *et al*., 2015; Igual *et al*., 2019). Increasing °Brix with RMD addition could improve the sweet profile and mouthfeel of orange juice, thus exhibiting interesting food technology applications together with its prebiotic function. For instance, prebiotics have been proposed as sugar replacers (Pimentel *et al*., 2015; Singla & Chakkaravarthi, 2017). Besides, pH was not altered ($p > 0.05$) either by RMD addition nor presence of orange pulp.

Table 1. Mean values (and standard deviations) of °Brix, pH, color coordinates (L*, a* and b*) and total color differences (ΔE) of pasteurized orange juice.

Sample	°Brix	pH	L*	a*	b*	ΔE*
OJP0	11.4 ± 0.2^{d}	3.64 ± 0.02^{a}	38.0 ± 0.8^{a}	-0.8 ± 0.4^{b}	28.8 ± 0.5^{bc}	-
OJP2.5	13.6 ± 0.2^{c}	3.63 ± 0.02^{a}	35.83 ± 0.03^{bc}	-0.6 ± 0.2^{b}	27.7 ± 0.8^{c}	2.7 ± 0.2^{b}
OJP5	16.4 ± 0.2^{b}	3.62 ± 0.02^{a}	35.8 ± 1.2^{b}	-0.12 ± 0.07^{a}	29.3 ± 1.6^{b}	2.9 ± 0.6^{b}
OJP7.5	18.2 ± 0.2^{a}	3.64 ± 0.02^{a}	35.2 ± 0.7^{bc}	-0.13 ± 0.08^{a}	29.4 ± 0.6^{b}	3.0 ± 0.6^{b}
OJWP0	11.4 ± 0.2^{d}	3.62 ± 0.02^{a}	38.1 ± 0.6^{a}	-0.19 ± 0.15^{a}	31.4 ± 0.5^{a}	-
OJWP2.5	13.3 ± 0.2^{c}	3.66 ± 0.02^{a}	37.6 ± 0.4^{a}	-0.16 ± 0.06^{a}	32.1 ± 0.8^{a}	1.0 ± 0.4^{c}
OJWP5	15.9 ± 0.2^{b}	3.66 ± 0.02^{a}	35.8 ± 0.6^{b}	-0.33 ± 0.05^{a}	29.4 ± 0.6^{b}	3.1 ± 0.3^{b}
OJWP7.5	18.1 ± 0.2^{a}	3.60 ± 0.02^{a}	34.8 ± 0.3^{c}	-0.23 ± 0.04^{a}	29.4 ± 0.9^{b}	4.0 ± 0.2^{a}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp; 0-7.5, resistant maltodextrin percentage.

Regarding color, which is one of the major attributes that affect the consumer perception of food quality (Sant'Anna et al., 2013), L*, a*, b* and ΔE* values are also shown in Table

1. Orange pulp did not show a significant effect ($p > 0.05$) on the L* values as OJP0 and OJWP0 got similar results. However, L* values significantly decreased ($p < 0.05$) due to RMD addition, meaning that orange juice turned slightly darker as higher RMD concentrations were added. In addition, OJWP0 marked a higher ($p < 0.05$) a* value than OJP0, meaning that pulp-free orange juice was slightly reddish than pulp-added orange juice. RMD addition on the OJWP samples did not have any significant effect ($p < 0.05$) on the a* values. However, it did have a protective effect ($p < 0.05$) on the reddish tones, as higher RMD concentrations led to higher a* values, especially from 5% RMD. This could be due to a protective effect on the carotenoids content of the orange pulp, which was found in our previous study (Arilla *et al*., 2021). For the b* values, OJWP0 samples obtained a higher ($p < 0.05$) value than OJP0, thus achieving a more yellowish color. RMD addition to OJWP samples slightly decreased ($p < 0.05$) b* values from 5%. On the other hand, RMD addition did not show any effect ($p > 0.05$) on the b* values of OJP samples. Thus, orange pulp could also interact with RMD to maintain yellowish tones. Color differences were higher ($p < 0.05$) as higher RMD concentrations were added in all OJP and OJWP samples.

Table 2 shows the mean values with standard deviations of AA, vitamin C, TP, TC and AC for all OJP and OJWP samples at the beginning of the storage period. Control samples (OJP0 and OJWP0) had almost the same vitamin C content. OJP and OJWP RMD-added samples had a similar behavior, as higher RMD concentrations slightly but not significantly ($p > 0.05$) increased vitamin C content. However, in the 7.5% RMD-added samples, the pulp-added sample obtained a significant ($p < 0.05$) higher vitamin C content than the rest. A similar behavior was also observed in our previous study (Arilla *et al*., 2021). Besides, the protective effect of other prebiotic fibers such as inulin or gluco-oligosaccharides on the vitamin C content of heat-treated juices has also been suggested by Alves Filho *et al*. (2018). In terms of AA content, OJWP0 got a higher ($p < 0.05$) value than OJP0. RMD addition maintained the AA content in the OJP samples ($p > 0.05$), while it did have a clear protective effect ($p < 0.05$) in the OJWP samples, especially at higher RMD concentrations ($p < 0.05$). Therefore, considering the vitamin C results, RMD addition seems to prevent DHAA degradation to other forms that no longer have a vitamin function.

OJP0 marked higher ($p < 0.05$) TP content than OJWP0, probably because orange pulp contains hesperidin, which is the main phenolic compound in oranges (Iglesias-Carres *et al*., 2019). Besides, orange pulp has been reported to contain 1.6 times higher TP concentration than orange juice (De Ancos *et al*., 2017), so its addition to orange juice is expected to increase TP content. This difference was even more noticeable in terms of

TC, where OJP0 also marked higher ($p < 0.05$) TC content than OJWP0. Rodrigo *et al.* (2015) also found an increase close to 40% in the TC content of orange pulp in comparison to freshly prepared orange juice. This enlightens the potential application of citrus by-products as an economic and natural way to increase health-related compounds content, as it has been previously suggested (Kaur *et al.*, 2021).

Table 2. Mean values (and standard deviations) of ascorbic acid (AA), vitamin C, total phenols (TP), total carotenoids (TC) content and antioxidant capacity (AC) of pasteurized orange juice, expressed in mg/100 $g_{\text{orange juice}}$.

Sample	AA	Vitamin C	TP	TC	AC (TEq)
OJP0	4.54 ± 0.13^{e}	4.88 ± 0.05^{cd}	50.4 ± 0.4^{e}	4.19 ± 0.02^{e}	51.9 ± 1.9^{c}
OJP2.5	4.69 ± 0.05^{d}	5.02 ± 0.12^{bc}	53.7 ± 0.4^{c}	4.54 ± 0.04^{c}	52.0 ± 2.4^{c}
OJP5	4.6026 ± 0.0007^{de}	4.97 ± 0.04^{bcd}	55.3 ± 0.5^{b}	4.756 ± 0.006^{b}	53.1 ± 0.4^{bc}
OJP7.5	4.66 ± 0.02^{de}	5.53 ± 0.02^{a}	56.7 ± 0.3^{a}	5.00 ± 0.08^{a}	54.1 ± 0.6^{bc}
OJWP0	4.71 ± 0.04^{cd}	4.85 ± 0.02^{d}	49.0 ± 0.6^{f}	3.25 ± 0.02^{i}	53.1 ± 0.6^{bc}
OJWP2.5	4.83 ± 0.05^{c}	5.10 ± 0.12^{b}	51.6 ± 0.4^{d}	3.573 ± 0.005^{g}	53.33 ± 0.12^{bc}
OJWP5	4.9991 ± 0.0009^{b}	4.977 ± 0.012^{bcd}	53.8 ± 0.6^{c}	3.86 ± 0.02^{f}	55.6 ± 0.3^{ab}
OJWP7.5	5.169 ± 0.017^{a}	4.95 ± 0.09^{bcd}	53.8 ± 0.4^{c}	4.36 ± 0.08^{d}	57.0 ± 1.2^{a}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp; 0-7.5, resistant maltodextrin percentage.

Also, RMD addition had a clearer impact on the TP and TC content that on the AA and vitamin C content of orange juice. It showed a protective effect ($p < 0.05$) of the TP and TC content of all OJP and OJWP samples, being this effect greater ($p < 0.05$) with higher RMD concentrations. Protecting TP from process degradation is of interest because their potential prebiotic-like effect (Dueñas *et al.*, 2015; Lima *et al.*, 2019). In addition, the protective effect of RMD on TP and TC content seems to be slightly greater in OJP samples than in OJWP samples, suggesting that orange pulp presence might also reinforce TP and TC protection. This agrees with the results in the a* and b* values (Table 1), where RMD addition showed a higher protective effect in the reddish and yellowish tones of OJP samples.

The AC is the result of each bioactive compound (AA, vitamin C, TP, and TC) contribution. OJPW0 showed a slight but not significant ($p > 0.05$) higher value than OJP0. RMD addition also sightly but not significantly ($p > 0.05$) increased the AC in OJP samples. However, it did exert a significant ($p < 0.05$) protective effect on the AC of OJWP samples, especially at 7.5% RMD concentration. A similar behavior was also observed in our previous study (Arilla *et al.*, 2021), where OJWP7.5 achieved the highest AC variation. It has been also reported that adding other prebiotic fiber such as inulin to acerola juice had a protective effect from thermal degradation on vitamin C and TP content, thus leading to higher AC (Fonteles *et al.*, 2021). Consequently, adding prebiotic fibers to pasteurized fruit-based beverages could be beneficial to better preserve health-related compounds from thermal degradation.

Orange juice stability

Figure 1 shows the mean values and standard deviation of pH and °Brix of all OJP and OJWP samples along storage. pH of all OJP and OJWP samples moved in the narrow range between 3.55 and 3.65 along the 170 days of storage. Consequently, not significant ($p > 0.05$) changes on the pH are appreciable during storage period due to orange pulp presence nor RMD addition. °Brix were also very stable over the course of the whole storage period, with no significant ($p > 0.05$) changes in any sample.

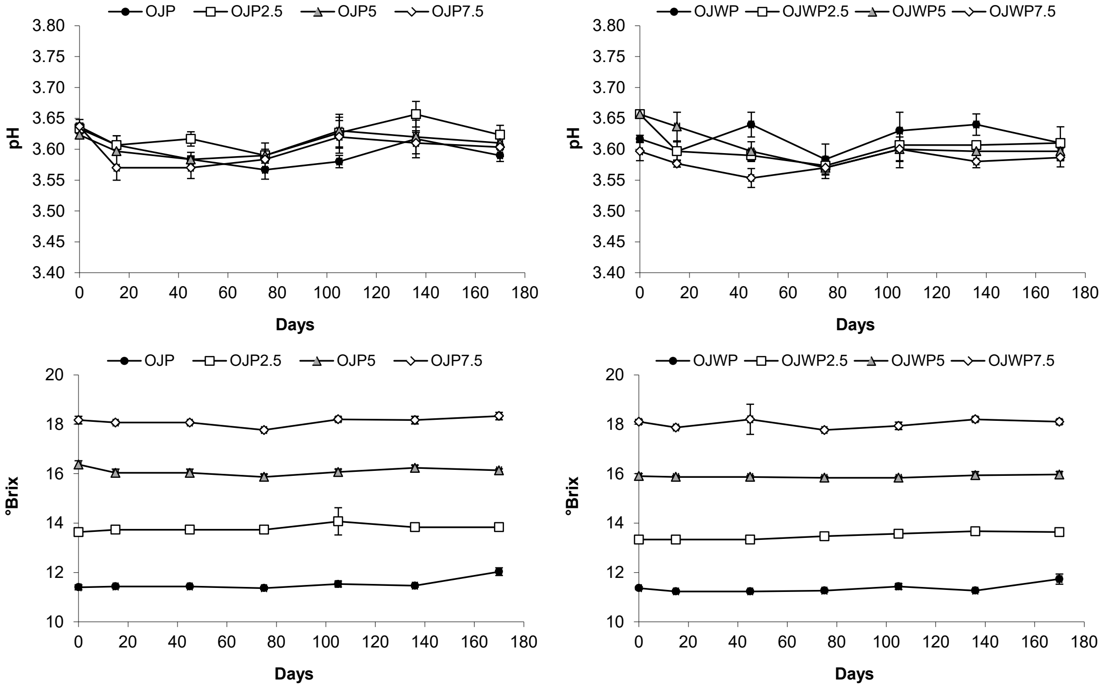

Figure 1. Mean values and standard deviation of pH and °Bx of studied juices during storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

The stability of °Brix and pH in citrus fruit juices along storage was also reported by another authors (Elez-Martínez *et al*., 2006; Igual *et al*., 2010; Agcam *et al*., 2016). A similar behavior was observed in fruit juice beverages fortified with another prebiotic fiber such as FOS, where pH and °Brix hardly changed in storage periods of 4–6 months (Renuka *et al*., 2009; Cascales *et al*., 2021). As explained before, °Brix and pH were measured as quality control parameters. Therefore, the stability of these parameters along the whole storage period indicates that no degradation reactions that altered the results of the study occurred, hence retaining the desirable physicochemical properties.

To evaluate the effect of the storage time to all samples, the variation of each component at each storage time (ΔM_i^t) was calculated referred to the original mass compound of each sample at the beginning of storage in the OJP and OJWP samples, respectively, according to Equation 1:

$$\Delta M_i^t = \frac{(M_i^t - M_i^0)}{M_i^0} \times 100 \qquad (1)$$

where, M_i^t: mass of compound i at storage time t for each sample obtained from 100 g of pasteurized orange juice (OJP and OJWP), and M_i^0: mass of compound i at the beginning of storage time (day 0) for each sample obtained from 100 g of pasteurized orange juice (OJP and OJWP).

Figure 2 shows the mean values and standard deviation of AA and vitamin C variations of all OJP and OJWP samples along storage. Despite both OJP0 and OJWP0 samples got similar AA variations along storage ($p > 0.05$), the initial drop in the AA content in OJWP0 is more noticeable than in OJP0. This suggests that orange pulp could play a role in better preserving the AA of pasteurized orange juice at the first stage of storage time. Also, RMD addition in OJWP samples, especially at higher doses, resulted in lower ($p < 0.05$) AA loss being this protective effect clearer at the first stages (days 15 to 75) of storage. On the contrary, all RMD-added OJP samples were losing AA at almost the same magnitude as OJP0, indicating that RMD addition did not exert a protective effect ($p > 0.05$) on the AA content of OJP samples.

Regarding vitamin C variations, OJP0 and OJWP0 had the same behavior, meaning that orange pulp did not display a significant ($p > 0.05$) impact on the vitamin C loss along storage. Besides, RMD addition to orange juice slightly improved ($p < 0.05$) vitamin C retention at the first stages of storage, especially in the OJP samples. However, from day 105 all samples had similar vitamin C loss. Other orange juice-based studies have also reported AA and vitamin C loss along storage (Elez-Martínez *et al*., 2006; Esteve & Frigola, 2008; Torres *et al*., 2011; Islam *et al*., 2014; Spira *et al*., 2018). Moreover, it has been suggested that AA and vitamin C degradation because dissolved oxygen presence may cause great color changes in heat-treated juices stored at room temperature (Elez-Martínez *et al*., 2006; Ros-Chumillas *et al*., 2007). Ascorbic acid acts as an oxygen scavenger for the removal of molecular oxygen. For this reason, deaeration has been suggested as a recommended process for juice industrial producers to improve juice quality along storage, as this process diminishes the oxygen consumption via oxidative reactions of ascorbic acid (Remini *et al*., 2015).

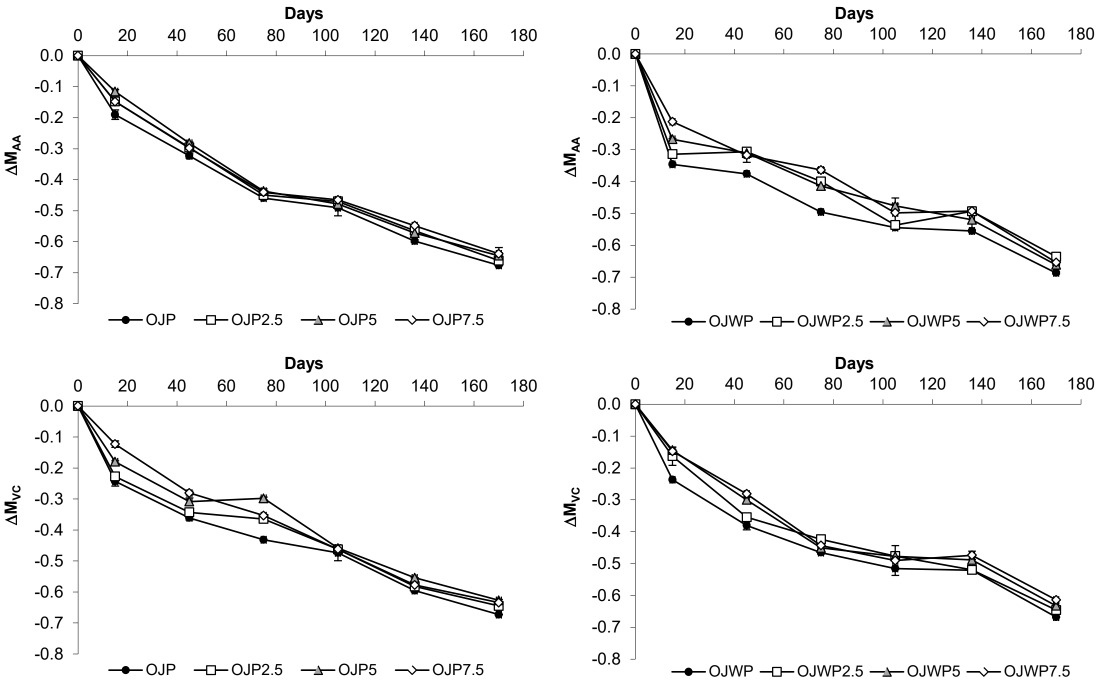

Figure 2. Mean values and standard deviation of ascorbic acid variation (ΔMAA) and vitamin C (ΔMVC) during storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

Concerning TP variation along storage (Figure 3), OJP0 and OJWP0 samples followed the same trend: both were losing TP by a similar magnitude over time. Thus, orange pulp did not have a significant ($p > 0.05$) impact on TP variation throughout storage. Contrarily, higher RMD concentrations led to lower ($p < 0.05$) TP variations. However, this protective effect of RMD on TP variations was limited, as the most noticeable differences were found on days 45, 75 and 105, from which time the differences narrowed. Other pasteurized orange-juice based studies also reported slight TP degradation during the shelf life of pasteurized orange juice (Ros-Chumillas *et al*., 2007; Spira *et al*., 2018). Pasteurization has been reported to generally reduce TP content in comparison to fresh juice, while non-thermal preservation methods as high hydrostatic pressure may even improve TP content, probably due to changes in the structure of vesicles in the orange juice that enables greater extraction of flavanones (Sánchez-Moreno *et al*., 2005; Esteve & Frigola, 2008).

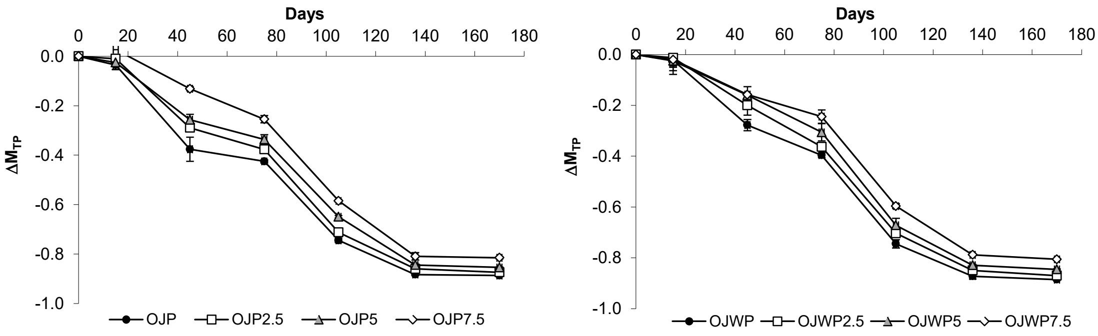

Figure 3. Mean values and standard deviation of total phenols variation (ΔMTP) during storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

Although the loss of TC during the first 15 days is greater in the OJP samples, from day 45 the evolution of TC variations was the same in both OJP and OJWP samples (Figure 4). Thus, the role of orange pulp in TC loss along storage is not significant ($p > 0.05$). RMD addition improved ($p < 0.05$) TC protection in all OJP and OJWP samples, being this effect clearer in the OJP samples, as all RMD-added OJP samples ended up with lower TC loss than the RMD-added OJWP samples. A slight decrease of TC content along storage period in pasteurized orange juice has also been reported (Esteve & Frigola, 2008).

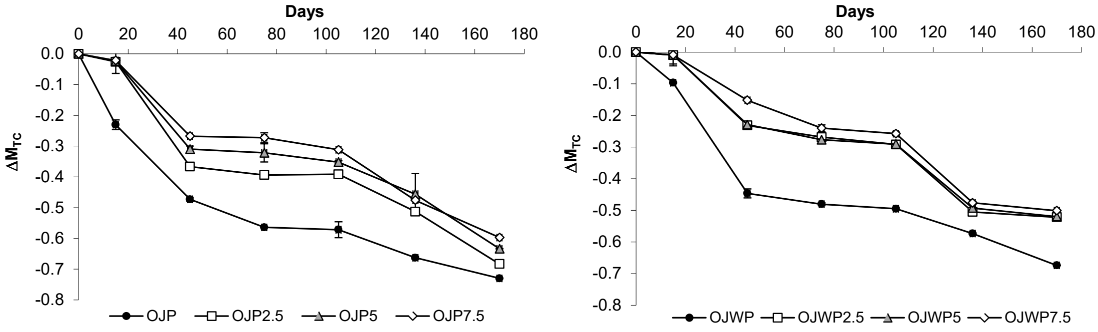

Figure 4. Mean values and standard deviation of total carotenoids variation (ΔMTC) during storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

Figure 5 shows the mean values and standard deviations of AC variation throughout the storage period. OJP0 had less ($p < 0.05$) AC variations along storage than OJWP0, meaning that orange pulp contributes to improve AC protection of orange juice. Also, all RMD-added samples marked lower ($p < 0.05$) AC variations in comparison to control samples. In the OJP samples, all RMD-added samples marked similar AC variations (except from day 75), indicating then that higher RMD concentrations in OJP samples did not lead to a significant higher ($p > 0.05$) protective effect along storage period. In the OJWP samples, however, higher RMD concentrations led to higher ($p < 0.05$) protective effect at the first stages of the storage time. At the end of storage time (from days 105 to 170), all RMD-added OJWP samples exhibited also similar AC variations.

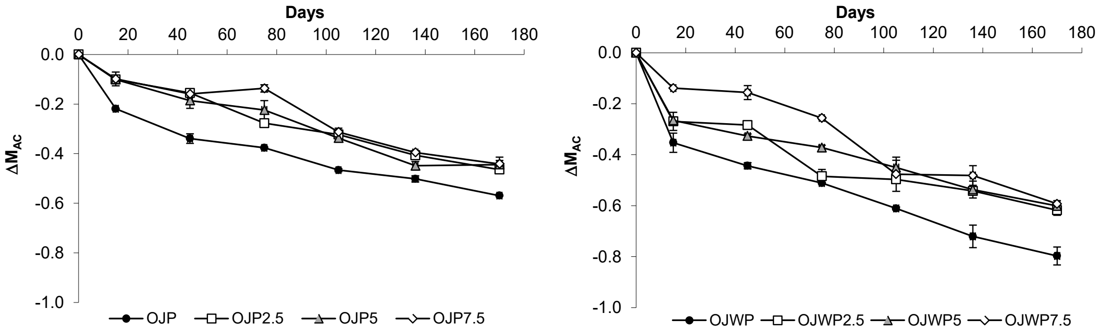

Figure 5. Mean values and standard deviation of antioxidant capacity variation (ΔMAC) during storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

To summarize, all samples were very stable along the whole storage period in terms of °Brix and pH. RMD addition to OJWP samples slightly improved AA loss at the first stages of storage, while it did not have any effect in the OJP samples. In addition, both OJP and OJWP RMD-added samples also increased vitamin C protection at the first stages of storage. Orange pulp did not play any effect on the TP variations along storage, while RMD addition improved TP retention. Regarding TC variations, OJP and OJWP samples had also similar behaviors, and RMD addition better preserved TC along storage in all samples. Finally, orange pulp helped to retain the AC of orange juice, and RMD addition increased AC retention in all samples.

Table 3 shows the mean values (and standard deviations) of studied bioactive compound and the AC at the end of storage period. OJP0 and OJWP0 marked almost the same AA ($p > 0.05$) at the end of storage time, indicating that orange pulp presence did not dampen AA loss over time. This contrasts to the results at the beginning of the study, where OJWP0 had a higher AA content than OJP0 (Table 2). RMD addition slightly improved ($p < 0.05$) AA retention, especially in the pulp-free samples. Probably this could be because RMD addition to OJWP samples before pasteurization process helped to maintain higher AA levels than in the OJP samples. Moreover, OJWP0 and OJP0 also marked similar ($p > 0.05$) vitamin C content at the end of the storage period. RMD addition improved ($p < 0.05$) vitamin C retention in all OJP and OJWP samples, being this effect higher with higher RMD concentrations.

In terms of TP content, orange pulp did not play a significant role ($p > 0.05$) at the end of the storage period, and higher RMD doses helped to maintain higher ($p < 0.05$) TP levels of all samples. This behavior agrees with the results at the beginning of the storage time shown in Table 2. Also, OJP0 had a higher TC content than OJWP0 at the first stage of the storage period. However, despite still showing significance ($p < 0.05$), this difference narrowed at the end of storage. RMD addition significantly ($p < 0.05$) improved TC retention in all OJP and OJWP samples, being this effect greater as higher RMD concentrations were added. At the beginning of the storage time, the difference between OJP0 and OJWP0 regarding AC was not significant. However, at the end of the study, OJWP0 marked a significant ($p < 0.05$) higher AC than OJP0. Therefore, although orange pulp has been suggested as an economic and natural way to add health-related compounds, is not enough to improve the AC of pasteurized orange juice over storage time by itself. However, RMD addition significantly improved ($p < 0.05$) the AC of all OJP and OJWP samples, being this effect higher in the pulp-added samples. Therefore, it seems that RMD addition could have better preserved the AC of orange pulp over storage time, so the combined addition of RMD and orange pulp resulted in higher AC retention. In addition, the highest AC were obtained at 5% RMD in both OJP and OJWP samples, suggesting that higher RMD concentrations does not necessarily help to maintain higher AC levels.

Table 3. Mean values (and standard deviations) of ascorbic acid (AA), vitamin C, total phenols (TP), total carotenoids (TC) content and antioxidant capacity (AC) of pasteurized orange juice after storage, expressed in mg/100 $g_{orange\ juice}$.

Sample	AA	Vitamin C	TP	TC	AC (TEq)
OJP0	1.47 ± 0.12^{e}	1.596 ± 0.003^{f}	5.70 ± 0.05^{d}	1.131 ± 0.003^{g}	2.71 ± 0.19^{f}
OJP2.5	1.591 ± 0.008^{de}	1.78 ± 0.02^{e}	6.77 ± 0.14^{c}	1.441 ± 0.004^{f}	27.9 ± 0.7^{b}
OJP5	1.63 ± 0.07^{cd}	1.854 ± 0.014^{c}	8.07 ± 0.12^{b}	1.743 ± 0.003^{d}	33.0 ± 1.2^{a}
OJP7.5	1.68 ± 0.02^{abc}	2.021 ± 0.015^{a}	10.5 ± 0.3^{a}	2.013 ± 0.007^{b}	30.20 ± 1.5^{b}
OJWP0	1.48 ± 0.02^{e}	1.609 ± 0.012^{f}	5.61 ± 0.14^{d}	1.059 ± 0.002^{h}	10.8 ± 1.9^{e}
OJWP2.5	1.76 ± 0.03^{ab}	1.81 ± 0.03^{de}	6.72 ± 0.17^{c}	1.707 ± 0.002^{e}	20.4 ± 1.05^{d}
OJWP5	1.69 ± 0.12^{abc}	1.828 ± 0.009^{cd}	8.32 ± 0.12^{b}	1.855 ± 0.003^{c}	24.99 ± 0.14^{c}
OJWP7.5	1.79 ± 0.012^{a}	1.909 ± 0.005^{b}	10.44 ± 0.15^{a}	2.1762 ± 0.0008^{a}	23.2 ± 0.7^{c}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$). OJP, orange juice with pulp; OJWP, orange juice without pulp; 0-7.5, resistant maltodextrin percentage.

To explain the influence of the different compounds quantified in this study on the AC of the samples, correlation statistical analyses were performed. All Pearson's correlation coefficient with AC were positives. TC played a major role in the antioxidant capacity of orange pasteurized juices (0.8949, $p < 0.05$), followed by the vitamin C (0.8410, $p < 0.05$), AA (0.8333, $p < 0.05$) and TP (0.8237, $p < 0.05$).

Figure 6 shows the mean values and standard deviations of L*, a*, b* and total color variations at days 105 and 170 with respect to the beginning of storage. All samples had a positive variation of all color parameters, being this clearer at the end of the storage time (day 170). L* values of OJP0 were lower ($p < 0.05$) than OJWP0, so orange pulp seems to help retaining the original brightness of orange juice. However, RMD addition to OJP samples significantly ($p < 0.05$) increased L* variations from a concentration of 5%, indicating that OJP5 and OJP7.5 obtained a lighter coloration at the end of storage. On the contrary, RMD did not play a clear role ($p > 0.05$) on the OJWP samples. Furthermore, OJP0 got a significant ($p < 0.05$) higher a* variation than OJWP0, meaning that orange pulp presence increased reddish tones of orange juice over storage time. RMD addition to OJP samples slightly decreased a* variations (except from OJP2.5), being this effect clearer with higher doses of RMD and especially at the end of storage (day 170). Therefore, RMD presence at high concentrations seems to dampen the effect of orange pulp on the reddish tones of orange juice. Conversely, RMD increased a* variations in the pulp-free samples. In fact, at the highest RMD concentration (7.5%), both OJP and OJWP samples at both storage times (days 105 and 170) did not show a significant ($p > 0.05$) difference in terms of a* values. Also, a* variations were relatively small. For example, at day 105 all samples except from OJP2.5 marked an a* variation lower than 1 unit, indicating that this color parameter is the most stable one in orange juice. Moreover, OJWP0 presented a higher ($p < 0.05$) b* variation than OJP0 at the end of storage. This indicates that orange pulp may help to maintain the original yellowness of orange juice. RMD addition to OJP samples increased ($p < 0.05$) b* variations over time, while in the OJWP samples it did not have a significant ($p > 0.05$) effect. In fact, the pulp-added samples with higher RMD concentrations (OJP5 and OJP7.5) obtained comparable b* variations to all OJWP samples. Therefore, RMD played a role in turning pulp-added orange juice more yellowish. Regarding total color differences, all samples marked variations larger than 8 units at days 105 and 170. This indicates that color difference at the end of storage was clearly perceptible to human eye in comparison to the color of the samples at the beginning of the study, as it needs at least a color difference of 3 units to be distinguished (Bodart *et al.*, 2008). OJP0 had a lower total color variation that OJWP0, indicating that orange pulp helped to better preserve ($p < 0.05$) the original color of orange juice. RMD addition to OJP samples at higher concentrations caused greater ($p < 0.05$) color variations. This also led to the fact that OJP5 and OJP7.5 presented a clear color difference in comparison to OJP0 and OJP2.5 at day 170. However, in the pulp-free samples RMD did not show a clear impact ($p > 0.05$) on the color differences, as all OJWP samples obtained similar total color variations.

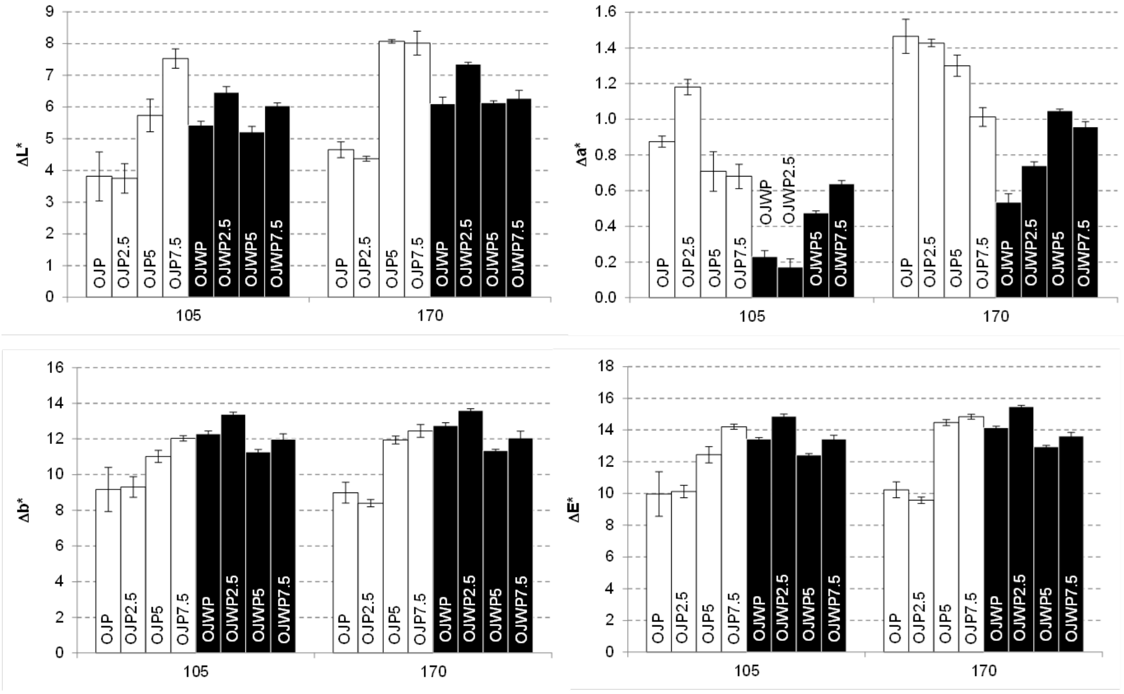

Figure 6. Mean values and standard deviation of L^*, a^*, b^* variation in orange juices (ΔL^*, Δa^*, and Δb^*) and total color differences (ΔE) at 105 and 170 days of storage. OJP, orange juice with pulp; OJWP, orange juice without pulp; 0–7.5, resistant maltodextrin percentage.

Establishing Pearson correlations between the color coordinates and the bioactive compounds on the days tested, a high correlation was observed between the AA content and the color coordinates. Pearson coefficients were for AA-L^* 0.8012 ($p < 0.05$), AA-a^* −0.7899 ($p < 0.05$) and AA-b^* 0.9449 ($p < 0.05$). According to these results, the color changes are probably due to oxidation reactions of AA.

CONCLUSIONS

RMD addition to orange juice before being pasteurized had a protective effect in all bioactive compounds at the beginning of the storage period, especially in the TP and TC content, which led to higher AC, especially in the OJWP samples. Despite orange pulp has been suggested as a natural way to increase bioactive compounds of food products, its effect over storage time was limited in the control samples, as OJWP0 achieved a higher AC than OJP0. However, RMD addition helped to improve all bioactive compounds retention in all OJP and OJWP samples, especially TP and TC. This led to higher AC in all samples, being this even clearer in the OJP. Therefore, this study enlightens the potential use of RMD to better preserve the health-related compounds of pasteurized orange juice along storage time, especially when orange pulp is added.

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CHAPTER 7. GENERAL CONCLUSIONS

The global juice industry has undergone significant transformation over the past decade, driven by evolving consumer preferences for healthier lifestyles and technological advancements. Adding prebiotics to orange juice emerge as a natural way to innovate and stimulate the fruit juice market. Most studies focus on the health effects of prebiotics in human body. However, how prebiotics fibers affect the food matrix into which they are added has barely been addressed. In our studies, the addition of RMD to orange juice, with and without pulp, before pasteurization process had many outcomes:

- The feasibility of incorporating RMD to orange juice without major challenges allows an easy adaptation in current production processes, which can encourage innovation and development of new functional beverages.
- Because its good water-dissolving properties, RMD can be added to orange juice in a wide range of concentrations. It is important to consider that, the higher RMD dosage is added, the more significant impact it displays in the intrinsic characteristics of orange juice.
- The varying effects of different RMD concentrations offer flexibility to customize products according to desired physicochemical properties and sensory profiles, catering to diverse consumer preferences.
- Orange pulp presence had very little impact on the physicochemical properties of orange juice, except for its rheology and turbidity, which can be expected since orange pulp represents the main insoluble fiber in a water and soluble solids matrix such as orange juice.
- Orange pulp could be used to increase bioactive compounds content, leading to higher antioxidant capacity. This shows the potential use of orange by-products as economic and sustainable raw materials to improve the nutrition quality of foodstuff.
- Adding RMD to orange juice before pasteurization played a role in preserving intrinsic health-promoting compounds with antioxidant capacity from thermal degradation. This protective effect was greater with higher RMD concentrations. Therefore, RMD could be added with other technological purposes other than its prebiotic benefits that also leads to health-related effects.
- The addition of RMD to orange juice enhanced its sensory attribute without affecting its aromatic profile, increasing its sweetness while reducing acidity and bitterness without any significant off-flavors. This led to higher overall ratings from panelists, especially in pulp-added samples. Consequently, RMD may be added to fruit juices to upgrade its sensory attributes.

- The protective action of RMD on the bioactive compounds of orange juice extended over storage time. This is relevant since RMD could be used to enhance the stability and retention of beneficial antioxidant compounds, such as phenols and carotenoids, during the shelf life of the juice, contributing to a better nutritional value. This can lead to the production and delivery of higher quality beverages.

In summary, the application of RMD in orange juice manufacturing could be of interest from both consumer and food industry perception, offering improved product quality, enhanced sensory attributes, and health-related benefits other than its prebiotic activity. This research could improve the marketability of fruit juices and open new pathways for innovation in the fruit juice market. By strategically incorporating prebiotics like RMD into day-to-day food products, the food technology sector could potentially redefine the standards for creating functional beverages.