Tesis Doctoral

Reformulación de productos horneados para disminuir el contenido en grasa y azúcar mediante la sustitución con inulina. Efectos sobre la estructura y propiedades físicas

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REFORMULACIÓN DE PRODUCTOS HORNEADOS PARA DISMINUIR EL CONTENIDO EN GRASA Y AZÚCAR MEDIANTE SUSTITUCIÓN CON INULINA. EFECTOS SOBRE LA ESTRUCTURA Y PROPIEDADES FÍSICAS

TESIS DOCTORAL

PRESENTADA POR:
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MI QU ALI
Grupo de Investigación
Microestructura y Química
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Dña. Isabel Hernando Hernando, Catedrática de Universidad del Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia,

HACE CONSTAR QUE:

El trabajo de investigación **“Reformulación de productos horneados para disminuir el contenido en grasa y azúcar mediante sustitución con inulina. Efectos sobre la estructura y propiedades físicas”** que presenta Dña. Julia Rodríguez García por la Universidad Politécnica de Valencia, y que ha sido realizado bajo mi dirección en el Grupo de Investigación de Microestructura y Química de Alimentos de la Universidad Politécnica de Valencia, reúne las condiciones para optar al grado de Doctor.

Valencia, Abril de 2014

Fdo. Dra. Isabel Hernando Hernando

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Con gratitud y admiración por todos vosotros,

Julia

PD: No es solo importante llegar, lo que importa es ir cantando al caminar

Índice

Resumen

La presente tesis doctoral se centra en el estudio de la estructura, las propiedades físicas y sensoriales de bizcochos y galletas en los que la grasa se ha reemplazado por inulina y el azúcar por oligofructosa, con la finalidad de reformular los productos horneados para reducir significativamente el contenido de estos ingredientes y conseguir así alimentos más saludables.

La caracterización de la estructura de la masa se llevó a cabo mediante técnicas de microscopía confocal laser de barrido y microscopía óptica, a través de las cuales se apreció que la masa era una matriz lipoproteica, formada por proteína y grasa procedentes principalmente de la harina y el huevo. En esta matriz los gránulos de almidón, el aceite en forma de glóbulos y las burbujas de aire se encontraron dispersos. Se observó que el aceite posee una funcionalidad compleja en estos sistemas ya que, aporta viscosidad, lo cual aumenta la estabilidad de la fase aire en la masa; actúa en la interfase de las burbujas, mejorando la estabilidad y retención de la fase aire durante el horneado; y además, se distribuye lubricando los componentes de la miga, influyendo así en la estructura, textura y palatabilidad del bizcocho final. La sustitución del aceite en los bizcochos se llevó a cabo con una inulina nativa y componente acuoso extra. Este reemplazo ocasionó una disminución significativa de la viscosidad de la masa, lo cual dio lugar a la oclusión de burbujas de tamaños mayores y más heterogéneos. Durante el horneado la menor estabilidad de la fase gas en este tipo de masas se tradujo en la pérdida de parte de las burbujas, en una expansión más limitada y por tanto en la obtención de bizcochos con menos alveolos e interconexiones alveolares, y menor altura. A mayor sustitución de grasa por inulina los bizcochos mostraron mayor dureza y menor elasticidad, debido a la estructura de miga compacta y poco lubricada. Se obtuvieron resultados satisfactorios reemplazando hasta el 70% de la grasa, sin obtener diferencias significativas en la aceptabilidad sensorial por parte de los consumidores.

El reemplazo de azúcar por oligofructosa afectó al mecanismo de solidificación de la masa. El azúcar retrasa las temperaturas de gelatinización del almidón y desnaturalización de proteínas. Al reducir su contenido, estos procesos se adelantan e incluso pueden darse de forma sucesiva afectando significativamente la formación de la estructura sólida del bizcocho. Las masas con sustitución de azúcar mostraron una viscosidad aparente menor y un mayor número de burbujas ocluidas. Durante el horneado se observó una ligera expansión de las burbujas. Los bizcochos con oligofructosa se caracterizaron por poseer baja altura y una miga formada por pocos alveolos y de pequeño tamaño. Estos bizcochos mostraron una dureza y elasticidad bajas. Los cambios en el proceso de solidificación de la masa dieron lugar a bizcochos más compactos pero más blandos. Con un reemplazo de hasta el 30% de azúcar se obtuvieron bizcochos con una aceptabilidad general similar a la del bizcocho control. Cuando se reemplazó de manera simultánea la grasa y el azúcar, la viscosidad aparente de las masas disminuyó significativamente y la expansión de las burbujas durante el horneado fue mayor. Los bizcochos resultantes mostraron una estructura alveolar más abierta y heterogénea. Su textura se vio influenciada sobre todo por la sustitución de azúcar, obteniéndose bizcochos de baja elasticidad. Los bizcochos con un reemplazo simultáneo del 50% de grasa y el 30% de azúcar recibieron una aceptabilidad general un poco menor al control pero semejante a los bizcochos con reemplazo sencillo de grasa (50%) o azúcar (30%). En general, los consumidores valoraron que la esponjosidad y dulzor de estos bizcochos debería mejorarse para conseguir una mayor aceptabilidad de estos productos.

El método de mezclado más empleado a nivel industrial para la elaboración de bizcochos consiste en un solo paso en el que todos los ingredientes se baten a la vez. Con el objetivo de escalar nuestra formulación a nivel de planta piloto se consiguió reemplazar un método de mezclado formado por múltiples fases por el método de una fase sin alterar las propiedades físicas y estructurales de los bizcochos.

Para mejorar la apariencia y estructura alveolar de los bizcochos con un contenido reducido en grasa (50% y 70% de reemplazo de grasa) se evaluaron los efectos de la incorporación de una enzima lipasa y un emulsionante comercial sobre sus propiedades físicas y estructurales. Los mejores resultados sobre la estructura de la miga se obtuvieron con 0.03% de lipasa y 0.5% de emulsionante. Cada mejorante ejerció un efecto diferente sobre la estructura de la masa; las masas con lipasa mostraron una menor estructuración que las masas control. La incorporación de emulsionante disminuyó significativamente la densidad relativa de las masas. Sin embargo durante el horneado, concentraciones de emulsionante mayores a 0.5% dieron lugar a un descenso de la viscosidad compleja de la masa, cuya estructura no fue capaz de retener las burbujas que expansionaron en esta fase, dando lugar a bizcochos colapsados. Los análisis de calorimetría diferencial de barrido mostraron que la formulación con mayores valores en los parámetros térmicos fue la formulación control, dando lugar a los bizcochos de mayor volumen. La adición de los mejorantes disminuyó los valores de los parámetros térmicos. Aunque el volumen de los bizcochos con 0.003% de lipasa y 0.5% de emulsionante fue menor, su estructura alveolar presentaba una apariencia más homogénea y la dureza de la miga fue similar a la del control. Por otro lado, aunque el reemplazo de grasa dio lugar a un aumento de la dureza durante el almacenamiento, la incorporación de lipasa ralentizó el incremento del valor de este parámetro durante los 14 días.

A través de microscopía confocal laser de barrido y de microscopía electrónica de barrido a bajas temperaturas, se observó la estructura de la masa de galletas como una matriz continua formada por azúcar y proteínas, donde los gránulos de almidón se dispersaban y la grasa se localizaba alrededor de ellos rompiendo la continuidad de la estructura. Al reemplazar parte de la grasa por inulina, se observó un aumento de la dureza de las masas y las galletas, lo cual se relacionó con un aumento de la hidratación de los componentes de la harina y una menor lubricación de la estructura. Reemplazar hasta el 20% de la grasa dió lugar a galletas con una estructura y propiedades texturales semejantes al control. Sin embargo, los consumidores sí que identificaron este producto como ligeramente diferente al control por ser un poco más duro y más dulce.

Resum

Aquesta tesi doctoral se centra en l'estudi de l'estructura i les propietats físiques i sensorials de bescuits i galetes en què el greix s'ha reemplaçat per inulina i el sucre per oligofructosa, amb la finalitat de reformular els productes fornejats per a reduir significativament el contingut d'aquests ingredients i aconseguir així aliments més saludables.

La caracterització de l'estructura de la massa s'ha dut a terme mitjançant tècniques de microscòpia confocal làser d'escombratge i microscòpia òptica, a través de les quals s'hi ha apreciat que la massa és una matriu lipoproteica, formada per proteïna i greix procedents principalment de la farina i l'ou. En aquesta matriu, els grànuls de midó, l'oli en forma de glòbuls i les bambolles d'aire s'hi troben dispersos. S'ha observat que l'oli posseeix una funcionalitat complexa en aquests sistemes, ja que aporta viscositat, la qual cosa augmenta l'estabilitat de la fase aire en la massa; actua en la interfase de les bambolles, i millora l'estabilitat i la retenció de la fase aire durant la fornejada. A més, s'hi distribueix i lubrica els components de la molla, de manera que influeix en l'estructura, la textura i la palatabilitat del bescuit final. La substitució de l'oli en els bescuits s'ha dut a terme amb una inulina nativa i component aquós extra. Aquest reemplaçament ha ocasionat una disminució significativa de la viscositat de la massa, fet que ha donat lloc a l'oclusió de bambolles de dimensions majors i més heterogenis. Durant la fornejada, la menor estabilitat de la fase gas en aquest tipus de masses s'ha traduït en la pèrdua de part de les bambolles, en una expansió més limitada i, per tant, en l'obtenció de bescuits amb menys alvèols i interconnexions alveolars, i menor altura. A major reemplaçament de greix per inulina, els bescuits mostren major duresa i menor elasticitat, a causa de l'estructura de molla compacta i poc lubricada. S'hi han obtingut resultats satisfactoris reemplaçant fins al 70% del greix, sense obtenir diferències significatives en l'acceptabilitat sensorial per part dels consumidors.

El reemplaçament de sucre per oligofructosa ha afectat el mecanisme de solidificació de la massa. El sucre retarda les temperatures de gelatinització del midó i desnaturalització de proteïnes. En reduir-ne el contingut, aquests processos s'avancen i fins i tot poden donar-se de forma successiva i afectar significativament la formació de l'estructura sòlida del bescuit. Les masses amb reemplaçament de sucre han mostrat una viscositat aparent menor i un major nombre de bambolles ocloses. Durant la fornejada s'ha observat una lleugera expansió de les bambolles. Els bescuits amb

oligofructosa s'han caracteritzat per posseir baixa altura i una molla formada per pocs alvèols i de petites dimensions. Aquests bescuits han mostrat duresa i elasticitat baixes. Els canvis en el procés de solidificació de la massa han donat lloc a bescuits més compactes però més blans. Amb un reemplaçament de fins al 30% de sucre, s'han obtingut bescuits amb una acceptabilitat general similar a la del bescuit control. Quan s'ha reemplaçat de manera simultània el greix i el sucre, la viscositat aparent de les masses ha disminuït significativament i l'expansió de les bambolles durant la fornejada ha sigut major. Els bescuits resultants han mostrat una estructura alveolar més oberta i heterogènia. La textura d'aquests s'ha vist influenciada sobretot pel reemplaçament de sucre, i així s'han obtingut bescuits de baixa elasticitat. Els bescuits amb un reemplaçament simultani del 50% de greix i el 30% de sucre han rebut una acceptabilitat general una mica menor al control, però semblant als bescuits amb reemplaçament senzill de greix (50%) o sucre (30%). En general, els consumidors han valorat que l'esponjositat i la dolçor d'aquests bescuits hauria de millorar-se per a aconseguir una major acceptabilitat d'aquests productes.

El mètode de mescla més emprat en la indústria per a l'elaboració de bescuits consisteix en un sol pas en el qual tots els ingredients es baten alhora. Amb l'objectiu d'escalar la nostra formulació a planta pilot, s'ha aconseguit reemplaçar un mètode de mescla format per múltiples fases pel mètode d'una fase sense alterar les propietats físiques i estructurals dels bescuits.

Per a millorar l'aparença i l'estructura alveolar dels bescuits amb un contingut reduït en greix (50% i 70% de reemplaçament de greix), s'han avaluat els efectes de la incorporació d'un enzim lipasa i un emulsionant comercial sobre les propietats físiques i estructurals d'aquests. Els millors resultats sobre l'estructura de la molla s'han obtingut amb 0,03% de lipasa i 0,5% d'emulsionant. Cada millorant ha exercit un efecte diferent sobre l'estructura de la massa; les masses amb lipasa han mostrat una menor estructuració que les masses control. La incorporació d'emulsionant ha disminuït significativament la densitat relativa de les masses. No obstant això, durant la fornejada, concentracions d'emulsionant majors a 0,5% han donat lloc a un descens de la viscositat complexa de la massa, l'estructura de la qual no ha sigut capaç de retenir les bambolles que han expansionat en aquesta fase, i això ha donat lloc a bescuits col·lapsats. Les anàlisis de calorimetria diferencial d'escombratge han mostrat

que la formulació amb majors valors en els paràmetres tèrmics ha sigut la formulació control, que ha donat lloc als bescuits de major volum. L'addició dels millorants ha disminuït els valors dels paràmetres tèrmics. Encara que el volum dels bescuits amb 0,003% de lipasa i 0,5% d'emulsionant ha sigut menor, l'estructura alveolar d'aquests presenta una aparença més homogènia i la duresa de la molla és similar a la del control. D'altra banda, encara que el reemplaçament de greix ha donat lloc a un augment de la duresa durant l'emmagatzematge, la incorporació de lipasa ha alentit l'increment del valor d'aquest paràmetre durant els 14 dies.

A través de microscòpia confocal làser d'escombratge i de microscòpia electrònica d'escombratge a baixes temperatures, s'ha observat l'estructura de la massa de galetes com una matriu contínua formada per sucre i proteïnes, en què els grànuls de midó es dispersen i el greix es localitza al voltant d'aquests i trenca la continuïtat de l'estructura. En reemplaçar part del greix per inulina, s'ha observat un augment de la duresa de les masses i les galetes, cosa que s'ha relacionat amb un augment de la hidratació dels components de la farina i una menor lubricació de l'estructura. Reemplaçaments de fins al 20% del greix han donat lloc a galetes amb una estructura i propietats texturals semblants al control. No obstant això, els consumidors sí que han identificat aquest producte com a lleugerament diferent al control en ser una mica més dur i més dolç.

Abstract

The research of this doctoral thesis has focused on the study of the structure, physical properties and sensory acceptability of cakes and biscuits, in which fat has been replaced by inulin and sugar has been replaced by oligofructose. The principal aim of this thesis was to develop reformulated bakery products with significantly less sugar and fat content and thus, with a healthier nutritional profile.

Batter structure was characterised using confocal laser scanning microscopy and light microscopy techniques. Cake batter was observed as a matrix constituted mainly by lipids and proteins from flour and egg. In this matrix starch granules, oil globules and air bubbles were dispersed. Oil showed a complex functionality in batters; increasing batter viscosity, forming an interface air-bubble and thus, increasing bubble stability in the batter and gas retention during baking. Moreover, the oil acted as a lubricant by coating and creating a continuous matrix; therefore it influenced the structure, texture, and palatability of the cake. In cakes, fat was replaced by inulin and extra aqueous component. Fat replaced batters showed a significant decrease in apparent viscosity, which led to a broad size distribution of occluded bubbles. During baking, the low stability of the gas phase gave place to a less gas retention and a lower cake expansion. Therefore, cakes showed a more compact crumb structure with less crumb cells and lower height and thus, harder cakes with less springiness were obtained. Cakes with 70% of fat replacement did not differ significantly from the control in the sensory attributes. In conclusion, high quality cakes with fat replacement up to 70% were obtained.

Sugar replacement by oligofructose affected the thermosetting mechanism of cake batters. Sugar plays an important role in delaying starch gelatinization and protein denaturation temperatures. When sugar content was reduced in cakes these processes came early and in successive steps, and thus the formation of the porous-solid structure was affected. Sugar-replaced batters showed a lower apparent viscosity and a higher number of air bubbles in comparison to control cake batter. During baking air bubbles slightly increased. Cakes with oligofructose were characterised by a crumb cell structure of few and little cells, and low height. These cakes showed low hardness and springiness values. Changes in the batter thermosetting mechanism gave place to cakes with more compact but softer crumbs. Cakes with sugar replacement up to 30% had similar consumer acceptability to control cakes. When sugar and fat were replaced simultaneously, batter apparent viscosity decreased significantly and a great bubble expansion was observed during heating. The resulting cakes had an open and uneven crumb cell structure. Sugar replacement had an important effect on cake texture giving place to cakes with low springiness. Cakes with simultaneous fat replacement of 50% and sugar replacement of 30% were scored by consumers slightly lower than the control cake. Nevertheless, these cakes were similar to cakes with simple replacement of 50% of fat and cakes with simple replacement of 30% of sugar. In general, consumer considered that sponginess and sweetness needed to be improved in these cakes to achieve a higher acceptability.

In large-scale cake manufacturing all-in mixing method is used for batter preparation on one stage. To scale up our cake production at pilot scale the traditional multi-stage mixing procedure was replaced by an all-in mixing method without modifying cake's physical and structural properties.

To improve the appearance and crumb cell structure of cakes with reduced fat content (50% and 70% of fat replacement) the effects of emulsifier and lipase incorporation were studied. The addition of 0.03% of lipase and 0.5% of emulsifier improved the crumb cell structure. Each improver had different effects in cake structure; batters with lipase showed a lower degree of system structuring than control batters. Emulsifier incorporation decreased significantly batter relative density. However, during heating, batters with high levels of emulsifier (> 0.5%) showed a decreased in batter complex viscosity. This batter could not retain the air bubble and thus, the obtained cakes were characterised by a collapsed structure. Differential scanning calorimetry studies showed that the control formulation displayed the highest thermal parameters values and thereby the control cake showed the highest volume. Lipase and emulsifier incorporation decreased the thermal parameter values and the obtained cakes showed lower volumes. Nevertheless, the crumb cell structure was improved showing a more uniform appearance and a similar hardness to the control cake. During storage time fat-replaced cakes showed higher hardness; however, lipase incorporation slowed down this increase.

Dough and biscuit microstructure was studied using confocal laser scanning microscopy and cryo scanning electron microscopy. Micrographs showed a continuous matrix composed mainly by sugar and proteins; starch granules were observed dispersed in the matrix and fat was located surrounding the granules and breaking the structure continuity. When fat was replaced by inulin an increase in dough and biscuit hardness was observed due to a higher hydration of flour components. Biscuits with fat replacement up to 20% were similar to control biscuits in terms of structure and textural properties. However, consumers were able to differentiate between control biscuits and biscuits with 20% of fat replacement reporting that the former was slightly less hard and sweet than the latter.

Introducción

1. Nutrición y salud

Los datos estadísticos revelan en los últimos años un deterioro paulatino de la calidad de la dieta ingerida en toda Europa así como una insuficiente práctica de la actividad física. De este modo, en las tres últimas décadas se ha producido un fuerte aumento del sobrepeso y la obesidad en el conjunto de la población de la Unión Europea; además, la obesidad y el sobrepeso presentes durante la infancia y la adolescencia pueden ser causa de hipertensión y complicaciones vasculares en etapas vitales posteriores (Álvarez-Martínez et al. 2013).

La Organización Mundial de la Salud (OMS) alerta de que la obesidad y el sobrepeso han alcanzado caracteres de epidemia a nivel mundial y se están convirtiendo en importantes problemas de salud pública en muchas partes del mundo. Los cambios en los hábitos alimentarios conducentes a una mayor densidad energética, donde la grasa y el azúcar añadido tienen un papel importante (Álvarez-Martínez et al. 2013), la influencia de la publicidad, la globalización de las dietas y la reducción generalizada de la actividad física han agravado en general los factores de riesgo y, probablemente, también las enfermedades sufridas en consecuencia (OMS/FAO 2003). Para invertir las tendencias actuales se requerirán políticas de salud pública multidimensionales. De este modo, en mayo de 2004 la 57ª Asamblea Mundial de la Salud aprobó la Estrategia Mundial de la Organización Mundial de la Salud sobre Régimen Alimentario, Actividad Física y Salud (OMS 2004). El reto para los Estados miembros fue, desde ese momento, la adaptación de la estrategia de la OMS a su entorno social y cultural. En este contexto, el año 2005 se puso en marcha en España la Estrategia para la Nutrición, Actividad Física y Prevención de la Obesidad (NAOS) desde el Ministerio de Sanidad y Consumo, a través de la Agencia Española de Seguridad Alimentaria y Nutrición, con el objetivo de sensibilizar a la población del problema que la obesidad representa para la salud, y de impulsar todas las iniciativas que contribuyan a lograr que los ciudadanos, y especialmente los niños y jóvenes, adopten hábitos de vida saludables, principalmente a través de una alimentación saludable y de la práctica regular de actividad física (AESAN 2005).

Una alimentación y nutrición adecuadas son importantes en todas las etapas de la vida, pero particularmente durante la infancia. La dieta de los niños y adolescentes españoles se caracteriza por un exceso de carnes, embutidos, productos lácteos y alimentos con alta densidad energética, como productos de bollería y bebidas carbonatadas (ricos en grasas y azúcares refinados) y por un déficit en la ingesta de frutas, verduras y cereales. Además, es preocupante que el 8% de los niños españoles acudan al colegio sin haber desayunado, ya que se ha demostrado que la prevalencia de obesidad es superior en aquellas personas que toman un desayuno escaso o lo omiten (AESAN 2005). En esta línea se han propuesto diversas líneas de actuación a nivel empresarial y de instituciones científicas, como la constitución de grupos de trabajo formados por tecnólogos para conocer, investigar y aplicar las posibilidades tecnológicas de ir sustituyendo los componentes grasos, o reduciendo su presencia (AESAN 2005). Según el Ministerio de Agricultura, Alimentación y Medio Ambiente (2004), la tendencia de la mayoría de estos productos será al alza; seguirá aumentando la oferta y con ello también la demanda. El consumidor es más exigente, se fija más en los ingredientes, en las propiedades de los diferentes productos y esto obliga a la industria a realizar grandes investigaciones.

2. Productos horneados

Los productos horneados como bizcochos y galletas tienen como ingredientes principales harina, grasa y azúcar, y como opcionales huevos, leche y otros componentes. Estos ingredientes y el proceso de elaboración dan lugar a productos con un dulzor, textura y palatabilidad características que son muy apreciadas por los consumidores. No obstante, el elevado porcentaje de grasa y azúcar que contienen hace que el aporte de calorías sea elevado y el perfil nutricional no muy saludable; por ello deben consumirse moderadamente. Por lo tanto, la disminución del contenido de grasa y azúcar en productos horneados para mejorar su perfil nutritivo es uno de los objetivos principales dentro de las áreas de actuación de los centros de investigación y la industria.

3. Bizcochos

Existen diferentes definiciones de bizcocho en diferentes partes del mundo, y la funcionalidad de los ingredientes también varía entre los distintos tipos de bizcocho. En función de las combinaciones de formulaciones y métodos de preparación, los bizcochos se clasifican en tres categorías: *batter*, *foam y chiffon*. Los bizcochos tipo *batter* se preparan mezclando primero la grasa y el azúcar hasta crear una espuma ligera, y al final se añade la harina. La preparación de los bizcochos tipo *foam* se caracteriza por la separación de las claras y las yemas para el batido; primero se baten las claras a punto de nieve y se les añade el azúcar. El resto de ingredientes se van añadiendo poco a poco hasta una correcta dispersión y disolución de todos ellos. El objetivo de este procedimiento es alcanzar el máximo volumen de la masa. La preparación de los bizcochos tipo *chiffon* consiste en una combinación de los métodos *batter* y *foam*; una masa con la harina, la yema, el aceite y el agua se añade a un merengue que se prepara inicialmente (Conforti 2006a; Wilderjans et al. 2013). En este trabajo se estudian los bizcochos tipo *foam,* cuya estructura y volumen dependen de las propiedades espumantes y de aireación del huevo.

La masa de bizcocho es una emulsión compleja de grasa en agua que consta de una fase discontinua de burbujas de aire y una fase continua de huevo, azúcar, agua y grasa, en la que se dispersan las partículas de harina (Kocer et al. 2007). Durante el mezclado se incorpora aire en la masa, el cual dependiendo del modo de incorporación de los ingredientes, puede encontrarse en la fase grasa o disperso como pequeñas burbujas en la fase acuosa continua (Kiosseoglou y Paraskevopoulou 2007). En masas de bizcocho tipo *foam* el aire se incorpora en la fase acuosa donde se encuentran los ingredientes hidrosolubles y los componentes tensoactivos como proteínas y lípidos polares (Sahi y Alava 2003). Las proteínas de la clara del huevo forman una espuma que ocluye y retiene el aire. Por tanto, la estabilización de las burbujas ocluidas tiene lugar en gran medida por material de naturaleza proteínica, posiblemente con alguna contribución por parte de lípidos polares propios del huevo y la harina (Sahi y Alava 2003). A medida que el mezclado continua las burbujas se vuelven cada vez más pequeñas (Bennion y Bamford 1997b). Al incorporar la yema, las lipoproteínas presentes en ésta llevan a cabo la emulsificación de la grasa presente ya que tienen la capacidad de adsorberse y reorganizarse rápidamente en la interfaz aceite-agua (Kiosseoglou y Paraskevopoulou 2007).

En el horneado a medida que la temperatura aumenta, la grasa se funde, los glóbulos de grasa coalescen y liberan lípidos que forman una capa en la superficie de las burbujas. El volumen de la masa comienza a aumentar debido a la expansión de los gases: el aire ocluido, el dióxido de carbono producido por el agente leudante, junto con la presión que crea el vapor de agua en las burbujas de aire (Bennion y Bamford 1997b). Por otra parte, tienen lugar los procesos de desproporción y coalescencia entre las burbujas sobre todo si son de gran tamaño y si existe una gran diferencia de tamaños entre ellas. Estos procesos se detienen debido a un aumento de la viscosidad de la masa, ya que a temperaturas elevadas tiene lugar la gelatinización del almidón y la coagulación de las proteínas.

La solidificación de la emulsión/espuma da lugar a una estructura porosa de textura esponjosa. La masa, es por tanto, un sistema activo, en el que los componentes pueden sufrir modificaciones o interaccionar unos con otros a medida que la temperatura aumenta durante el horneado (Pateras et al. 1989). Además, cada ingrediente tiene una funcionalidad particular en la calidad del producto final: esponjosidad, estructura, sabor, etc… (Conforti 2006a).

3.1. Ingredientes y funcionalidad

3.1.1. Harina

Los tipos de trigo se diferencian por su contenido en proteínas. Las harinas usadas para bizcochos son harinas blandas, las cuales tienen un contenido bajo de proteínas (8%-11%), un alto contenido en almidón y un tamaño de partícula pequeño. De este modo la harina proporciona a los bizcochos una estructura delicada, una textura suave y un sabor característico (Conforti 2006a).

El almidón y las proteínas de la harina participan en la formación de la estructura de la miga. La miga se forma parcialmente durante el horneado y durante el enfriamiento del bizcocho. La estructura de la miga depende del número y tamaño de los alveolos que se forman, el grado de gelatinización del almidón y la cantidad de proteína coagulada.

En la harina, las principales proteínas son las que forman el gluten; las gluteninas, responsables de la elasticidad de la masa, y las gliadinas, responsables de la extensibilidad de la masa. Durante la fase de mezclado, las proteínas forman una red de gluten débil, que no se refuerza demasiado durante el batido, pero que es suficientemente resistente para mantener la estructura de espuma de la masa (Conforti 2006b). El desarrollo de esta red de gluten se ve limitado por el elevado contenido de grasa y azúcar en la masa. La habilidad de la masa para expandirse se debe a la presión de los gases durante el horneado: el aire, el vapor de agua y el dióxido de carbono, combinado con la elasticidad de las gluteninas y la fluidez de las gliadinas (Conforti 2006a). Por tanto, el gluten participa en el desarrollo y mejora de la estructura del producto final: suprime el colapso, da lugar a una distribución homogénea de alveolos y mejora el volumen final del bizcocho (Wilderjans et al. 2008).

Aunque el desarrollo del gluten es importante para la estructura del bizcocho, la capacidad de los gránulos de almidón intactos para gelatinizar es esencial para el refuerzo de la estructura (Conforti 2006b). El proceso de gelatinización, tiene lugar durante el horneado y se verá influenciado por la cantidad de agua y azúcar presente en la formulación de la masa. Cuando el almidón comienza a hincharse y gelatinizar, la viscosidad de la masa aumenta significativamente; este proceso, junto con la desnaturalización de proteínas, imparte a la masa un carácter sólido (Wilderjans et al. 2010). Es importante que el mecanismo de solidificación de la masa tenga lugar en una fase más tardía que temprana durante el horneado, para que permita una expansión óptima de la estructura y así conseguir un volumen elevado en el bizcocho final. Por otro lado, la capacidad del almidón de gelificación durante el atemperado del bizcocho tras el horneado es decisiva para evitar el colapso de la estructura. El almidón tiene un papel importante en la estructura final del las paredes de los alveolos; aparentemente, el gel de almidón contribuye a la dureza de las mismas (Wilderjans et al. 2013; Wilderjans et al. 2010). Por lo tanto, la combinación de la formación de una red proteica, formada durante el horneado, con un gel de almidón, formado durante el enfriamiento, da lugar a una estructura estable con mayor resistencia al colapso (Wilderjans et al. 2010).

3.1.2. Azúcar

Aparte de contribuir al dulzor, el azúcar también influencia el volumen, la humedad, la firmeza, el color, la apariencia y el contenido en calorías del bizcocho.

Durante la fase de mezclado el azúcar controla la viscosidad de la masa limitando la cantidad de agua libre (Pateras y Rosenthal 1992). La masa necesita una viscosidad suficiente para atrapar y retener las burbujas de aire.

Durante el horneado, el azúcar compite con el almidón por el agua necesaria para la hidratación de las proteínas y el almidón. Debido a la escasa agua disponible, la presencia de azúcar eleva la temperatura de gelatinización del almidón y la temperatura de desnaturalización de proteínas (Conforti 2006a; Pateras y Rosenthal 1992). Todo esto permite que el gluten se expanda durante más tiempo y por lo tanto aumente así aun más el volumen del bizcocho contribuyendo además a una textura fina y homogénea. Por otra parte, el azúcar participa en las reacciones de caramelización y Maillard de la superficie del bizcocho durante el horneado. Finalmente, la naturaleza higroscópica del azúcar aumenta la humedad del producto horneado (Conforti 2006a).

El papel del azúcar en la nutrición y la salud ha sido muy discutido; en general, la atención se ha dirigido hacia la salud dental, la obesidad y la calidad de la dieta (Bennion y Bamford 1997c). Se sabe que un elevado consumo de azúcares amenaza la calidad nutricional de la dieta al suministrar un elevado aporte energético sin nutrientes específicos (OMS/FAO 2003). Por ello en este trabajo se estudia el reemplazo de parte del azúcar por oligofructosa.

3.1.3. Huevo

La incorporación de huevo en bizcochos aumenta el valor nutricional del producto y mejora su color y apariencia final. Además, el huevo posee interesantes propiedades funcionales: actúa como humectante debido a la elevada cantidad de agua presente; facilita la incorporación de aire debido a la capacidad espumante de las globulinas al ser batidas; enriquece ya que en la yema hay una alta proporción de grasa; actúa como emulsionante debido a la presencia de lecitina en la yema; y aporta estructura ya que las proteínas de la yema y la clara coagulan con el calor formando un gel firme (Cauvain y Young 2007; Conforti 2006a).

Los productos horneados, sobre todo los bizcochos, son sistemas en los que varias proteínas, que difieren en estructura y funcionalidad, interaccionan entre ellas para dar lugar a una red de proteínas coaguladas de fases separadas, que posee una estructura y características de textura únicas (Kiosseoglou y Paraskevopoulou 2007). Por una parte, durante el horneado, las interacciones entre proteínas pueden llegar a ser importantes para la estructura del bizcocho; esta agregación de proteínas proporciona material estructural a las paredes de los alveolos contribuyendo a aumentar la resistencia al colapso (Wilderjans et al. 2008). Por otra parte, la estructura final del bizcocho se puede considerar como un sistema en red mixto basado en el desarrollo del gluten, pero modulado significativamente por la capacidad de fijación por calor de las proteínas de huevo (Wilderjans et al. 2008).

3.1.4. Leche

El agua es un ingrediente esencial en la elaboración de productos horneados; sin embargo normalmente se adiciona esta agua en forma de leche. La leche aporta sabor, nutrientes y contiene ciertos componentes que ayudan a producir una textura suave, una miga blanca y una superficie dorada. La fase líquida realiza diversas funciones en el proceso de elaboración de bizcochos: hidrata el almidón y durante el calentamiento da lugar a la gelatinización del mismo; contribuye al desarrollo de la red de gluten; disuelve ingredientes como el azúcar, la sal y el agente leudante, el cual reacciona en presencia de agua y produce dióxido de carbono; produce vapor de agua durante el calentamiento participando en la expansión de la masa (Conforti 2006a).

3.1.5. Grasa

La funcionalidad de la grasa en productos horneados es muy versátil. Las principales funciones de la grasa en los bizcochos son: contribuye a una buena aireación de la masa dando bizcochos de mayor volumen y mejores características de textura; imparte el efecto *shortening* al lubricar los componentes de la matriz limitando el desarrollo de la red de gluten y dando lugar con ello a una textura más suave y blanda en el producto final (Bennion y Bamford 1997a); al lubricar la matriz reduce y retrasa el fenómeno de retrogradación del almidón y el endurecimiento del gluten, y por otra parte mejora la retención de humedad en el producto final, aumentando así la vida útil del producto (Lai y Lin 2007);

La elección del tipo de grasa adecuado para cada producto depende de su sabor, capacidad emulsionante, punto de fusión etc. En la elaboración de bizcochos las grasas tipo *shortening* son las más empleadas por su funcionalidad en la aireación de la masa y expansión del producto. Sin embargo, tradicionalmente en España el uso de aceites como el de oliva o de girasol es muy común en la elaboración de bizcochos caseros. El perfil nutricional de estos aceites es más saludable que el del *shortening* y los bizcochos son más tiernos y menos secos. No obstante, la capacidad de los aceites para ocluir y retener aire durante el batido y horneado es menor que la del *shortening*. En este trabajo se elaboran bizcochos empleando aceite de girasol.

La creciente demanda de los consumidores de productos bajos en grasa y bajos en calorías ha incrementado la investigación y desarrollo de sustitutos de grasa y la reformulación de productos para mejorar su perfil nutricional. Por este motivo, en este trabajo se estudia el efecto del reemplazo de grasa por inulina en bizcochos.

3.1.6. Agente impulsor

El agente impulsor de naturaleza química está formado por un ácido y una base. Cuando estos compuestos se ponen en contacto con agua o calor tiene lugar una reacción química en la que se produce dióxido de carbono, el cual hace aumentar el volumen de las burbujas de aire ocluidas durante el batido de la masa.

El bicarbonato sódico ha sido el agente impulsor más eficaz en productos horneados durante más de un siglo. Este se descompone por el calor en dióxido de carbono, agua y carbonato de sodio. En la mayoría de los casos se combina con un ácido para acelerar la reacción y la producción de gas a temperaturas más bajas.

Tradicionalmente unos de los ácidos más empleados eran el vinagre (ácido acético) y el limón (ácido cítrico). Estos ácidos reaccionan con el bicarbonato sódico en cuanto se ponen en contacto en un medio acuoso.

3.1.7. Sal

En productos como los bizcochos, con elevado contenido en azúcar, la sal ayuda a modificar el dulzor del producto y mejora su sabor.

3.2. Reemplazo de grasa y/o azúcar en productos horneados de alta humedad

Se han desarrollado numerosos estudios sobre el reemplazo de grasa y/o azúcar en productos horneados de alta humedad. En general, al disminuir el contenido de grasa se ha observado una disminución en la estabilidad de las burbujas de aire en la masa, las cuales coalescen y suben a la superficie, dando lugar a bizcochos de menor volumen y una textura más dura (Barker y Cauvain 1994). Al usar dextrinas en la sustitución de grasa, las masas mostraron mayor número de burbujas de aire ocluidas que las masas control; las maltodextrinas debido a la baja viscosidad que aportaron a la masa dieron lugar a una menor retención de aire durante el horneado y por tanto el volumen de los bizcochos resultantes fue menor; sin embargo, las amilodextrinas proporcionaron mayor viscosidad a las masas durante el horneado, mejorando la retención del aire ocluido y el volumen del bizcocho (Kim et al. 2001). La fibra de cacao también ha sido empleada como sustituto de grasa en madalenas (Martínez-Cervera et al. 2010); las masas con fibra ocluyeron más aire, pero su elevada consistencia limitó la expansión de la matriz, obteniendo madalenas con menor volumen, una estructura alveolar más compacta y una textura blanda y de fácil rotura. Cuando se utilizó fibra de melocotón como sustituto de grasa, su adición aumentó significativamente la dureza de las madalenas (Grigelmo-Miguel et al. 2001).

Al reemplazar el azúcar en productos horneados como bizcochos y madalenas, el dulzor, sabor y el color de estos cambian; además, el proceso de expansión y solidificación de la matriz durante el horneado también se altera dando lugar a una estructura y textura en el producto final muy variable (Barker y Cauvain 1994). El estudio del reemplazo de azúcar por polidextrosa en bizcochos se ha desarrollado en las últimas décadas. Esta sustitución amplió la distribución de tamaños de burbujas en la masa (Pateras et al. 1989) y aumentó la temperatura de gelatinización del almidón pero no la de las proteínas de huevo dando lugar a una miga más débil (Pateras y Rosenthal 1992). Además, los bizcochos con polidextrosa presentaron menor volumen y miga más compacta, con menor interconectividad entre alveolos (Hicsasmaz et al. 2003; Martínez-Cervera et al. 2012). Actualmente se están estudiando otros sistemas para el reemplazo del azúcar, ya que el uso de combinaciones de sustitutos del azúcar con agentes de carga como fibras, dextrinas o polioles pueden ayudar a reemplazar la multifuncionalidad del azúcar (Manisha et al. 2012; Zahn et al. 2013).

El estudio del efecto de la sustitución simultaneo de grasa y azúcar en bizcochos es más complejo, ya que se disminuye el contenido de dos de los ingredientes fundamentales de la estructura del bizcocho. En los trabajos que se han llevado a cabo se llega a resultados parecidos tras haber empleado diferentes ingredientes y técnicas de elaboración y análisis; destaca la inestabilidad de la fase aire en la masa, la alteración del proceso de solidificación de la matriz durante el horneado y la obtención de un producto de menor volumen y con una textura más dura (Khouryieh et al. 2005; Kocer et al. 2007; Pong et al. 1991).

3.3. Mejorantes

Existen diversos ingredientes, denominados mejorantes, que se emplean en la industria de productos horneados para simplificar el proceso de elaboración, mejorar la dispersión de los ingredientes, la incorporación de aire, la estabilidad de la fase aire, etc. Ejemplos de estos ingredientes son los emulsionantes y las enzimas.

3.3.1. Emulsionantes

Los emulsionantes son agentes surfactantes; la palabra surfactante se usa para denominar a los agentes activos en las superficies, los cuales migran a las interfases entre dos fases físicas. La característica clave a nivel molecular de los surfactantes es que son amfifílicos, la parte lipófila de la molécula se orienta hacia un ambiente lipídico y la parte hidrófila se orienta hacia un ambiente acuoso. La preferencia de cada parte de la molécula de orientarse hacia un tipo de medio quiere decir que la energía libre termodinámica del sistema es mínima cuando la parte lipofílica de la molécula se encuentra en la fase grasa (o aire) y la parte hidrofílica en fase acuosa. Al someter al sistema a una energía mecánica de modo turbulento (por ejemplo: en la fase de mezclado) hace que una fase se subdivida y se incremente el área y energía total de la interfase; cuanto menor es la energía libre de interfase por unidad de área, mayor es el área de interfase que se crea por unidad de energía aplicada. Esta relación es básica para el uso de surfactantes en productos horneados (Stauffer 1995). Los surfactantes que se usan en productos horneados normalmente se conocen como emulsionantes, entre los que se encuentran el monostearato de glicerol (GMS; E471) y los esteres de poliglicerol de ácidos grasos (PGE; E475); en este trabajo se ha empleado una mezcla comercial de ambos. Los emulsionantes mejoran la dispersión de la grasa de la formulación, ayudan en la oclusión de aire durante el mezclado y en la subdivisión de las partículas de la fase discontinua; además aumentan la viscosidad del sistema y así mejoran la estabilidad de la fase gas (Bennion y Bamford 1997a). Por otra parte, también interaccionan con otras moléculas del sistema como el gluten y el almidón.

Aunque siempre se ha considerado que el almidón y el gluten son moléculas hidrófilas, son moléculas amfifílicas. La amilosa forma una hélice, cuyo centro tiene carácter lipofílico. Ciertos emulsionantes, particularmente los monoglicéridos tienen una configuración estérica que formar un complejo con las regiones helicoidales del almidón. Este complejo insoluble helicoidal aumenta la temperatura de gelatinización del almidón y disminuye los gránulos totales gelatinizados. Además, este fenómeno retarda la retrogradación del almidón. Por otra parte, este complejo no participa en el transporte de humedad desde la red proteica de alrededor, y por tanto no aumenta tanto la rigidez y dureza de la miga (Podmore 2002; Stauffer 1995).

Puesto que el gluten contiene un 40% de amino ácidos hidrofóbicos, éste interacciona con moléculas lipídicas. Por ejemplo, en el caso de la masa de pan, cuando se adicionan emulsionantes como acondicionadores de la masa, estos interaccionan con el gluten para mejorar la retención de gas y la elasticidad de la masa (Podmore 2002).

La adición de emulsionantes permite el uso de métodos de mezclado de una sola etapa, en el que los ingredientes en polvo se ponen en la amasadora, los líquidos se adicionan sobre estos, se amasa a velocidades bajas para que se mezclen y seguidamente se amasan a velocidades altas para favorecer la incorporación de aire (Stauffer 1995). En el método de mezclado de una sola fase, el aire se incorpora en la fase acuosa y queda estabilizado por proteínas del huevo. Es importante prevenir la desestabilización de la espuma por la presencia de grasa. Esto se logra adicionando emulsionantes, los cuales rodean el aceite en la interfase aceite-agua, evitando que desestabilicen la espuma (Stauffer 1995, 1996).

3.3.2. Enzima lipasa

La aplicación de enzimas en productos horneados para mejorar el procesado y/o calidad de la harina, la masa y el producto final está ampliamente consolidada. Existe una amplia diversidad de tipos de enzimas, de diferentes clases, especificidad y funcionalidades (Goesaert et al. 2007). El uso de tecnología enzimática se ha propuesto como una alternativa a emulsionantes y agente oxidantes por las mayores restricciones que existen en el uso de aditivos químicos. (Goesaert et al. 2007; Miguel et al. 2013). Además, el uso de enzimas en lugar de otros aditivos permite presentar etiquetas más limpias reduciendo la cantidad de números E- presentes.

Las lipasas se llevan usando desde hace más de dos décadas, junto con otras enzimas y emulsionantes para mejorar las características de los productos horneados. Estas enzimas hidrolizan los esteres de los triglicéridos y producen mono o diglicéridos, glicerol y ácidos grasos libres.

La primera generación de lipasas hidrolizaban los enlaces éster entre el glicerol y los ácidos grasos en posición 1 y 3. Esta lipasa fortalecía la red de gluten, pero en exceso daban lugar a una masa muy sólida, dando lugar a un producto de menos volumen. La segunda generación de lipasas actúa sobre lípidos polares y no polares de la harina de trigo, produciendo más componentes polares. Se han observado mejores resultados en el volumen, la estabilidad de la masa y la estructura de la miga. La tercera generación de esta enzima son lipasas más concentradas, que toleran mejor cambios en el tipo de harina y dosis (Moayedallaie et al. 2010). Estas lipasas mejoran las características reológicas de la masa, mejoran el volumen y la calidad de producto final. Además se ha observado que las lipasas modifican las interacciones entre los lípidos de la harina y el gluten, así como las interacciones entre pentosanos y almidón; por otro lado, el incremento de monoglicéridos que forman complejos amilosa-lípido juega un papel importante retardando la retrogradación del almidón (Stojceska y Ainsworth 2008).

4. Galletas

La estructura de la masa de galletas de masa corta es una suspensión de proteínas, partículas de harina y gránulos de almidón aislados en una fase continua que es una solución concentrada de azúcar en la que los lípidos están emulsionados. El proceso de horneado transforma la masa en una estructura alveolar sólida con una textura característica (Chevallier et al. 2000).

Las galletas de masa corta forman un sistema que consiste en tres ingredientes mayoritarios: harina, azúcar y grasa, y una pequeña proporción de agua. Este tipo de galletas tiene una elevada aceptabilidad y son muy populares en muchos países (Laguna et al. 2011).

4.1. Ingredientes y funcionalidad

4.1.1. Harina

La textura de las galletas se puede interpretar en función del estado de cada uno de sus principales ingredientes, entre ellos la harina.

Las galletas se elaboran con harinas blandas (11% de proteína) en las que el nivel de almidón dañado es bajo, lo que permite que la extensión de la masa durante el horneado sea mayor y se obtengan galletas de diámetros mayores (Pareyt y Delcour 2008). Los gránulos de almidón permanecen en su forma nativa durante el horneado sin formar una estructura continua (Kulp et al. 1991). Esto se debe a que la grasa puede ejercer un efecto de protección física de los gránulos de almidón contra el efecto del calor húmedo, a que otros solutos como el azúcar compiten por el agua disponible (Flint et al. 1970), y a que la elevada concentración de azúcar en la matriz, el bajo contenido en agua y las bajas temperaturas de horneado retrasan o impide la gelatinización del almidón; obteniéndose así una estructura crujiente (Laguna et al. 2011).

En el caso de galletas de masa corta, que son con las que se trabaja en este estudio, el desarrollo de gluten ha de ser limitado. Para ello la cantidad de agua que se adiciona a la formulación es muy baja; además el efecto del azúcar y otros solutos, así como la adición de grasa, es determinante en la actividad del agua. Aparte de tener en cuenta el efecto de los ingredientes y sus concentraciones, el método de mezclado también ha de tenerse en cuenta para limitar el desarrollo de la red de gluten. Normalmente, para ello se emplea el método denominado *creaming*, en el que primero se mezclan todos los ingredientes, excepto la harina. En esta primera etapa, los azúcares y otros componentes se disuelven y la disolución resultante se dispersa en la grasa. La harina se mezcla con la disolución anterior, dando lugar a una masa suave que carece de una formación de gluten significativa por la dificultad de que se hidraten las proteínas del gluten, por la baja humedad de la formulación y el poco tiempo de mezclado al que se somete la masa (Cauvain y Young 2009).

4.1.2. Grasa

La grasa es un ingrediente esencial en las galletas de masa corta y es el segundo componente en mayor proporción en la formulación después de la harina (Manohar y Rao 1999). En la masa corta de galletas, se ha observado que la grasa no se distribuye como pequeños glóbulos, sino que forma parte de la matriz almidón-proteína (Flint et al. 1970). La grasa tiene una función lubricante (hace falta muy poca agua para conseguir la consistencia deseada) y restringe el desarrollo de la red de gluten; lo cual implica que las características físicas de la masa dependen de la distribución del agua y la grasa en este sistema (Olewnik y Kulp 1984). Además de rodear las proteínas, la grasa envuelve y aísla los gránulos de almidón, impidiendo así la continuidad de las estructuras de proteína y almidón, y favoreciendo una estructura más friable, suave y blanda (Ghotra et al. 2002).

4.1.3. Azúcar

El azúcar juega un papel muy importante en la producción y en las características finales de las galletas (Laguna et al. 2012). Durante el mezclado, el azúcar compite con la harina por el agua, inhibiendo así el desarrollo de la red de gluten (Gallagher et al. 2003). El efecto del azúcar es importante en la etapa de laminado de la masa porque influye en la consistencia de la masa; por otro lado, durante el horneado el azúcar ejerce un efecto sobre la gelatinización del almidón, la formación del gluten, la expansión de la galleta y las reacciones de pardeamiento; además, influye en la textura crujiente y acabado superficial de las galletas (Laguna et al. 2012).

4.1.4. Agua

La funcionalidad del agua en la producción de galletas es muy similar a la que ejerce en las masas de bizcocho, con respecto a la dispersión e hidratación de ingredientes (Cauvain y Young 2009).

En la formulación de galletas la cantidad de agua que se añade siempre es reducida (normalmente menos del 15% en base harina), porque la mayoría se pierde durante el horneado para obtener una textura quebradiza y una vida útil prolongada (Cauvain y Young 2009).

4.1.5. Leche en polvo desnatada

La leche en polvo se utiliza, como ingrediente menor, por el sabor delicado que aporta a la galleta, para mejorar la textura y ayudar a la coloración de la superficie. La lactosa, principal azúcar de la leche, es un disacárido reductor con poder edulcorante; además se combina con las proteínas según las reacciones de Maillard, en la superficie de la galleta durante la cocción, produciendo un atractivo tono pardo-rojizo (Manley 2000b).

4.1.6. Agente impulsor

Estos agentes forman un grupo de sales predominantemente inorgánicas que añadidas a la masa, bien una sola, o en combinación, reaccionan produciendo gases que forman los núcleos para el desarrollo de la textura dentro de la galleta.

En presencia de humedad, el bicarbonato reacciona con cualquier sustancia ácida, produciendo anhídrido carbónico, al formase la correspondiente sal sódica y agua. Al calentarse, el bicarbonato libera algo del dióxido de carbono y permanece como carbonato sódico. Resulta conveniente utilizar bicarbonato sódico para ajustar el pH, que puede afectar al esparcimiento de la masa y al color de las galletas (Manley 2000a).

El bicarbonato amónico es un agente esponjante, extraordinariamente útil en galletería. Durante el calentamiento se descompone completamente desprendiendo anhídrido carbónico, amoniaco gaseoso y agua. Como es un carbonato, reacciona rápidamente con otros ingredientes ácidos, pero la alcalinidad conferida a la masa no permanece en la pieza y se necesita recurrir al bicarbonato sódico para controlar el pH de la misma (Manley 2000a).

4.1.7. Sal

La sal tiene un notable efecto potenciador sobre la mayoría de los sabores. Su concentración más eficaz se sitúa alrededor de 1-1.5 % del peso de la harina, pero a niveles superiores a 2.5 % se hace desagradable (Manley 2000a). Además, al ser higroscópica mejora la vida útil del producto final.

4.2. Reemplazo de grasa en productos horneados de baja humedad

El reemplazo de grasa se puede conseguir al reformular el alimento incorporando ingredientes de tipo lipídico, proteico o carbohidratos, de forma individual o en combinación. Los sustitutos de la grasa representan una amplia variedad de estructuras químicas con diversidad de funciones, propiedades sensoriales y efectos fisiológicos (Akoh 1998).

Los carbohidratos forman en presencia de suficiente agua una matriz tipo gel, que tiene propiedades lubricantes y de flujo similares a la grasa (Sensus Operations 2000; Sudha et al. 2007).

La masa de las galletas se vuelve más dura a medida que se reduce el contenido graso. Esto se debe a que la harina absorbe más agua y el desarrollo de la red de gluten aumenta. Por ejemplo, al adicionar sustitutos de la grasa como maltodextrina o polidextrosa, la cantidad de grasa que queda presente está mucho más diluida y su efecto lubricante se reduce (Sudha et al. 2007).

Entre los efectos que se observan en las galletas al reemplazar la grasa por miméticos tipo carbohidrato, como la polidextrosa, maltodextrina o la inulina, es que la humedad y actividad de agua aumentan. La incorporación de polidextrosa da lugar a galletas más duras y muy quebradizas. Sin embargo, usando maltodextrina o inulina como sustitutos de la grasa se obtienen galletas de dureza y fragilidad semejantes a la control (Zoulias et al. 2002).

5. Fructanos

Fructano es el término general que se usa para denominar a los hidratos de carbono, polímeros u oligómeros, lineales o ramificados, en los que la mayoría de los enlaces intermoleculares son fructosil-fructosa. Los fructanos son, o bien inulinas con enlaces β-(2,1) o levanos con enlaces β-(2,6) (Roberfroid y Delzenne 1998). Por tanto, la inulina es un tipo de fructano formado mayoritariamente por enlaces fructosil-fructosa β-(2,1). Puede contener también una molécula de α-D-glucosa inicial (Roberfroid 2005).

Las especies de plantas que contienen fructanos forman parte de familias de mono y dicotiledóneas como *Liliaceae, Amaryllidaceae, Gramineae y Compositae*. Algunas partes de las plantas que contienen fructanos se consumen, por ejemplo: espárrago, ajo, puerro, cebolla, alcachofa, endivias, etc. Los fructanos tipo inulina se consumen ampliamente en la dieta a través de estos vegetales; la ingesta diaria estimada en Europa es de 3-11 g (Roberfroid y Delzenne 1998).

Solo un número limitado de especies son adecuadas para la extracción de inulina en la industria de alimentos. En las familias *Liliaceae, Amaryllidaceae* y *Compositae* los fructanos se almacenan mayoritariamente en los bulbos, tubérculos y raíces tuberosas, los cuales debido a la ausencia de otros componentes que interfieran se pueden extraer fácilmente y procesar a productos purificados (Roberfroid y Delzenne 1998). Actualmente en la industria de alimentos la inulina se extrae mayoritariamente de dos especies, la aguaturma (*Helianthus tuberosus*) y sobre todo de la achicoria (*Cichorium intybus*). Tanto la inulina como la oligofructosa están reconocidas oficialmente como ingredientes naturales en los países europeos y se clasifican como compuestos GRAS (*generally regarded as safe*) en los Estados Unidos (Franck 2002; Roberfroid y Delzenne 1998). La inulina se extrae de la achicoria por un proceso de difusión con agua caliente, se purifica y finalmente obtiene seca por aspersión (Sensus Operations 2000). De este modo se obtiene la inulina nativa, cuyo grado de polimerización promedio es de 12 unidades y tiene una distribución de moléculas de longitudes de entre 2 a 60 unidades. Estos productos se comercializan como un polvo blanco, sin olor y de sabor neutro, que al combinarse con otros ingredientes no modifica los sabores más delicados (Franck 2002; Niness 1999; Sensus Operations 2000). La oligofructosa se obtiene por hidrólisis enzimática parcial de la inulina, obteniéndose moléculas con grado de polimerización promedio de 4 unidades (Franck 2002).

Estos ingredientes presentan características tecnológicas que los hacen muy interesantes para el desarrollo de productos. Aportan tan solo 2 kcal/g y en función de la estructura molecular del fructano, especialmente de su grado de polimerización, se definirá su facilidad de dispersión, solubilidad en agua, viscosidad y capacidad de formar una textura cremosa (Roberfroid y Delzenne 1998). En particular, la inulina tiene la capacidad de formar una estructura tipo gel o crema en combinación con el agua, cuyas características reológicas y sensoriales lo hacen una opción excelente como sustituto de grasa en gran cantidad de alimentos (Sensus Operations 2000). La oligofructosa, permite la sustitución de azúcar, a la vez que mejora la retención de la humedad y aumenta el contenido en fibra del producto reformulado.

A parte de sus ventajas tecnológicas los fructanos tipo inulina ejercen propiedades beneficiosas en nuestra salud; son compuestos que forman parte de la fibra dietética alimentaria, son fermentables y ayudan a mejorar las funciones intestinales, especialmente ayudando a la regularidad; son compuestos bifidogénicos y prebióticos; mejoran la absorción del calcio; y reducen el nivel de triglicéridos en sangre en individuos con niveles elevados de grasa en sangre. Por todo ello, los fructanos tipo inulina están clasificados como ingredientes alimentarios funcionales, cuyo objetivo es la funcionalidad del tracto digestivo, específicamente del intestino y su microflora, y de las funciones sistémicas relacionadas con la salud y el bien estar (Roberfroid 2005, 2007). Para asegurar los efectos bifidogénicos se deben adicionar 3-8 g de inulina por porción de alimento (Meyer et al. 2011).

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Objetivos

El objetivo general de la presente tesis doctoral es evaluar el efecto del reemplazo de grasa por inulina y la sustitución de azúcar por oligofructosa en productos horneados de alta humedad (bizcochos), sobre la micro y macroestructura, propiedades físicas y sensoriales de los sistemas antes y después del horneado. También se evalúan los efectos del reemplazo de grasa por inulina en un producto horneado de baja humedad (galletas). Todo ello permitirá conseguir reformular productos horneados de contenido reducido en grasa y azúcar, con un valor nutricional añadido al contener una fibra dietética prebiótica como es la inulina.

Para la consecución de este objetivo general se establecen los siguientes objetivos parciales:

- Estudiar la distribución (microestructura) y funcionalidad (interrelación estructura-propiedades físicas) de los ingredientes en la masa y el bizcocho final.
- Evaluar los cambios a nivel de la micro y macroestructura (distribución de ingredientes, evolución de la fase gas, estructura alveolar) y de las propiedades físicas (viscosidad, dimensiones, textura) en masas y bizcochos con reemplazo de grasa por inulina y sustitución de azúcar por oligofructosa. Estudiar la aceptación sensorial de los bizcochos obtenidos.
- Reemplazar el proceso de mezclado tradicional por un mezclado en una sola etapa. Evaluar los efectos del cambio en el método de elaboración de bizcochos desde escala de laboratorio a escala planta piloto, sobre las propiedades físicas de masas y bizcochos.
- Mejorar la calidad de los bizcochos con contenido reducido en grasa. Estudiar el efecto de dos mejorantes (enzima lipasa y emulsionante comercial) sobre la estructura y textura del producto final.
- Estudiar el efecto del reemplazo de grasa por inulina en galletas de masa corta. Analizar los principales cambios en su estructura y textura.

Estructura de la tesis

La presente tesis doctoral se enmarca dentro del proyecto del Ministerio de Ciencia e Innovación titulado "Reformulación de alimentos por adición de nuevos ingredientes comerciales para disminuir los contenidos en azúcar o grasas. Efectos sobre la reología, microestructura, propiedades sensoriales y aceptación". En este proyecto se han estudiado los cambios estructurales que tienen lugar cuando se reemplaza la grasa o el azúcar de la formulación de productos horneados, la relación de estos cambios con la aptitud tecnológica de los sistemas (comportamiento durante el amasado y horneado) y con las propiedades físicas y sensoriales del producto final. Se han evaluado los mecanismos e interacciones que determinan una buena incorporación de los nuevos ingredientes y la obtención de sistemas equilibrados para conseguir mejorar la calidad de los productos horneados con contenido reducido en grasa o azúcar. El proyecto aborda dos modelos de matrices alimentarias, uno de baja humedad, que corresponde a un tipo de galleta denominado de masa corta y uno de alta humedad como son los bizcochos.

La presente tesis aborda el estudio del reemplazo de grasa y azúcar en bizcochos y galletas, utilizando como ingredientes sustitutos fructanos; en particular se ha empleado inulina nativa de alta dispersibilidad, para reemplazar la grasa, y oligofructosa como sustituto del azúcar. Todo proceso de reformulación de alimentos implica una inversión económica, de tiempo y conocimiento que se corresponde con un incremento en el precio del producto final. Sin embargo, también hay que tener en cuenta que al emplear ingredientes funcionales, como son los fructanos, incorporarnos un valor añadido al alimento debido a sus efectos beneficiosos sobre nuestra salud.

En primer lugar, debido a la elevada complejidad de las matrices (masa y bizcocho) el primer objetivo fue la comprensión y el conocimiento de la funcionalidad de los ingredientes que las integran y sus interacciones. A continuación, el trabajo se centró en la reformulación de un bizcocho tradicional, para desarrollar un nuevo producto con contenido reducido de grasa. El desarrollo de nuevos productos se amplió con un estudio sobre la sustitución de parte del azúcar, así como el reemplazo simultáneo de grasa y azúcar en estos bizcochos. Llegados a este punto, la investigación se enfocó en el proceso de elaboración de los bizcochos; el objetivo fue escalar la elaboración en laboratorio a una producción a nivel de planta piloto y estudiar las modificaciones que este cambio conlleva para optimizar el método de mezclado. Una vez definido el proceso de producción a escala de planta piloto y a partir de los resultados del estudio de reemplazo de grasa por inulina, el trabajo se centró en la mejora de la calidad de los bizcochos de contenido reducido en grasa a partir de la incorporación de mejorantes, una enzima lipasa y una mezcla comercial de emulsionantes. Por último, se estudió la sustitución de grasa por inulina en galletas, la segunda matriz alimentaria objeto de estudio en este proyecto.

El contenido de la tesis se divide en cinco capítulos que recogen los objetivos principales de este trabajo. Las publicaciones científicas derivadas de esta tesis se presentan a lo largo de los capítulos en el siguiente orden:

Capítulo 1: Bizcochos, funcionalidad de los ingredientes

Functionality of several cake ingredients: a comprehensive approach Julia Rodríguez-García, Ana Puig, Ana Salvador and Isabel Hernando. Czech Journal of Food Sciences (2013) 31(4), 355-360.

Capítulo 2: Reemplazo de grasa y azúcar por inulina y oligofructosa en bizcochos

Optimization of a sponge cake formulation with inulin as fat replacer: structure, physicochemical, and sensory properties Julia Rodríguez-García, Ana Puig, Ana Salvador and Isabel Hernando. Journal of Food Science (2012) 77(2), C189-C198.

Replacing fat and sugar with inulin in cakes: bubble size distribution, physical and sensory properties. Julia Rodríguez-García, Ana Salvador and Isabel Hernando. Food and Bioprocess Technology (2014) 7(4), 964-974.

Capítulo 3: Escalado y optimización del proceso de elaboración de bizcochos

Optimising mixing during the sponge cake manufacturing process. Julia Rodríguez-García, Sarabjit S Sahi, Isabel Hernando. Cereal Foods World. En revisión.

Capítulo 4: Mejora de la formulación de bizcochos con contenido reducido de grasa

Functionality of lipase and emulsifiers in low-fat cakes with inulin. Julia Rodríguez-García, Sarabjit S Sahi, Isabel Hernando. LWT-Food Science and Technology. DOI: 10.1016/j.lwt.2014.02.012.

Capítulo 5: Reemplazo de grasa por inulina en galletas

Effect of fat replacement by inulin on textural and structural properties of short dough biscuits. Julia Rodríguez-García, Laura Laguna, Ana Puig, Ana Salvador and Isabel Hernando. Food and Bioprocess Technology (2013) 6(10), 2739-2750.

Resultados y discusión

Capítulo 1

Bizcochos, funcionalidad de los ingredientes

Functionality of several cake ingredients: a comprehensive approach

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Czech Journal of Food Sciences (2013) 31(4), 355-360

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Abstract

The roles of some cake ingredients –oil, leavening agent, and inulin– in the structure and physicochemical properties of batter and cakes were studied in four different formulations. Oil played an important role in the batter stability, due to its contribution to increasing batter viscosity and occluding air during mixing. The addition of the leavening agent was crucial to the final height and sponginess of the cakes. When inulin was used as a fat replacer, the absence of oil caused a decrease in the stability of the batter, where larger air bubbles were occluded. Inulin dispersed uniformly in the batter could create a competition for water with the flour components: gluten was not properly hydrated and some starch granules were not fully incorporated into the matrix. Thus, the development of a continuous network was disrupted and the cake was shorter and softer; it contained interconnected air cells in the crumb, and was easily crumbled. The structure studies were decisive to understand the physicochemical properties.

Keywords: fat replacement, inulin, physical properties, structure

1. Introduction

Bakery products constitute one of the most consumed foods in the world. Among them, cakes are popular and are associated in the consumer's mind with a delicious sponge product with desired organoleptic characteristics (Matsakidou et al. 2010). The physicochemical properties of cakes are largely dependent on the batter and cake structure. Therefore, understanding the internal macro- and microstructure of bakery products is essential (Turabi et al. 2010). Cake batter is a complex emulsion and foam system. Flour, milk, fat, sugar, eggs and the leavening agent are the main ingredients used in its elaboration; each ingredient has an important function in the cake structure. For that reason, several microstructural techniques were applied in this work to correlate the batter and cake structures with the physicochemical properties. Also, image analysis and quantification of the relative features were applied as they are the basis of the modern food microscopy (Turabi et al. 2010). The objective of this study was to understand the functionality of oil and inulin as structural ingredients of the sponge cake. The role of the leavening agent was also evaluated as it is an ingredient which contributes to the formation of the proper structure. For this purpose, four different formulations were examined, with or without the inclusion of oil, the leavening agent, and inulin.

2. Materials and methods

2.1. Ingredients

The ingredients used in the preparation of the cake batters were: wheat flour (Harinera Belenguer, S.A., Valencia, Spain; composition provided by the supplier: moisture ≤ 15%, proteins ≥ 10% s.s.s., ash ≤ 0.6% s.s.s., dry gluten ≥ 10.8%); sugar (Azucarera Ebro, Madrid, Spain); liquid pasteurised egg white and yolk (Ovocity, Llombay, Spain); skimmed milk (Puleva Food, Granada, Spain); refined sunflower oil (Coosol; Coosur S.A., Jaen, Spain); inulin Frutafit® HD of average chain length 8-13 (Sensus, Roosendaal, the Netherlands); sodium bicarbonate and citric acid (A. Martinet, Cheste, Spain); salt.

2.2. Batter and cake preparation

Four batters (B1, B2, B3, and B4) were prepared as given in Table 1. The batters were prepared according to Baixauli et al. (2008). Promptly, within 5 min after the end of the batter mixing, the batter was analysed.

For the preparation of the cakes, 800 g of batter were placed in a Pyrex baking pan (diameter 20 cm) and baked at 160°C for 40 min in a conventional oven (2CF-3V 'Elegance', Fagor, Guipuzkoa, Spain) preheated to 160°C during 20 minutes. Four cakes (C1, C2, C3, and C4) were obtained, corresponding to the batters formulations showed in Table 1. The cakes were kept at room temperature for 1 h and then analysed. All the batters and cakes were prepared in triplicates.

Table 1. Formulations of the different batters (B) and cakes (C) (% in wheat flour basis)

Ingredients	B1/C1	B2/C2	B3/C3	B4/C4
Wheat flour	100.00	100.00	100.00	100.00
Egg yolk	27.00	27.00	27.00	27.00
Egg white	54.00	54.00	54.00	54.00
Skimmed milk	50.00	50.00	50.00	50.00
Sugar	100.00	100.00	100.00	100.00
Sunflower oil	0.00	46.00	46.00	0.00
Frutafit® HD	0.00	0.00	0.00	14.50
Sodium bicarbonate	0.00	0.00	4.00	4.00
Citric acid	0.00	0.00	3.00	3.00
Salt	1.50	1.50	1.50	1.50

2.3. Apparent viscosity

The viscosity of the batter was determined using Haake Viscotester 6 R Plus (Thermo Scientific, Walthman, USA) equipped with spindle 4 at 6 rpm. The measurements were performed in duplicates.

2.4. Confocal laser scanning microscopy (CLSM)

A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) was used. An Ar laser line (488 nm) was employed as light source to excite fluorescent dyes rhodamine B and nile red. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 580 nm was solubilised in distilled water at 0.2%. This dye was used to stain proteins and carbohydrates. Nile red (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 515 nm was solubilised in PEG 200 at 0.1 g/L. A 60× /1.40NA/Oil/ Plan Apo VC Nikon objective lens was used.

For sample visualization a microscopy slide was elaborated with 2 razor blades (platinum coated double edge blades with 100 μm thickness) stuck to a glass (Sahi and Alava 2003). A drop of batter was placed in the microscope slide, within the central gap of the blades, rhodamine B solution and nile red solution were added and the cover slide was carefully positioned to exclude air pockets. By this means, it was possible to visualize the sample without deforming native sample structure when putting the cover. Observations were performed 10 min after diffusion of the dyes into the sample. Images were observed and stored with 1024×1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

2.5. Light microscopy (LM) and image analysis of batter bubbles

The batter samples were observed in a microscope (Nikon Eclipse E800, Nikon Co., Ltd., Tokyo, Japan) at 10× magnification (objective lens 10×/0.30 DIC M Plan Flour, Nikon, Tokyo, Japan).

One drop of the sample was placed on the glass slide as described previously. By this means, it was possible to assure a sufficient and constant specimen thickness, to allow the comparison of the sizes and number densities of the bubbles. Images were captured and stored in a format of 1280×1024 pixels using the microscope software (Nikon ACT-1, Version 2.62, Nikon, Tokyo, Japan)

All the bubbles from a total of 20 images were measured for each sample, using the software ImageJ (Natl. Inst. of Health, Bethesda, Mariland., USA.). Computed parameters included bubble area (μm^2) and bubble size distribution.

2.6. Weight loss during baking

Weight loss (WL) during baking was calculated by using the following equation (Sumnu et al. 2005):
$WL (\%) = (W_{batter} – W_{cake} / W_{batter}) \times 100$

Where: W = weight (g)

2.7. Cake height

The maximum cake height was measured in the cross section of the product using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The baked product was cut with a stainless steel knife and photographed with a digital camera (E-510 Olympus, Hamburg, Germany). The measurements were performed in triplicates.

2.8. Image analysis of cellular structure of the crumb

The cakes were cut vertically with a stainless steel knife into 4 slices of 15 mm thickness. The cut side of each slice was scanned using a scanner (Epson Perfection 1250; Epson America, Inc., Long Beach, USA).

The scanned images were analysed using the software ImageJ. The image was cropped in a 50×50 mm section, on which the analysis was performed. The cell area (mm^2) and total cell area within the crumb (%) were calculated. The data were obtained by measuring cells in twelve different images for each formulation.

2.9. Cake texture

The texture profile analysis (TPA) was carried out according to Sanz et al. (2009) with slight modifications. The test was performed in four cubes (40 mm side), the strain was 40% of the original cube height, and a 50 mm diameter aluminium plate (P/50) was used. The parameters obtained from the curves were hardness, adhesiveness, springiness, cohesiveness, and chewiness.

2.10. Cryo scanning electron microscopy (Cryo-SEM)

Cryo-SEM studies of the crumbs of the different cakes were carried out according to the method described by Llorca et al. (2007).

2.11. Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Plus 5.1 software package (Statistical Graph Co., Rockville, USA). Least Significant Difference (LSD) Fisher's test was used to evaluate the mean values differences ($P < 0.05$).

3. Results and discussion

3.1. Apparent viscosity

There were significant differences between all batter viscosity values (Table 2). B2 had a significantly ($P < 0.05$) higher apparent viscosity values than B1, while B3 was even more viscous ($P < 0.05$). The increase in viscosity in B2 and B3, in comparison to B1, was caused by the addition of sunflower oil. Oil in B4 was replaced by Frutafit® HD, a polysaccharide that easily disperses in the aqueous phase, and acts as fat mimetic imparting viscosity as observed previously by Akalin and Erisir (2008). However, B4 apparent viscosity values were significantly ($P < 0.05$) lower than those of B3, thus the inulin addition did not increase the batter viscosity as much as the oil did.

3.2. Confocal laser scanning microscopy (CLSM)

The distribution of ingredients in cake batters is shown in Figure 1. A lipoprotein matrix was observed in B1 consisting of protein and fat (mainly contributed by eggs and flour) and starch granules within the matrix. The oil was observed in B2 and B3, stained with nile red, as green globules. Some small oil globules could be observed around the air bubbles. These oil globules located at the interface matrix-air could increase the bubble stability. Moreover, they could provide some of the physical properties that several authors have stated for solid fats, such as stabilising air bubbles and improving gas retention (Mousia et al. 2007). B4 showed inulin homogenously dispersed in the lipoprotein matrix where the starch granules were embedded.

Table 2. Mean values of apparent viscosity of batters (B1, B2, B3, and B4), weight losses during baking and heights of the cakes (C1, C2, C3, and C4)

Physical parameters	B1/C1	B2/C2	B3/C3	B4/C4
Apparent viscosity (mPa·s)	7030.00^{a} (28.28)	8780.00^{b} (254.56)	11425.00^{c} (190.92)	10730.00^{d} (84.85)
Weight loss (%)	12.14^{a} (1.19)	8.67^{b} (0.70)	6.67^{c} (0.68)	7.76^{bc} (1.03)
Height (cm)	2.60^{a} (0.05)	1.86^{b} (0.19)	12.31^{c} (0.46)	8.74^{d} (0.40)

Values in parentheses are the standard deviations Means in the same row without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test

3.3. Light microscopy (LM) and image analysis of batter bubbles

The distribution and size of bubbles can be compared in the micrographs shown in Figure 2. The bubbles in B1 were small and clustered together. This clustered distribution could be due to the low viscosity of B1 that enabled the bubbles mobility throughout the batter. The addition of oil in B2 and B3 affected the stability of the batter and enabled a homogenised distribution of air. Hicsasmaz et al. (2003) reported that an even distribution of bubbles within the cake batter is a function of the fat. B1, B2, and B3 contained a narrow size distribution of small air bubbles. B4 had a wider range of bubble sizes and larger bubble areas. This batter contained no oil, which is one of the components that act as stabilising air bubbles.

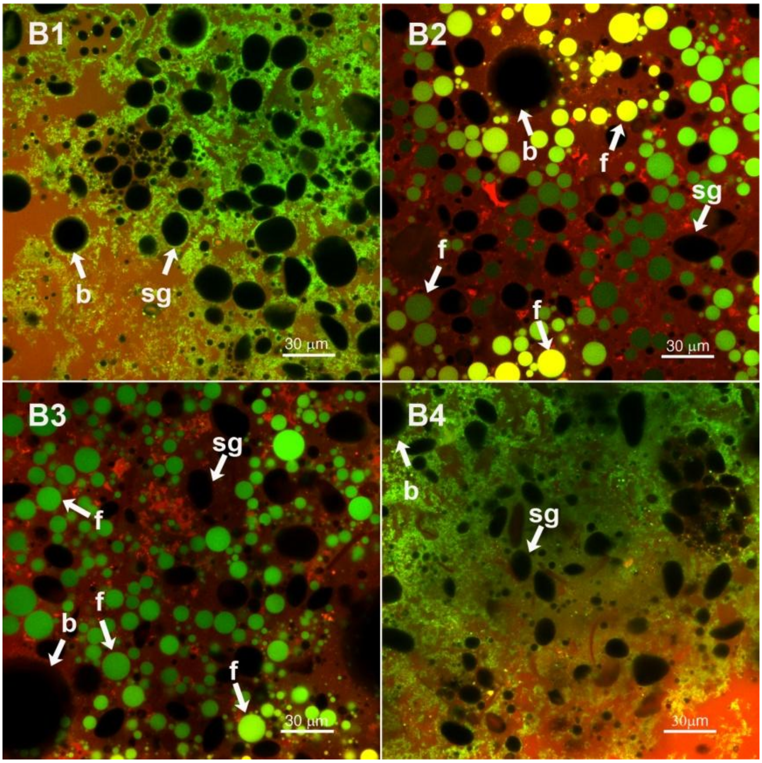

Figure 1. Confocal laser scanning microscopy. Batter images (B1, B2, B3, and B4) (60×). Arrows show sg: starch granule, b: air bubble, f: fat globule

3.4. Weight loss during baking (WL) and cake height

Table 2 shows the weight loss during baking. In B1 and B2, the low batter viscosity allowed bubble movement increasing the gas diffusion to the external surface and water evaporation. So, a large volume of gas was created leaving behind large central holes (Figure 3, C1 and C2) and increasing the WL. Similar results were observed by Turabi et al. (2010) in rice flour cakes prepared with different types of gum, where in low apparent viscosity batters the air bubbles could easily rise to the surface and get lost into the atmosphere. However, the weight loss during baking was significantly higher in B1 ($P < 0.05$) than in B2 where, according to the pore-sealing hypothesis, the fats seal the pores in the structure to retard the escape of gas (Mousia et al. 2007).

B3 revealed a significantly lower weight loss ($P < 0.05$) than B1 and B2. In addition, B3 had a higher standing height than B1, B2, and B4 (Table 2). These results could be due to the leavening agent effect during the baking expansion and a better gas retention. These results were in concordance with those by Sanz et al. (2008) who suggested that the batter viscosity has an important effect on the bubble incorporation and movement which are considered controlling factors in the final cake volume.

B4 did not significantly differ ($P < 0.05$) in WL from B3, due to the water binding capacity of carbohydrate-based fat replacers. However, the lower apparent viscosity of B4, as compared with that of B3, and the large bubble size variation in B4 led to a significantly lower cake height ($P < 0.05$). These results were in accordance with other studies (Zahn et al. 2010) in which muffins with 50% of fat replaced with inulin showed a volume reduction without affecting significantly the mass loss during baking.

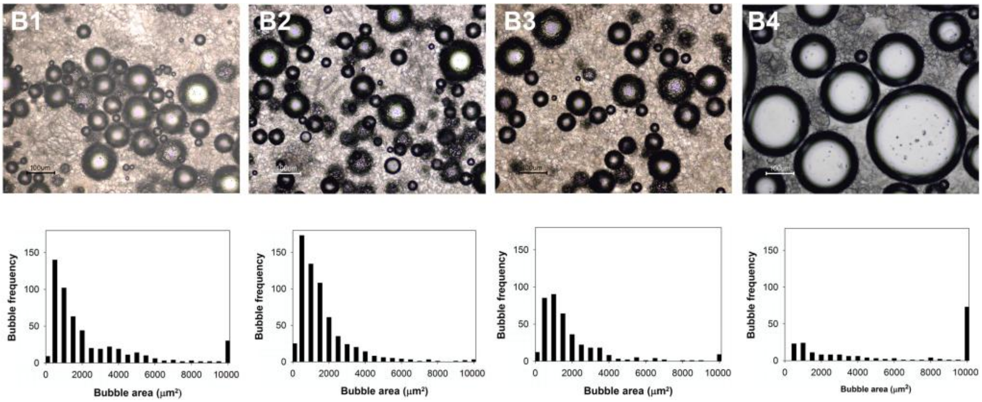

Figure 2. Batter microstructure. 1st row: light microscopy images (B1, B1, B3, and B4) (10×). 2nd row: bubble size distribution histograms

3.5. Image analysis of the cellular structure of the crumb

The complexity and heterogeneity of the cake structure mean that the physical properties differ greatly from one cake to another. In our study, the crumb structures were very different between the four types of cake (Figure 3). C1 and C2 corresponded to the model formulations, in which the leavening agent is not added, and so the development of the cake was limited. As C3 and C4 corresponded to the fully developed cakes, our work focuses on the analysis and comparison of C3 and C4 in this and the following sections.

Figure 4 shows the scanned and binarised images and the cell area distribution histograms of C3 and C4. Moreover, quantitative information in terms of the cell area and total cell area within the crumb were obtained from the binarised images using image analysis (data not shown).

There were significant differences ($P < 0.05$) in the cell areas, as well as the total cell areas within the crumbs –with the highest values corresponding to the cake made with inulin (C4). As stated before, fat replacement by inulin in B4 resulted in the occlusion of larger bubbles, which could expand more, and had more mobility. Thus, large interconnected cells and crack-like diffusion pathways dominated C4 structure as seen in Figure 4. As Kocer et al. (2007) showed, a higher mobility of the gas phase enhanced the formation of diffusion pathways.

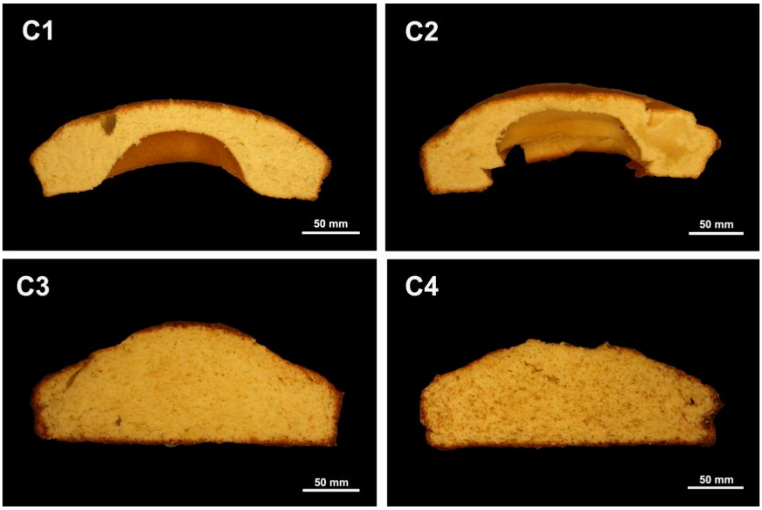

Figure 3. Appearance of the sponge cakes C1, C2, C3, and C4 (digital photographs of the cakes)

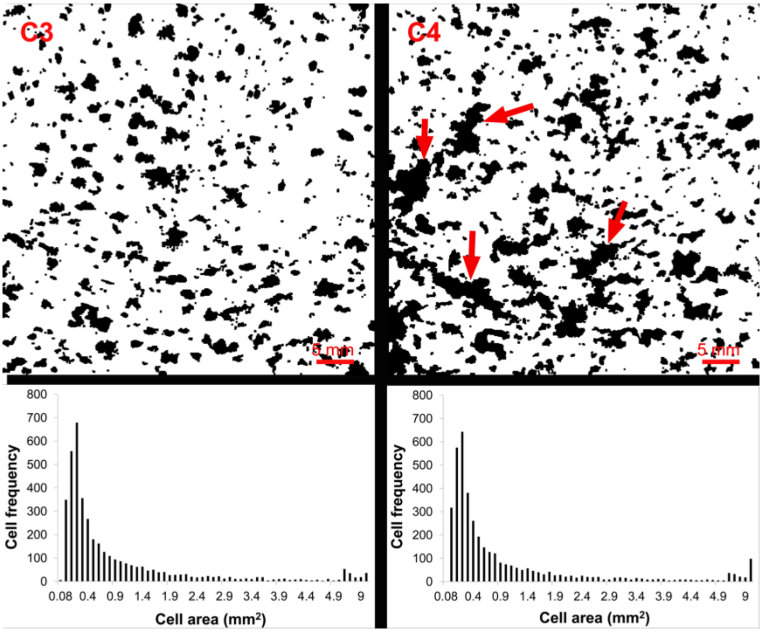

Figure 4. Cellular structure of crumb cakes C3 and C4. 1st row: binarised images of scanned crumbs (arrows: interconnected air cells). 2nd row: cell size distribution histograms

3.6. Cake texture

TPA parameters are shown in Table 3. The incorporation of inulin and oil absence significantly decreased ($P < 0.05$) the crumb hardness. This result could be related with the cell crumb structure of C4: a higher percentage of the cell area and diffusion pathways. C4 was more adhesive; fructan molecules during baking were involved in caramelisation and Maillard reactions; thus, some products of these reactions would confer adhesiveness not only on the internal structure, but also on the external surface. C3 and C4 revealed similar springiness values. Significantly higher values of cohesiveness were obtained for C4 ($P < 0.05$). The uneven cell crumb structure where large air cells and compact crumb areas were found, gave this product higher cohesiveness. C4 was significantly less ($P < 0.05$) chewy than the control. This observation indicates that the structure was more compact than the control.

Table 3. Mean values of texture profile analysis parameters of cakes C3 and C4

TPA parameters	C3	C4
Hardness (N)	8.73^{a} (2.31)	6.7^{b} (1.32)
Adhesiveness (N·s)*	0.01^{a} (0.01)	0.04^{b} (0.03)
Springiness	0.85^{a} (0.01)	0.85^{a} (0.02)
Cohesiveness	0.69^{a} (0.03)	0.72^{b} (0.02)
Chewiness (N)	5.12^{a} (1.13)	4. 08^{b} (0.81)

Values in parentheses are the standard deviations. Means in the same row without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test. *Absolute values.

3.7. Scanning electron microscopy at low temperatures (Cryo-SEM)

The differences between cakes C3 and C4 microstructures can be seen in the micrographs obtained by Cryo-SEM technique (Figure 5). The C3 structure was mainly formed by a well-developed and hydrated protein network, where other components such as partially gelatinised starch granules were embedded. The oil acted as a lubricant and created a continuous and flexible structure. During baking, the oil globules released fat, which created a film that coated and smoothened the surface, as shown in Figure 5. In comparison, the microstructure of C4 was irregular and with less integrity. Inulin could absorb part of the water creating competition for water with other ingredients such as gluten or starch. C4 was basically a protein structure in which gluten had difficulties in hydrating which affected its development into a continuous network. In addition, some starch granules were not fully incorporated into the matrix. So, in Figure 5, starch granules are identifiable as separate structures on the surface of the sample.

4. Conclusions

Oil increased the batter viscosity and helped to retain small air bubbles as observed in the interface air-matrix. During baking, oil was distributed as a coating layer linking the components and enabling the development of a homogeneous cell crumb structure. The leavening agent contributed to the optimal expansion of the structure in terms of height and sponginess. Inulin imparted less viscosity to the batter than oil. Bigger air bubbles were occluded, giving rise to a cake with an aerated structure and soft texture.

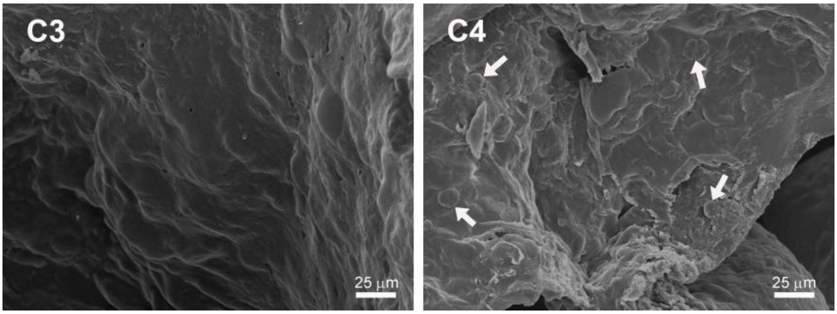

Figure 5. Cryo-SEM micrographs of cakes C3 and C4 (arrows: starch granules) (500×)

5. Acknowledgements

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Capítulo 2

Reemplazo de grasa y azúcar por inulina y oligofructosa en bizcochos

Optimization of a sponge cake formulation with inulin as fat replacer: structure, physicochemical, and sensory properties

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Abstract

The effects of several fat replacement levels (0%, 35%, 50%, 70%, and 100%) by inulin in sponge cake microstructure and physicochemical properties were studied. Oil substitution for inulin decreased significantly ($P < 0.05$) batter viscosity, giving heterogeneous bubbles size distributions as it was observed by light microscopy. Using confocal laser scanning microscopy the fat was observed to be located at the bubbles' interface, enabling an optimum crumb cake structure development during baking. Cryo-SEM micrographs of cake crumbs showed a continuous matrix with embedded starch granules and coated with oil; when fat replacement levels increased, starch granules appeared as detached structures. Cakes with fat replacement up to 70% had a high crumb air cell values; they were softer and rated as acceptable by an untrained sensory panel ($n = 51$). So, the reformulation of a standard sponge cake recipe to obtain a new product with additional health benefits and accepted by consumers is achieved.

Keywords: batter, cake, fat replacement, inulin, structure

1. Introduction

In developed countries, nutrition-related diseases are increasing because, among others, energy intake is too high and dietary fibre intake is below recommendations (Zahn et al. 2010). Because of diet and health concerns, consumers have been urged to consume less fat and more food containing complex carbohydrates (Khalil 1998). As fat has the highest energetic value of all major food constituents, a promising way for the food industry to provide advantageous food is to replace fat by dietary fibre (Zahn et al. 2010). So, in response to dietary guidelines and health goals, the food industry has introduced a variety of innovative food products designed to help consumers lower their fat intake (Khalil 1998).

Cakes typically contain 15% to 25% fat on a batter weigh basis. The fat assists in the incorporation of air bubbles into the batter during mixing, helps to leaven the product, tenderizes the crumb, imparts moistness, and enhances mouthfeel (Khalil 1998; Matsakidou et al. 2010). A prerequisite of fat replacers based on dietary fibre is that these are of low energetic value but mimic sufficiently the techno-functional properties of fat (Zahn et al. 2010).

Chemically, native inulin is a mixture of oligomer and polymer chains with a variable number of fructose molecules, joined by β (2–1), which usually include a glucose molecule at the end of the chain (Bayarri et al. 2011). Inulin chain lengths vary from 2 to 60 units with an average degree of chain polymerization of approximately 10 (Niness 1999). Inulin offers a unique combination of nutritional and technological advantages (Franck 2002). In recent years, dietary fibre has received increasing attention from researchers and industry due to the likely beneficial effects on the reduction of coronary heart-related diseases, diabetes incidence and gut neoplasia (Peressini and Sensidoni 2009). Long-chain inulin can be used to structure low-fat foods; its property to act as fat mimetic or fat replacer is based on its capacity to form micro-crystals, which interacts with each other, thereby forming small aggregates that occlude a great amount of water and that ultimately may agglomerate into a gel network (Bayarri et al. 2011).

Inulin has been used to replace fat in several foods, as meat products (Mendoza et al. 2001; Jánváry 2007) and dairy products (Ipsen et al. 2001; Tárrega and Costell 2006; Modzelewska-Kapituła and Kłebukowska 2009; Bayarri et al. 2010). Franck (2002) gathered inulin uses in several products as table spread, butter like products, frozen desserts, sauces, and soups. In baked foods, several studies dealing with the effect of inulin on physical and sensory properties can be found in the literature. Zoulias et al. (2002) used 4 types of fat mimetics –polydextrose, maltodextrine, inulin, and a blend of microparticulate whey proteins and emulsifiers– to partially replace fat in sugar free cookies. Physical, textural, and sensory properties of cookies were evaluated. They achieved a fat replacement up to 50% using the inulin, maltodextrine or the blend of whey proteins with emulsifiers. However, the resulting cookies were hard, brittle, and did not expand properly after baking. Devereux et al. (2003) used inulin as fat replacer, achieving a significant reduction in the fat content (20% to 80%) of carrot and chocolate cakes; these products were rated as acceptable by an untrained taste panel. Also, Zahn et al. (2010) studied the effects of inulin as fat replacer on texture and sensory properties of muffins showing that a replacement up to 50% of fat by inulin is possible in muffins. O'Brien et al. (2003) examined the effect of powdered and gel inulin as fat replacers in wheat bread and dough; differences in rheological, textural, and colour properties were found depending on the type of inulin added. However, no literature has been found related to microstructural studies on fat-replaced-by inulin cakes.

The aim of this study was to correlate the microstructure of batter and cakes with their final physicochemical properties; for this purpose, inulin was used to replace oil on a weight basis at 0%, 35%, 50%, 70%, and 100% replacement levels.

2. Materials and methods

2.1. Ingredients

The ingredients used in the preparation of the cake batters were: wheat flour (Harinera Belenguer, S.A., Valencia, Spain; composition provided by the supplier: 15% moisture, 10% proteins); sugar (Azucarera Ebro, Madrid, Spain); liquid pasteurized egg white and yolk (Ovocity, Llombay, Spain); skimmed milk (Puleva Food, Granada, Spain); refined sunflower oil (Coosol, Coosur S.A., Jaen, Spain); refined sunflower oil (Coosol, Coosur S. A., Jaen, Spain); inulin Frutafit® HD (Sensus, The Netherlands, Europe; specifications provided by the supplier: average chain length 8 to 13, 2 kcal/g, sweetness of 10% compared to sucrose (100%)); sodium bicarbonate and citric acid (A. Martinet, Cheste, Spain); and salt.

2.2. Batter and cake preparation

Five batters (B) were prepared according to Table 1, in order to obtain the correspondents cakes (C). A control formulation (B0/C0) and 4 formulations, where sunflower oil was replaced by inulin, were prepared. The replacement levels were: 35% (B35/C35), 50% (B50/C50), 70% (B70/C70), and 100% (B100/C100). In order to achieve the proper dispersion of inulin to act as fat mimetic, the appropriate amounts of water (from skimmed milk) were added on the basis of manufacturer suggestions, being the inulin-to-water ratio 1 to 2. It is known that the high water-binding capacity formulations with carbohydrate-based fat replacers, as inulin, require a higher amount of water in the batter (Zahn et al. 2010).

The batters (B0, B35, B50, B70, and B100) were prepared according to Baixauli et al. (2008). Within 5 min after the end of the batter mixing, the batter was analysed.

For the preparation of the cakes (C0, C35, C50, C70, C100), 800 g of batter were placed in a Pyrex baking pan (diameter = 20 cm) and baked in a conventional oven (Fagor, 2CF-3V 'Elegance', Guipuzkoa, Spain), preheated at 170 ºC during 20 min, at 170 ºC during 35 min. Five cakes were obtained, corresponding to the batter formulations shown in Table 1. Cakes were kept at room temperature for 1 h and then analysed. All the batters and cakes were prepared in triplicate.

2.3. Apparent viscosity

The viscosity of the batter was determined using a viscosimeter (Haake Viscotester 6 R Plus, Thermo Scientific, Walthman, USA) equipped with spindle 4, at 6 rpm at room temperature. Measurements were performed in duplicate.

2.4. Confocal laser scanning microscopy (CLSM)

A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) was used. An Ar laser line (488 nm) was employed as light source to excite fluorescent dyes rhodamine B and nile red. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{\text{ex max}}$ 488 nm and $\lambda_{\text{em max}}$ 580 nm was solubilised in distilled water at 0.2%. This dye was used to stain proteins and carbohydrates. Nile red (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 515 nm was solubilised in PEG 200 at 0.1 g/L. A 60× /1.40NA/Oil/ Plan Apo VC Nikon objective lens was used.

For sample visualization a microscopy slide was elaborated with 2 razor blades (platinum coated double edge blades with 100 µm thickness) stuck to a glass (Alava et al. 1999; Sahi and Alava 2003). A drop of batter was placed in the microscope slide, within the central gap of the blades, rhodamine B solution and nile red solution were added and the cover slide was carefully positioned to exclude air pockets. By this means, it was possible to visualize the sample without deforming native sample structure when putting the cover. Observations were performed 10 min after diffusion of the dyes into the sample. Images were observed and stored with 1024×1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

Table 1. Formulations of the different batters and cakes (% in wheat flour basis)

Ingredients	B0/C0	B35/C35	B50/C50	B70/C70	B100/C100
Wheat flour	100.00	100.00	100.00	100.00	100.00
Egg yolk	27.00	27.00	27.00	27.00	27.00
Egg white	54.00	54.00	54.00	54.00	54.00
Skimmed milk	50.00	61.00	66.50	72.00	81.50
Sugar	100.00	100.00	100.00	100.00	100.00
Sunflower oil	46.00	30.00	22.00	14.00	0.00
Frutafit® HD	0.00	5.00	7.50	10.00	14.50
Sodium bicarbonate	4.00	4.00	4.00	4.00	4.00
Citric acid	3.00	3.00	3.00	3.00	3.00
Salt	1.50	1.50	1.50	1.50	1.50

2.5. Light microscopy (LM) and image analysis of batter bubbles

The batter samples were observed in a microscope (Nikon Eclipse E800, Nikon Co., Ltd., Tokyo, Japan) at 10× magnification (objective lens 10×/0.30 DIC M Plan Flour, Nikon, Tokyo, Japan).

One drop of the sample was placed on the glass slide as described previously. By this means, it was possible to assure a sufficient and constant specimen thickness, to allow the comparison of the sizes and number densities of the bubbles. Images were captured and stored in a format of 1280×1024 pixels using the microscope software (Nikon ACT-1, Version 2.62, Nikon, Tokyo, Japan).

All the bubbles from a total of 20 images were measured for each sample, using the software ImageJ (Natl. Inst. of Health, Bethesda, Mariland., USA.). Computed parameters included bubble area (μm^2) and bubble size distribution.

2.6. Weight loss during baking

Weight loss (WL) during baking was calculated taking into account the initial water content in each formulation. Equation 1 was used for the calculation:

WL (%) = (B – C / IW) × 100

Where:
WL = weight loss during baking
B = weight (in grams) of batter before baking.
C = weight (in grams) of cake after baking
IW = initial water content (in grams).
Initial water content was the sum of the initial water content of each ingredient in each formulation.

2.7. Image analysis of cellular structure of the crumb

Cakes were cut in 4 slices of 1.5 cm thickness, horizontally. The cut side of each slice was scanned using a scanner (Epson Perfection 1250, Epson America Inc., Long Beach, California, USA). Scanning was performed with a resolution of 300 dpi.

The scanned images were analysed using the software ImageJ (National Institutes of Health). The image was cropped in a 6×12 cm section, on which the analysis was performed. First, the image was split in colour channels, then the contrast was enhanced and finally the image was binarised after grey scale threshold. Cell density (number of cells per field [6×12 cm]), total cell area within the crumb, cell area, and cell circularity were calculated. The data were obtained by measuring cells in 4 different images for each formulation.

2.8. Cake height

The maximum cake height was measured from the cross section of the product using the software ImageJ (Natl. Inst. of Health). The baked product was cut and photographed with a digital camera (E-510 Olympus, Hamburgo, Germany) with a varifocal lens (14 to 24 mm ED, Olympus, Zuiko Digital, Hamburg, Germany) and a maximum aperture ratio of 1: 3.5 to 5.6. The images were stored in a format of 3648×2736 pixels. Measurements were performed in triplicate.

2.9. Cake texture

Texture profile analysis (TPA) was carried out using a TA-TXT plus Texture Analyser (Stable Microsystem, Godalming, U.K.) using the Texture Exponent Lite 32 software (version 4.0.8.0, Stable Microsystems). The test was performed in 4 cubes (4 cm side) taken from the central crumb of each cake. Texture profile analysis was performed using a test speed of 1 mm/s with a strain of 40% of the original cube height and a 5 s interval between the 2 compression cycles. A trigger a force of 5 g was selected. The double compression test was performed with a 50 mm diameter aluminium plate (P/50). The parameters obtained from the curves were hardness, chewiness, springiness, adhesiveness, and cohesiveness.

2.10. Cryo scanning electron microscopy (Cryo-SEM)

A Cryostage CT-1500C (Oxford Instruments Ltd., Witney, U.K.) was used, coupled to a JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan). The sample was immersed in slush N_2 (-210 ºC) and then quickly transferred to the Cryostage at 1 kPa where fracture of the sample took place. Sublimation (etching) was carried out at -95 ºC. The sample was coated with gold at 0.2 Pa, applied for 3 min, with an ionization current of 2 mA. Observation in the scanning electron microscope was carried out at 15 kV at a working distance of 15 mm, temperature ≤-130 °C. Samples were taken from the crumb of the cake.

2.11. Colour measurements

The instrumental measurements of crust and crumb cake were carried out with a chroma meter CR-400. (Konica Minolta Sensing Americas, Inc., Ramsey, N.J., USA.). The results were expressed in accordance with the CIELAB system with reference to illuminant C and a visual angle of 2º. The parameters determined were L^*(L^* = 0 [black] and L^* = 100 [white]), a^* ($-a^*$ = greenness and $+a^*$ = redness), b^* ($-b^*$ = blueness and $+b^*$ = yellowness), C_{ab}^* chroma [$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$] and h_{ab} hue [h_{ab} = arctan (b^*/a^*)].

Total color difference (ΔE^*) was calculated as follows (Francis and Clydesdale 1975):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The values used to determine whether the total colour difference was appreciable by the human eye were the following (Bodart et al. 2008):

$E^* < 1$ colour differences are not obvious for the human eye.
$1 < E^* < 3$ colour differences are not appreciate by the human eye.
$E^* > 3$ colour differences are obvious for the human eye.
Measurements were performed in triplicate.

2.12. Sensory analysis

Consumers were recruited among employees of the Institute of Agrochemistry and Food Technology (CSIC). A total of 51 untrained panellists (consumers) aged 22 to 63 were used for the study.

The samples were assessed in a standardized tasting room equipped with individual booths (ISO 1988). Each consumer received 5 pieces of cakes (the control with no inulin and 1 piece of each fat-replaced cake) monadically in a single session following a balanced complete block experimental design. The pieces of cakes were coded by three-digit random numbers. The pieces of cakes were served at room temperature in random order. Water was supplied to clean the consumers' mouths between each sample.

Consumer acceptance testing was done using a successive-category scale to score the 'appearance', 'texture', 'colour', 'taste' acceptability, and 'overall acceptance' of the product. The scale was a nine-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely). Data acquisition was performed using Compusense 5 releases 4.6 software (Compusense Inc., Guelph, Ont., Canada).

2.13. Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Plus 5.1 software package (Statistical graph Co., Rockville, Md., USA). Least significant difference (LSD) Fisher's test was used to evaluate mean values differences ($P < 0.05$). Pearson correlation was calculated for the apparent viscosity and the total cell air within the crumb.

3. Results and discussion

3.1. Apparent viscosity

Viscosity of batters decreased as fat replacement level increased (Table 2). The aqueous component was incorporated as skimmed milk (inulin-to-water ratio 1:2) to allow the dispersion of the inulin in fat-replaced batters. Some of this skimmed milk can be contributing to increase the free water in the batters. So, the higher amount of inulin added, the more amount of skimmed milk was used and less viscosity was obtained. As stated by Sahi and Alava (2003), this free water is important as it influences the viscosity of the batter.

3.2. Confocal laser scanning microscopy (CLSM)

The distribution of ingredients in cake batters was studied using the confocal laser scanning microscopy technique (Figure 1). By using staining agents rhodamine B and nile red, the components of the batters were observed as follows: protein and carbohydrate in red, fat in green, and starch in black.

Table 2. Mean values of apparent viscosity of batters

Batter	Apparent viscosity (mPa·s)
B0	11173.33^{a} (212.13)
B35	7081.11^{b} (820.99)
B50	5648.89^{c} (162.63)
B70	4914.44^{c} (386.97)
B100	3796.67^{d} (103.33)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test

The batters stained with rhodamine B are observed as a continuous matrix constituted mainly by egg, milk, and flour proteins. Three different structures can be observed in black: the big and round structures (b) corresponding to air bubbles, the small and round structures (f) corresponding to fat globules from oil, and the lenticular structures (sg) corresponding to starch granules from wheat flour.

When inulin is progressively added to the batter as fat is being replaced, less fat globules can be observed as it was expected (Figure 1, nile red staining).

Some small fat globules can be observed around the air bubbles (Figure 2). Oil globules formed an interface that increased bubble stability and could provide some of the physical properties that several researchers have stated for solid fats (Brooker 1996; Mousia et al. 2007).

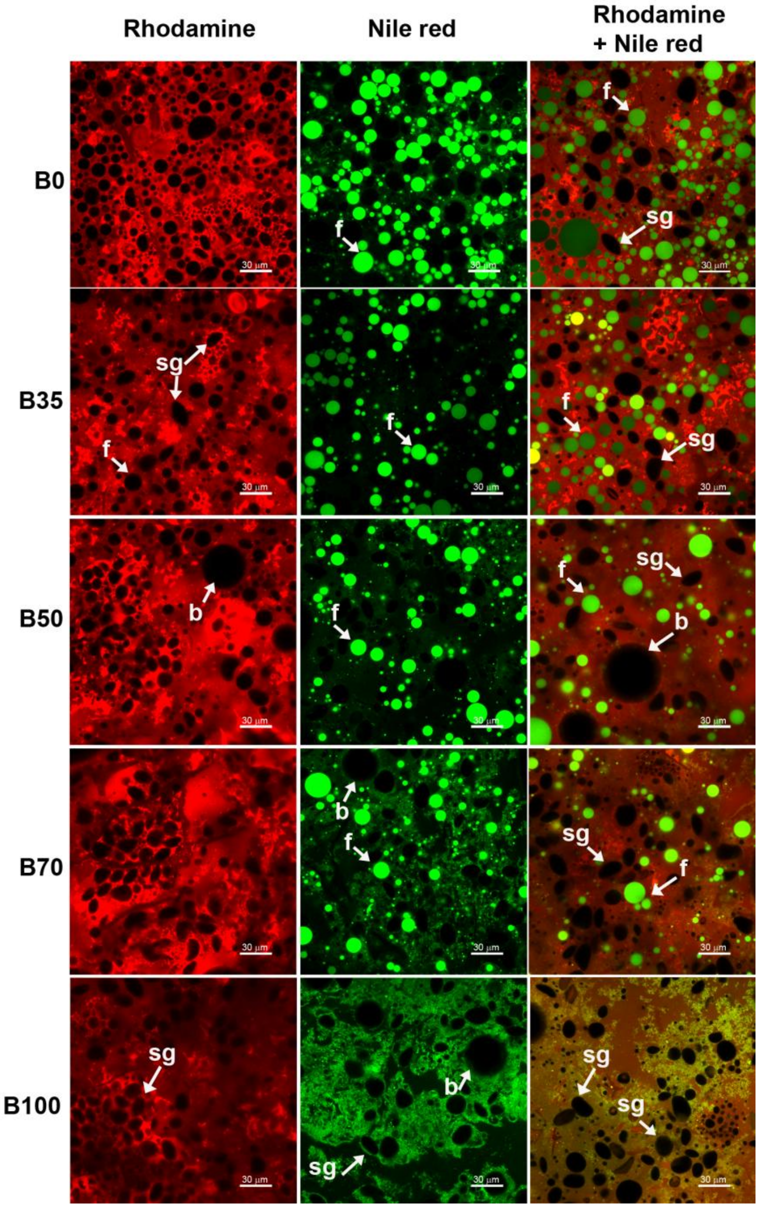

Figure 1. Confocal laser scanning microscopy (CLSM). Batter images (B0, B35, B50, B70, B100) stained with Rhodamine and Nile Red (proteins and carbohydrates in red, fat in green and starch in black). sg: starch granule, b: air bubble, f: fat globule. (60×)

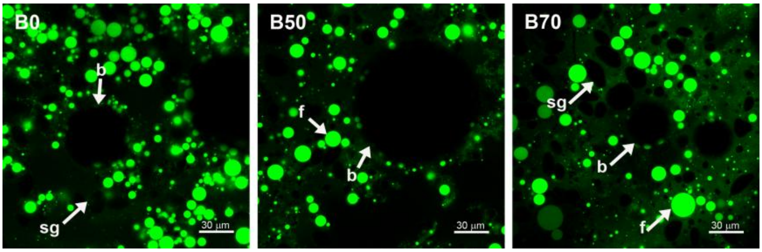

Figure 2. Fat distribution in batters B0, B50 and B70. sg: starch granule, b: air bubble, f: fat globule. (60×)

3.3. Light microscopy (LM) and image analysis of batter bubbles

The images of the batters without fat replacement or with low fat replacement levels showed a narrow bubble size distribution, with small areas (Figure 3). These batters had the higher viscosity values (Table 2). For high replacement levels, a broad size distribution of bubbles areas was observed, in accordance with a decrease in batter viscosity values. The batter with no oil and the lower viscosity value, B100, contained a broad bubble size distribution with an increase in bubble size.

As it was previously described in the confocal laser scanning microscopy section, the oil would act as bubble stabilizer by forming a film in the interface air-matrix. So, batters with higher concentration of oil had a narrow bubble size distribution and small area sizes.

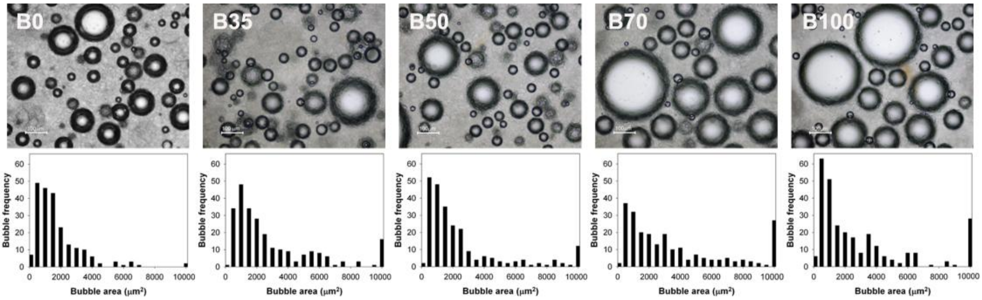

Figure 3. Batter microstructure. First row: light microscopy images of batters (B0, B35, B50, B70, and B100) (10×)- Second row: bubble size distribution histograms

3.4. Weight loss during baking

Weight loss (WL) values did not present significant differences among formulations (Table 3).

Table 3. Mean values of weight loss during baking of cakes

Cake	Weight loss (%)
C0	19.24^a (1.05)
C35	18.78^a (1.12)
C50	18.44^a (0.80)
C70	18.37^a (0.85)
C100	18.23^a (1.38)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test.

3.5. Image analysis of the cellular structure of the crumb and cake height

Table 4 shows the cell structure characteristics (cell density, total cell area within the crumb, cell area, and cell circularity) and cake height measured using image analysis from the binarised images of the different cake crumbs (Figure 4).

Cell density is the number of cells per area of crumb analysed (6×12 cm). It can be observed that cakes with higher inulin content had lower cell density values. Total cell area is the percentage of cells in the crumb area analysed. In concordance with cell density values, the cakes with higher fat replacement had significantly ($P < 0.05$) lower total cell area percentage. Cell area is the size of the cells in mm^2 and cell circularity describes the shape of the cell. As inulin content increased in the formulations cells became smaller and significantly ($P < 0.05$) more circular. This could be due to the fact that cakes with more oil content occluded a large number of air bubbles, which coalesced during baking forming continuous air channels and therefore giving cells with low circularity values. The formation of continuous channels is found to be essential in maintaining the volume of the cake in cooling once removed from the oven (Matsakidou et al. 2010). So, cakes with higher air volume occluded had significantly higher heights. Thus, control cakes had higher height. These results could be due to a higher apparent viscosity that might help the entrapment of air into the cake batters and cause significantly higher volumes ($P < 0.05$) and porosity values ($P < 0.05$) as Turabi et al. (2010) observed in rice cakes.

Pearson coefficient = 0.977 showed a significant correlation between apparent viscosity and the total cell air within the crumb ($P = 0.004$). Figure 5 shows the relationship between apparent viscosity and the total cell air within the crumb in the five cakes. Thus, differences between the cell structure of the control cake (C0), partially fat-substituted cakes (C35, C50, C70), and totally fat substituted cake (C100) must be due to an inadequacy in bubble expansion in batters with high fat replacement levels.

Table 4. Mean values of crumb cell structure characteristics and height of cakes

Cake	Cell density	Total cell rea (%)	Cell area (mm^2)	Cell circularity	Height (cm)
C0	1045.25^a (132.06)	50.52^a (5.29)	1.21^a (0.05)	0.77^a (0.00)	12.18^a (0.45)
C35	868.50^{ab} (133.02)	42.82^{ab} (3.90)	1.24^a (0.08)	0.78^a (0.01)	10.62^b (0.15)
C50	982.25^a (101.36)	41.40^{bc} (3.31)	1.06^{ab} (0.14)	0.79^a (0.01)	10.63^b (0.39)
C70	920.25^{ab} (155.50)	37.22^{bc} (8.23)	1.01^b (0.13)	0.81^b (0.01)	10.42^b (0.21)
C100	773.25^b (67.82)	33.94^c (5.84)	1.10^{ab} (0.14)	0.81^b (0.02)	9.07^c (0.11)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test

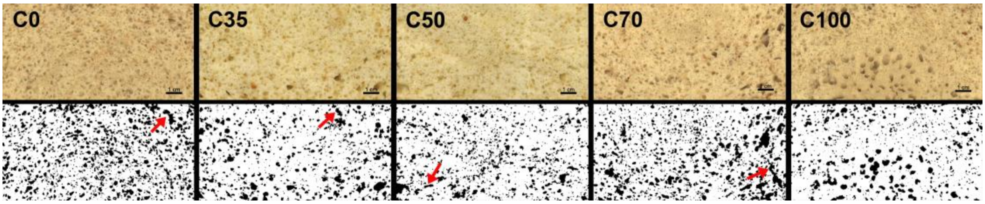

Figure 4. Cellular structure of the crumb of the cakes. 1st row: scanned crumbs of cakes (C0, C35, C50, C70 and, C100). 2nd row: binarised images of scanned crumbs. Arrows: diffusion pathways

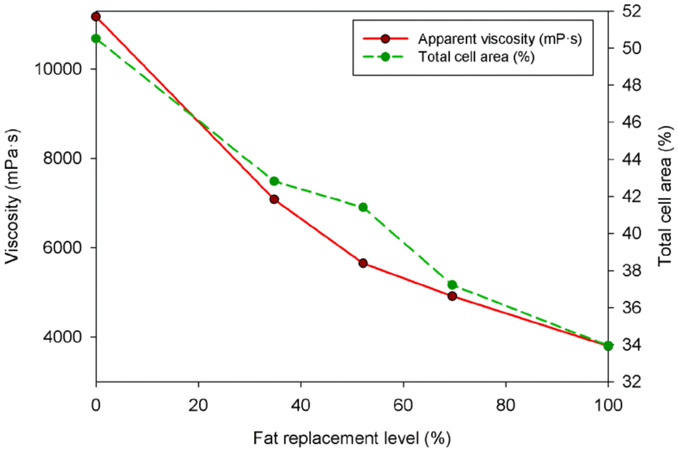

Figure 5. Viscosity values in contrast to total cell area within the crumb in the different cakes

3.6. Texture profile analysis

Table 5 shows TPA parameters values of the different cakes. The hardness of the cakes was highly sensitive to the air bubble size distribution. B0, B35, and B50 with a narrow bubble size distribution in small areas resulted in significantly ($P < 0.05$) softer cakes. The opposite occurred in B100 where a large variation in the size distribution of the bubbles resulted in a significantly ($P < 0.05$) harder crumb. Alava et al. (1999) observed that the cake had a softer texture when a homogeneous distribution of bubbles was observed in the batter, whereas when the bubble size distribution was broad, the cake tended to have a harder texture. Also, as it was observed in previous sections (image analysis of the cellular structure of the crumb and cake height) substitution of oil by inulin reduced the number of crumb cells, diminishing the height of the cakes, thereby increasing the force needed for compression. Other researchers also observed that inulin or fibre-based fat replacers increased crumb firmness (hardness) in muffins (Grigelmo-Miguel et al. 2001; Zahn et al. 2010).

Springiness varied in an irregular manner with fat replacement. At 50% of fat replacement with inulin (C50) springiness increased significantly ($P < 0.05$). Similarly, Zahn et al. (2010) observed that the replacement of 50% fat significantly increased springiness of the crumb, indicating that the strength of the bonds in the 3-dimensional crumb network was increased. At full fat replacement (C100) springiness decreased significantly ($P < 0.05$). Sanz et al. (2009) reported that the decrease in springiness was associated to a decrease in the number of muffin air bubbles and the existence of a denser matrix, as previously described for C100 in crumb cell structure section.

The cohesiveness values were seen to increase with fat replacement level. These results might be due to a denser crumb cell structure in replaced cakes. At higher levels of fat replacement, the number of cells decreased, the sphere-like cells increased and crumb areas without cells appeared. Thus, the energy required for the second compression increased.

Adhesiveness increased significantly ($P < 0.05$) with inulin addition. C50, C70, and C100 had higher adhesiveness because of the presence of fructan molecules, from inulin, and the partial absence of oil that could act as a lubricant.

3.7. Cryo Scanning electron microscopy (Cryo-SEM)

Figure 6 presents the micrographs obtained by the Cryo-SEM technique. The starch granules are partially gelatinized and immersed in a continuous matrix formed mainly by denatured proteins. During baking, the oil melted and coated the surface, creating a smoothened appearance (see Figure 6: C0 and C35).

Table 5. Mean values of texture profile analysis parameters of cakes

Cake	Hardness (N)	Chewiness (N)	Springiness	Cohesiveness	Adhesiveness (g·s)*
C0	8.80^{a} (1.78)	5.17^{ab} (1.09)	0.85^{a} (0.01)	0.69^{ab} (0.03)	1.85^{a} (0.77)
C35	8.48^{ab} (1.21)	4.98^{ab} (0.68)	0.86^{b} (0.01)	0.68^{a} (0.01)	4.16^{ab} (2.79)
C50	8.91^{ab} (0.51)	5.59^{a} (0.51)	0.88^{c} (0.01)	0.71^{b} (0.05)	8.79^{bc} (6.35)
C70	7.60^{b} (0.77)	4.56^{b} (0.39)	0.85^{ab} (0.01)	0.70^{ab} (0.02)	9.89^{c} (4.90)
C100	14.91^{c} (2.66)	8.51^{c} (1.06)	0.81^{d} (0.04)	0.71^{ab} (0.01)	66.79^{d} (14.52)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test. *Absolute values.

As the fat replacement level increased, cake matrix became more irregular, the starch granules were not fully embedded and they were observed as detached structures on the matrix surface. These facts might be due to the lack of oil, which acted as lubricant interconnecting and surrounding all the ingredients in a continuous matrix. Also, the presence of inulin would limit gluten and starch hydration during mixing and baking giving place to a less developed structure. These results were in accordance with the macroscopic appearance as C100 was crumbled during hand manipulation.

3.8. Colour

The effect of fibre (inulin) addition on crumb and crust colour of the cakes is summarized in Table 6. In general, L^* values of cake crumb increased significantly ($P < 0.05$) with the addition of inulin in the formulation. However, C100 did not show significant ($P < 0.05$) differences if compared to C0, C50, and C70. On the other hand, in cake crust the presence of inulin decreased significantly ($P < 0.05$) L^* and b^* values and increased significantly ($P < 0.05$) a^* values. These results might be due to inulin, which contains reducing sugars. The reducing sugars are involved in Maillard reaction during baking; further hydrolysis of low molecular weight fructan to fructose during the baking process may also favour nonenzymatic browning (Peressini and Sensidoni 2009). These observations were in agreement with those of Khalil (1998) who found that cakes prepared with fat replacers show a tendency toward darker colour compared to control, possibly because of a higher degree of Maillard browning reactions as a consequence of the carbohydrate nature of the fat replacer.

b^*, C_{ab}^*, and h_{ab} crumb colour parameters were similar, with slightly significant differences. Thus, only for C35 and C70 the colour differences were appreciable by the human eye ($E^* > 3$). On the contrary, in cake crust the replacement of oil by inulin led to a decrease of crust hue (h_{ab}) and chrome (C^*). These values might be due to the decrease of b^* parameter, which was more noticeable than the increase of a^* parameter. So, for cake crust E^* parameters were higher than 3 implying that their colour in comparison to the control was obvious for the human eye.

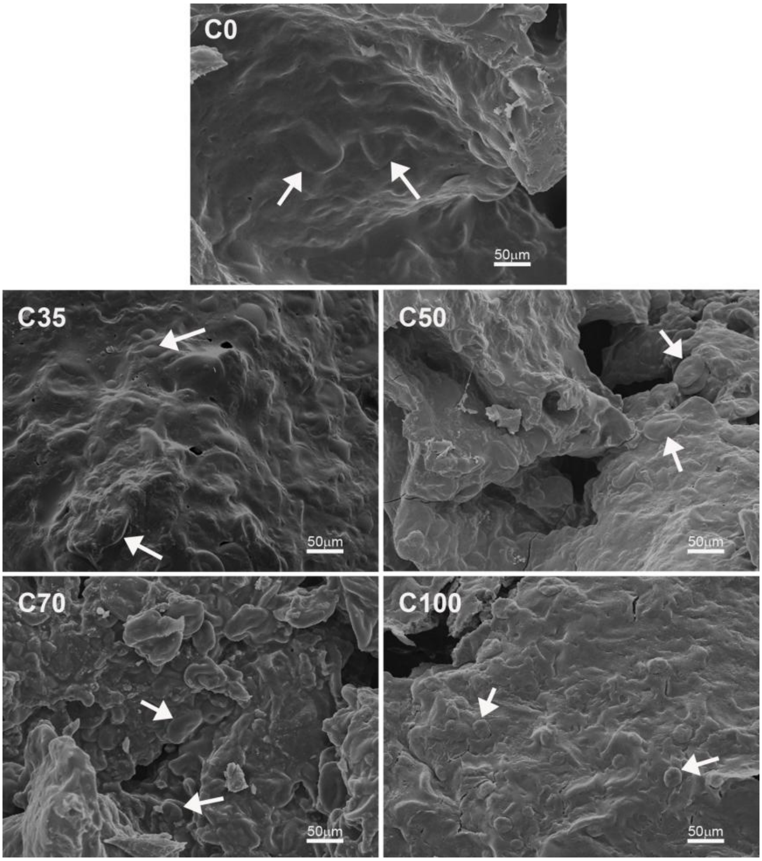

Figure 6. Cryo-SEM micrographs of the cakes Arrows: starch granules.(500×)

3.9. Sensory acceptance

The mean sensory acceptance scores for the 'appearance', 'colour', 'texture', 'taste', and 'overall acceptance' of the control cake and the cake with the different fat replacement levels are presented in Figure 7.

Statistical analysis showed that the control cake and cakes with 35%, 50%, and 70% of fat replacement levels did not differ significantly ($P < 0.05$) in all the attributes scored. This fact revealed that quality differences due to fat replacement by inulin were not considered as negative factors.

Full fat replaced cake was scored significantly ($P < 0.05$) lower for all the sensory attributes. The lowest scores of C100 for appearance and texture could be explained due to the crumbling and the irregular crumb cell structure. So, the comparison of the texture hedonic results with the instrumental measurements indicated that the highest hardness, adhesiveness, chewiness, and the lowest springiness may have had an important negative influence in hedonic acceptability. Regarding the taste, C100 showed the lower score if compared to other samples. The lower score could be due to the higher amount of inulin. Frutafit® HD has a sweet flavour, which can be perceived by the consumers when added to the cakes, lowering their scores for C100 in taste attribute.

4. Conclusions

Oil substitution for inulin decreased batter viscosity, which leads to an increase in air bubble size. The absence of oil –which acts as an interface, stabilizing air bubbles– gave place to a less aerated structure after baking. Cakes with fat replacement up to 70% did not differ significantly from the control, in all the sensory attributes scored. So, a good quality cake with a 70% of oil replacement can be achieved, and it can be labelled as 'reduced in fat' according to U.S. FDA (2009) and EU regulations (European Union 2006). Further studies could be conducted to investigate ingredients that stabilize air bubbles in fat replaced cakes elaborated with inulin.

Table 6. Mean values of color parameter of cakes

	Crumb color					Crust color				
	C0	C35	C50	C70	C100	C0	C35	C50	C70	C100
L*	64.48^{a} (1.95)	68.26^{c} (1.50)	67.44^{bc} (1.22)	67.54^{bc} (1.21)	66.24^{ab} (1.92)	54.00^{a} (1.56)	44.20^{bc} (1.83)	44.62^{bc} (1.89)	43.49^{c} (1.61)	45.72^{b} (1.05)
a*	-1.40^{ab} (0.23)	-1.23^{b} (0.30)	-1.37^{ab} (0.30)	-1.56^{a} (0.14)	-1.11^{b} (0.27)	11.91^{a} (1.26)	15.74^{b} (0.83)	14.93^{b} (0.99)	15.88^{b} (0.42)	15.55^{b} (0.52)
b*	23.33^{a} (1.05)	23.29^{a} (0.86)	23.33^{a} (1.63)	23.01^{a} (1.01)	23.92^{a} (1.26)	34.89^{a} (0.47)	29.95^{b} (1.95)	30.53^{b} (1.76)	28.89^{b} (1.80)	29.20^{b} (1.56)
h_{ab}*	93.43^{ab} (0.61)	93.03^{a} (0.71)	93.40^{ab} (1.02)	93.89^{b} (0.40)	92.66^{a} (0.64)	71.16^{a} (2.02)	62.22^{b} (2.14)	63.49^{b} (2.24)	61.13^{b} (2.00)	61.92^{b} (1.46)
C_{ab}*	23.38^{a} (1.05)	23.32^{a} (0.86)	23.37^{a} (1.61)	23.07^{a} (1.01)	23.95^{a} (1.27)	36.88^{a} (0.34)	33.86^{b} (1.71)	33.50^{b} (0.89)	32.98^{b} (1.45)	33.09^{b} (1.42)
ΔE*		3.78	2.95	3.08	1.87		11.63	10.78	12.74	10.69

Values in parentheses are the standard deviations. Means in the same row without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test.

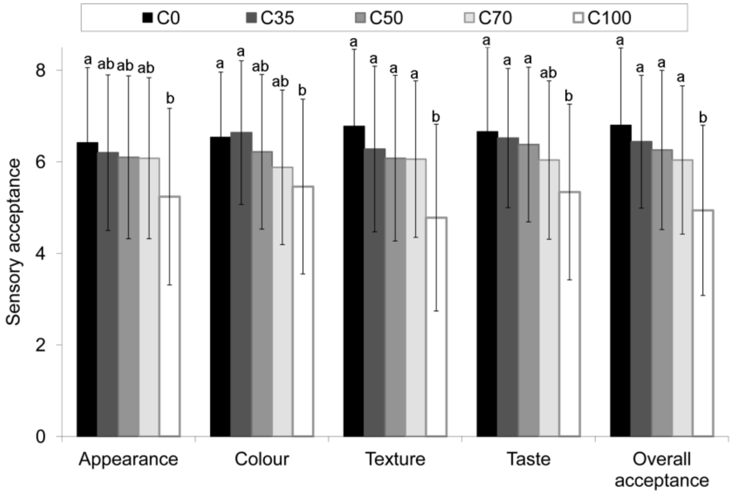

Figure 7. Mean consumer acceptance scores for the different cakes. Same letter indicates no significant differences between means for the same attribute

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Replacing fat and sugar with inulin in cakes: bubble size distribution, physical and sensory properties

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Abstract

The replacement of fat and sugar in cakes is a challenge as they have an important effect on the structural and sensory properties. Moreover, there is the possibility to incorporate an additional value using novel replacers. In this work, inulin and oligofructose were used as fat and sugar replacers, respectively. Different combinations of replacement levels were investigated: fat replacement (0% and 50%) and sugar replacement (0%, 20%, 30%, 40% and 50%). Simulated microbaking was carried out to study bubble size distribution during baking. Batter viscosity and weight loss during baking were also analysed. Cake characteristics were studied in terms of cell crumb structure, height, texture and sensory properties. Fat and sugar replacement gave place to batters with low apparent viscosity values. During heating, bubbles underwent a marked expansion in replaced cakes if compared to the control cake. The low batter stability in fat-replaced samples increased bubble movement, giving place to cakes with bigger cells and less height than the control. Sugar-replaced samples had smaller and fewer cells and lower height than the control. Moreover, sugar replacement decreased hardness and cohesiveness and increased springiness, which could be related with a denser crumb and an easily crumbled product. Regarding the sensory analysis, a replacement up to 50% of fat and 30% of sugar –separately and simultaneously– did not change remarkably the overall acceptability of the cakes. However, the sponginess and the sweetness could be improved in all the replaced cakes, according to the Just About Right scales.

Keywords: cake, fat replacement, inulin, oligofructose, structure, sugar replacement

1. Introduction

Cakes are baked products relished by consumers and are available worldwide. The main problem of such baked goods is their high fat and sugar content, which turns them into high-calorie products (Schirmer et al. 2012). Dietary imbalances, for instance inadequate intakes of dietary fibre or excessive energy intakes, have been associated with diseases such as obesity, non-insulin-dependent diabetes and others (FAO and WHO 1992).

Fat and sugar perform multiple functions in this kind of baked products. The lipids disrupt the structure of the gluten network –shortening effect– and contribute to increase volume and to form a soft and even-textured crumb grain. Furthermore, the polar lipids stabilize the gas bubbles, thanks to their surface-active properties, and fill the gaps in proteinaceous films, thus preventing the escape of gas (Sikorski and Sikorska-Wisniewska 2006). Sugar contributes in providing energy and sweetness. Moreover, during the mixing stage, it controls the viscosity of the batter by limiting the amount of free water, and during baking the low available water elevates the starch gelatinization temperature. Sugar also rises the egg white protein denaturation temperature (Pateras and Rosenthal 1992).

Interest in nutrition is driving consumer demands for less fat, sugar and calories; thus, the food industry is being challenged to redesign traditional foods for optimal nutritional value (Frye and Setser 1991). Nevertheless, the reduction of fat and sugar levels in cake systems affects the structural and sensory properties.

Inulin is a polydisperse β(2–1) fructan consisting of a mixture of fructose oligomers and polymers often terminated by a glucose molecule (GFn), with an average degree of polymerization of approximately 10. Oligofructose is also a mixture of molecules linked with the β(2–1) bond, but these range in molecular length from 2 to 10 (Niness 1999a). Inulin and oligofructose are used worldwide to add fibre to food products. The differences in chain length between inulin and oligofructose account for their distinctly different functionality attributes: inulin has been used successfully to replace fat in table spread, baked goods, etc.; oligofructose possesses functional qualities similar to sugar or glucose syrup (Niness 1999b).

In a previous work (Rodríguez-García et al. 2012) the effect of fat replacement by inulin in sponge cake microstructure and physicochemical properties was studied. Batters and cakes with 50% of fat replacement did not show significant differences if compared to the control in most of the parameters studied. Also, cakes with a fat replacement up to 70% did not differ significantly from the control and were rated as acceptable by an untrained sensory panel. Other authors have studied the impact of fat replacement by inulin on the baking characteristics, texture and sensory properties of muffins (Zahn et al. 2010); they conclude that a replacement of up to 50% is possible in muffins or related products. Moreover, Devereux et al. (2003) reformulated several baked foods –blueberry muffin, carrot cake and chocolate cake– by replacing some fat with inulin. A significant reduction in fat content was achieved: 50%, 38% and 20%, respectively.

Several authors have studied the replacement of sugar with inulin or oligofructose in combination with other sweeteners. Zahn et al. (2013) evaluated whether steviol glycosides may be used for partial replacement of sugar in muffins in combination with several fibres. They stated that a combination of inulin or polydextrose with steviol glycosides resulted in products with characteristics close to those used as the reference. The effects of oligosaccharides, such as oligofructose and polydextrose, together with polyols on the quality of sugar-free sponge cake were studied by Ronda et al. (2005); best results were obtained with xylitol and maltitol, leading to sponge cakes more similar to the control one. In addition, other sugar replacers and combinations of sweeteners have been studied on cakes and bakery products in recent years: sucralose and polydextrose in muffins (Martínez-Cervera et al. 2012b), polydextrose in cakes (Hicsasmaz et al. 2003; Schirmer et al. 2012; Siti Faridah and Noor Aziah, 2012), and fructose and sucralose on different 'croissant-type' formulations (Mariotti and Alamprese 2012). Moreover, the use of polyols is widely studied: stevioside and liquid sorbitol in cakes (Manisha et al. 2012), erythritol in chocolate cakes (Lin et al. 2003), erythritol in Spanish muffins (Martínez-Cervera et al. 2012a), and erythritol and maltitol in short-dough biscuits (Laguna et al. 2012). Other researchers focused their study on the effects of sugar replacement on the structure of batters and cakes before, during and after baking (Hicsasmaz et al. 2003; Pateras et al. 1994; Pateras and Rosenthal 1992; Pateras et al. 1989). In addition, few studies dealing with simultaneous fat replacement and sugar replacement in cakes have been found in the literature. Kocer et al. (2007) studied the effect of polydextrose as sugar and fat replacer in terms of the batter structure and expansion characteristics of the cake. The high-ratio cake system allowed 25% fat and 22% sugar replacement. Khouryieh et al. (2005) developed a no-sugar-added/low-fat muffin by incorporating xanthan gum, maltodextrine and sucralose. Sensory analyses showed that no-sugar-added/low-fat muffin was significantly higher in taste liking and lower in chewiness liking than the commercial muffin. Pong et al. (1991) evaluated the potential use of a commercial emulsifier and a sweetening system to replace fat and sugar in cupcakes. A lower standing height and firmer texture were observed in variations prepared with the substitutes.

The present study evaluates the functionality of two types of inulin as replacers for fat and sugar on the development of low-fat/low-sugar cakes. Simulated microbaking was developed to study the batter characteristics before and during baking in terms of bubble size, bubble size distribution and expansion rate. Cake characteristics were also studied in terms of cake height, cell crumb structure and textural properties. The effect of simultaneous fat and sugar substitution was also evaluated by a sensory analysis: successive-category scale.

2. Materials and methods

2.1. Ingredients

The ingredients used in the preparation of the cake batters were: wheat flour (Harinera Belenguer, S. A., Valencia, Spain; composition provided by the supplier: 15% moisture, 10% proteins); sugar (Azucarera Ebro, Madrid, Spain); Frutafit® CLR (an oligofructose with high solubility; Sensus, Roosendaal, the Netherlands; specifications provided by the supplier: average chain length, 7-9, 2 kcal/g, sweetness of 30% compared to sugar (100%)); liquid pasteurized egg white and yolk (Ovocity, Llombay, Spain); skimmed milk (Puleva Food, Granada, Spain); refined sunflower oil (Coosol, Coosur S. A., Jaen, Spain); Frutafit® HD (a highly dispersible native inulin; Sensus, Roosendaal, the Netherlands; specifications provided by the supplier: average chain length, 8-13, 2 kcal/g, sweetness of 10% compared to sugar (100%)); sodium bicarbonate and citric acid (A. Martinet, Cheste, Spain); and salt.

2.2. Batter and cake preparation

Ten batters (B) were prepared according to Table 1 in order to obtain the corresponding cakes (C). A control formulation (B0-0/C0-0) and four formulations, where sugar was replaced 1:1 with Frutafit® CLR, were prepared. The sugar replacement levels were: 20% (B0-20/C0-20), 30% (B030/C0-30), 40% (B0-40/C0-40) and 50% (B0-50/C0-50).

Moreover, another formulation where 50% of fat was replaced by Frutafil® HD was prepared (B50-0/C50-0). In order to achieve the proper dispersion of inulin to act as fat mimetic, the appropriate amounts of water (from skimmed milk) were added on the basis of manufacturer suggestions, the inulin-to-water ratio being 1:2. Four more formulations where sugar was replaced by Frutafit® CLR were also prepared: 20% (B50-20/C50-20), 30% (B50-30/C50-30), 40% (B50-40/C50-40) and 50% (B50-50/C50-50). The batters were prepared according to Baixauli et al. (2008b). Within 5 min after the end of batter mixing, the batter was analysed.

For the preparation of the cakes, 800 g of batter was placed in a Pyrex baking pan (diameter, 20 cm) and baked in a conventional oven (Fagor, 2CF-3V 'Elegance', Guipuzkoa, Spain), preheated at 160 ºC for 20 min, at 160 ºC during 40 min. Cakes were kept at room temperature for 1 h and then analysed. All the batters and cakes were prepared in triplicate.

2.3. Apparent viscosity

The viscosity of the batter was determined using a viscosimeter (Haake Viscotester 6 R Plus, Thermo Scientific, Walthman, USA) equipped with spindle 4 at 6 rpm at room temperature. The samples were placed in a thermostatic bath to maintain the temperature (25 ºC). Measurements were performed in duplicate.

2.4. Microscopic examination and image analysis of the batters

The equipment used for the microscopical examination during simulated microbaking was set up as follows: a temperature-controlled stage (Analysa-LTS350, Linkam, Surrey, UK) was mounted under the lens of a light microscope (Nikon ECLIPSE 80i, Nikon Co., Ltd., Tokyo, Japan).

Table 1. Formulations of the different batters and cakes (per cent in wheat flour basis)

Fat-sugar replacement (%)*	0-0	0-20	0-30	0-40	0-50	50-0	50-20	50-30	50-40	50-50
Wheat flour	100.00	100.00	100.00	100.00	100.00	100.00	100.00	1000.0	100.00	100.00
Egg yolk	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00
Egg white	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Skimmed milk	50.00	50.00	50.00	50.00	50.00	66.50	66.50	66.50	66.50	66.50
Sugar	100.00	80.00	70.00	60.00	50.00	100.00	80.00	70.00	60.00	50.00
Frutafit® CLR	0.00	20.00	30.00	40.00	50.00	0.00	20.00	30.00	40.00	50.00
Sunflower oil	46.00	46.00	46.00	46.00	46.00	22.00	22.00	22.00	22.00	22.00
Frutafit® HD	0.00	0.00	0.00	0.00	0.00	7.50	7.50	7.50	7.50	7.50
Sodium bicarbonate	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Citric acid	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50

*The first digit corresponds to the fat replacement level (%) and the second digit corresponds to the sugar replacement level (%)

One drop of the sample was placed in the concavity of a glass slide and placed in the temperature-controlled stage. In the heating experiments, the rate was controlled with a liquid nitrogen pump cooling system (Linkam, Surrey, UK) and a system controller for the heating and freezing stages (T95, Linkam, Surrey, UK). The temperature profile used was 1.5 ºC/min from room temperature (25 ºC) to 105 ºC; this temperature was kept for 1 min. Batter samples were observed at 4× magnification (objective lens 4×/0.13∞/– WD 17.1, Nikon, Tokyo, Japan). A camera (ExWaveHAD, model no. DXC-190, Sony, Tokyo, Japan) was attached to the microscope and connected to the video entry port of a computer. During the simulated microbaking, a video film was recorded and images were acquired each 10 s. Images were captured and stored in a format of 640×540 pixels using the microscope software (Linksys 32, Linkam, Surrey, UK). The software was directly interfaced with the microscope, enabling temperature control and image recording control.

B0-0, B0-50, B50-0 and B50-50 were assessed for initial bubble size distributions and then baked on the hot stage of the microscope. These four samples were chosen as the most representative formulations: B0-0 as the control, B0-50 as the maximum sugar replacement level, B50-0 as the maximum fat replacement level and B50-50 as the highest simultaneous replacement level of fat and sugar. Three specimens from each batter were examined. The heating process was stopped when expansion was arrested by the setting of the cake matrix. The images were analysed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA).

2.5. Weight loss during baking

Weight loss (WL) during baking was calculated taking into account the initial water content in each formulation. WL was calculated using the following equation:
$\mathrm{WL}\ (\%) = (B - C / \mathrm{IW}) \times 100$

Where WL is the weight loss during baking, B is the weight (in grams) of batter before baking; C is the weight (in grams) of cake after baking; and IW is the initial water content (in grams). Initial water content was the sum of the initial water content of each ingredient in each formulation. WL was measured six times for each formulation.

2.6. Cake height

The maximum cake height was measured from the cross-section of the product using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The baked product was cut and photographed with a digital camera (E-510 Olympus, Hamburg, Germany) with a varifocal lens (14–24 mm ED, Olympus, Zuiko Digital, Hamburg, Germany) and a maximum aperture ratio of 1:3.5-5.6. The images were stored in a format of 3648×2736 pixels. Measurements were performed in triplicate.

2.7. Image analysis of cellular structure of the crumb

Cakes were cut into four slices of 1.5 cm thickness, horizontally. The cut side of each slice was scanned using a scanner (Epson Perfection 1250, Epson America Inc., Long Beach, California, USA). The scanned images were analysed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The image was cropped in a 12×12 cm section, on which the analysis was performed. First, the image was split into colour channels, then the contrast was enhanced and, finally, the image was binarised after grey scale threshold. Cell area (mm^2) and total cell area within the crumb (%) were calculated. Measurements were performed in duplicate.

2.8. Cake texture

Texture profile analysis was carried out using a TA-TXT plus Texture Analyser (Stable Micro Systems, Godalming, UK) using the Texture Exponent Lite 32 software (version 4.0.8.0, Stable Micro Systems). The test was performed in four cubes (4 cm side) taken from the central crumb of each cake. Texture profile analysis was performed using a test speed of 1 mm/s with a strain of 40% of the original cube height and a 5 s interval between the two compression cycles. A trigger a force of 5 g was selected. The double compression test was performed with a 50 mm diameter aluminium plate (P/50). The parameters obtained from the curves were hardness, chewiness, springiness and cohesiveness. Measurements were performed in duplicate.

2.9. Sensory analysis

A total of 110 untrained panellists (consumers) aged 22-63 years were used for the study. As a sensory evaluation of ten samples is highly complicated to perform, each consumer received six samples, corresponding to the four formulations chosen for the microscopic examination (B0-0, B0-50, B50-0 and B50-50) and two more formulations with intermediate replacements levels (B0-30 and B50-30). The samples were coded by three-digit random numbers and were served at room temperature in random order. Water was supplied to clean the consumers' mouths between each sample.

Consumer acceptance testing was done using a successive-category scale to score the 'appearance', 'texture', 'colour', 'taste' acceptability and 'overall acceptance' of the product. A nine-point hedonic scale (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5=neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2 = dislike very much; 1 = dislike extremely) was used.

The adequacy of 'sponginess' and 'sweetness' level was scored with the use of bipolar Just About Right (JAR) scales (from 1=much too little to 5=much too much, 3 being just about right) of each sample.

Consumers also evaluated the intention to purchase on five-point category scales with the ends anchored with 'I would definitely buy it' through to 'I would definitely not buy it' and a neutral central point 'maybe I would or maybe I would not buy it'.

2.10. Statistical analysis

A categorical multifactorial experimental design with two factors –level of fat replacement and level of sugar replacement– was used. Analysis of variance was performed on the data using the Statgraphics Centurion XVI, version 16.1.11, software package (Statistical graph Co., Rockville, USA). Least significant difference (LSD) Fisher's test was used to evaluate mean value differences ($P < 0.05$). The JAR results were analysed by penalty analysis using XLSTAT 2010.5.02 statistical software (Microsoft, Mountain View, California, USA).

3. Results and discussion

3.1. Apparent viscosity

The interaction plot with LSD intervals for the apparent viscosity, where significant interactions between factors ($P < 0.05$) were observed, is shown in Figure 1A. Batters with 0% of fat replacement had significantly higher apparent viscosity values ($P < 0.05$) than batters with 50% of fat replacement. The incorporation of the extra aqueous component could significantly decrease batter viscosity, as was confirmed in a previous research (Rodríguez-García et al. 2012).

In batters with 0% of fat replacement, as sugar replacement increased, batter apparent viscosity significantly decreased ($P < 0.05$). This effect could be due to a lower water binding capacity of Frutafit® CLR than sugar. Abbasi and Farzanmehr (2009) studied this effect on the rheological properties of milk chocolates where sugar was replaced with different combinations of inulin (Frutafit® IQ, TEX and CLR), polydextrose, maltodextrine and sucralose. These authors found the lowest levels of viscosity when sugar substitutes were used, particularly inulin. They reported that this behaviour could be related to the low hygroscopicity of inulin and its low water binding capacity.

In batters with 50% of fat replacement, when sugar was replaced by Frutafit® CLR, no significant differences were observed in the apparent viscosity values ($P > 0.05$). The effect of extra aqueous component, due to oil replacement by inulin (Frutafit® HD), on batter apparent viscosity was significant enough to mask the effect of sugar replacement on fat-reduced batters.

3.2. Microscopic examination and image analysis of the batters

Figure 2A shows the images of batters with different fat and sugar replacements during simulated microbaking in the microscope. Visual analysis of the batter images showed a clear effect on bubble expansion due to sugar and fat replacement at 50% by inulin: Frutafit® CLR and Frutafit® HD, respectively. In batter with no replacement (B0-0), neither of fat or sugar, bubble size increased in a controlled and uniform rate. When sugar and/or fat were replaced at 50%, higher numbers of small bubbles were occluded during mixing. When temperature increased, there was a marked expansion and the bubble size distribution tended to widen.

Images were analysed to quantify the microscopy results. Bubble size distribution histograms at different temperatures are shown in Figure 2B. B0-0 occluded less bubbles than the other batters and showed a higher percentage of small bubbles during all the microbaking. This behaviour could be due to the higher apparent viscosity of this batter, which retards bubble movement in the batter and slows down the disproportionation process, hence aiding batter stability (Sahi 1999). In general, a lower apparent viscosity of the replaced batters may have allowed occluding more air during mixing; thus, more number of bubbles per field is observed at the beginning of the baking procedure (Figure 2A).

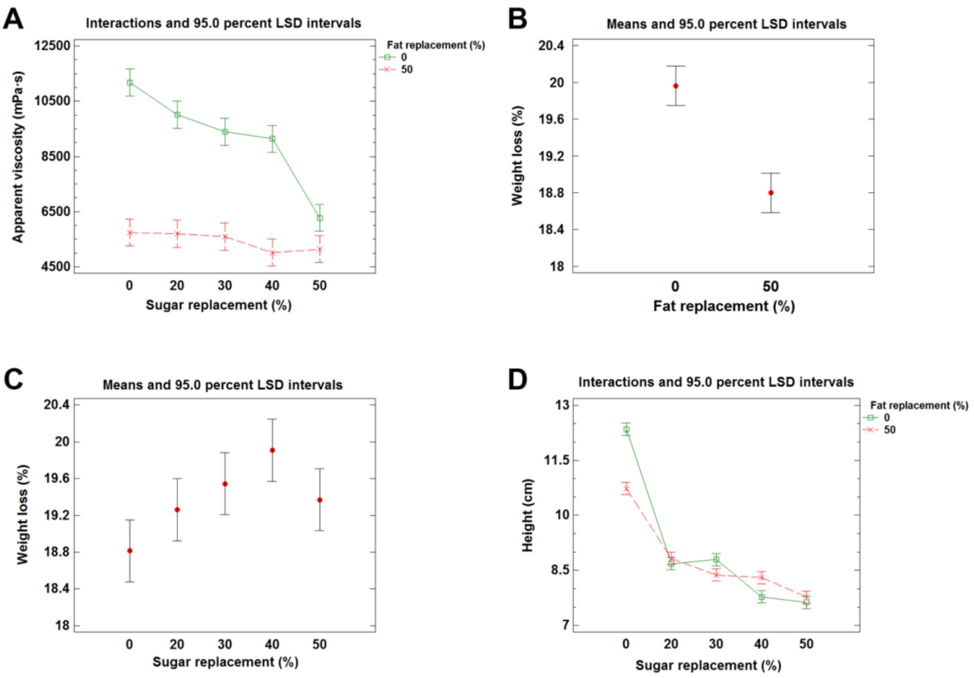

Figure 1. Interactions and mean plots with LSD intervals. A: interaction between sugar and fat replacement for the apparent viscosity of batters. B, C: mean values for the weight loss according to the fat and sugar replacement, respectively. D: interaction between sugar and fat replacement levels for the height of cakes

In batters with 50% of sugar replacement (B0-50), disproportionation processes were observed during heating; the number of bubbles decreased and bubble size slightly increased. Fat-replaced batter (B50-0) underwent a marked change in expansion at high temperatures, resulting in a reduction of the number of bubbles per field and in a broad distribution of bubble sizes. The decrease in apparent viscosity would increase the migration of air bubbles in the batter; this would decrease bubble stability by increasing the rate of disproportionation and coalescence. Kocer et al. (2007) also observed that the basic effect of both sugar and fat replacement was a decrease in batter stability during heating. Fat reduction could affect batter physical properties, but also starch and protein hydration and interactions during mixing and baking. Furthermore, sugar has major influences on the structure of the cakes, retarding the setting of the structure and allowing longer time for the gases to expand the structure (Pateras et al. 1989). Therefore, fat and sugar replacement could remarkably change bubble expansion during heating.

3.3. Weight loss during baking

No significant interactions ($P > 0.05$) were observed between the sugar replacement level and the fat replacement level for the WL during baking; the mean plots with LSD intervals are shown in Figure 1B, C. Cakes with 50% of fat replacement had significantly lower WL ($P < 0.05$) than cakes with 0% of fat replacement (Figure 1B). Inulin (Frutafit® HD) as a fibre could bind more water than oil, helping to retain moisture during baking. When sugar was replaced at different levels (Figure 1C), cakes with no sugar replacement showed significant differences ($P < 0.05$) if compared to cakes with 30 and 40% of sugar replacement. As stated before, it could be due to the higher water binding capacity of sugar than Frutafit® CLR.

3.4. Cake height

Significant interactions were observed ($P < 0.05$) between fat replacement level and sugar replacement level when the height of the cakes was studied (Figure 1D). The control cake (C0-0) showed the highest height when compared to cakes elaborated with inulin. In general, cakes with 0% of fat replacement had similar or higher height than cakes with 50% of fat replacement. Furthermore, as sugar replacement increased (>40%), cake height decreased significantly ($P < 0.05$). The final volume of the cakes is not only dependent on the initial air incorporated in the batter but also on its capacity to retain air during baking, as Zhou et al. (2011) stated when they studied the influence of different types of fats on high-ratio layer cakes. The control batter (B0-0) showed less bubbles occluded during mixing, a better gas retention and a more controlled structure development during microbaking (Figure 2), which helped in the obtention of a higher cake. In contrast, in fat-replaced cakes, the decrease in expansion seemed to be related to the decrease in batter stability during baking – related to the batter viscosity decrease and the bubble size increase.

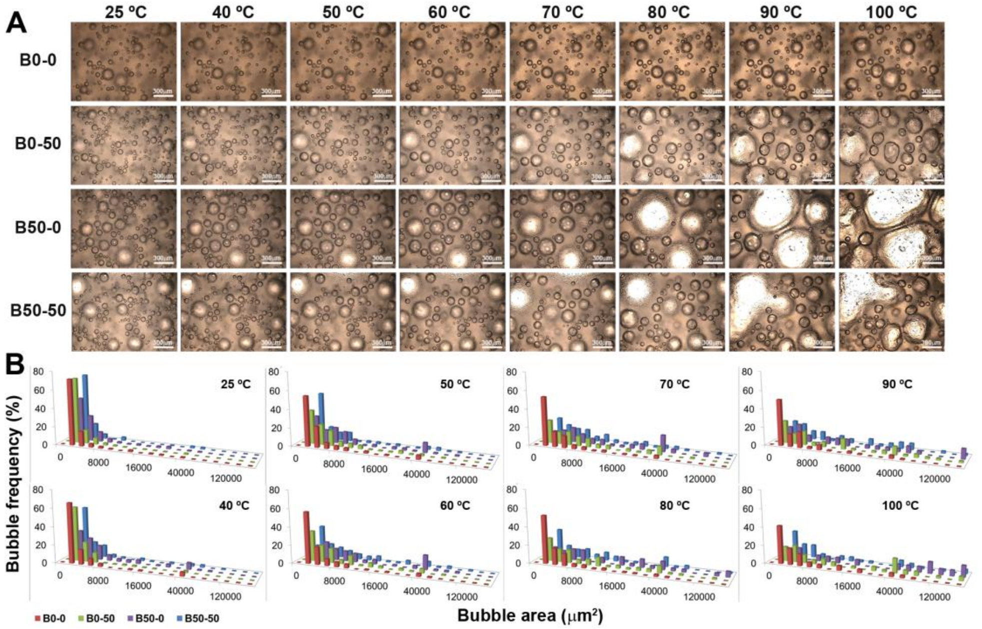

Figure 2. Batter microstructure during simulated microbaking. A: light microscopy images of bubble expansion at different temperatures during baking (4×). B: bubble size distribution histograms at different temperatures during baking. The first digit corresponds to the fat replacement level (%) and the second digit to the sugar replacement level (%)

Non-reduced sugar cakes resulted in highly aerated structures which had higher height if compared to sugar-replaced cakes. According to Schirmer et al. (2012), the expansion of the crumb cells is mainly dependent on the CO_2 production, the heating rate, and the thermal change of the structure due to protein denaturation and starch gelatinization. Sugar plays an important role in delaying starch gelatinization during cake baking so that the air bubbles can be properly expanded by carbon dioxide and water vapour before the cake sets (Manisha et al. 2012). Therefore, sugar-reduced cakes had less time to expand and to achieve a proper height.

3.5. Cellular structure of the crumb

Figure 3 shows the scanned and binarised images of the cakes. Image analysis was carried out to measure several crumb characteristics. The sugar replacement level did not show interactions with the fat replacement level for any parameter; the mean plots with LSD intervals are shown in Figure 4. Cakes with 0% of fat replacement had significantly smaller cells and significantly higher percentage of cells ($P < 0.05$) than cakes with 50% of fat replacement (Figure 4A, C). The control batter (B0-0) showed a more constant narrow bubble size distribution and controlled baking expansion. Thus, the final cake showed a high number of small cells within the crumb. As was observed during simulated microbaking, when fat was replaced at 50%, a noticeable bubble expansion and widening of the bubble size distribution took place. The resulting crumb had bigger and fewer cells (Figure 4A, C) distributed within the crumb in an irregular manner. Pateras et al. (1989) stated that a large variation in bubble size resulted in an irregular cake crumb.

Sugar replacement also affected significantly the crumb cell structure. When sugar was replaced, the cell area and the total cell area within the crumb decreased significantly ($P < 0.05$) (Figure 4B, D). Sugar replacement could cause changes in the thermosetting mechanism, which could affect the expansion and retention of the bubbles.

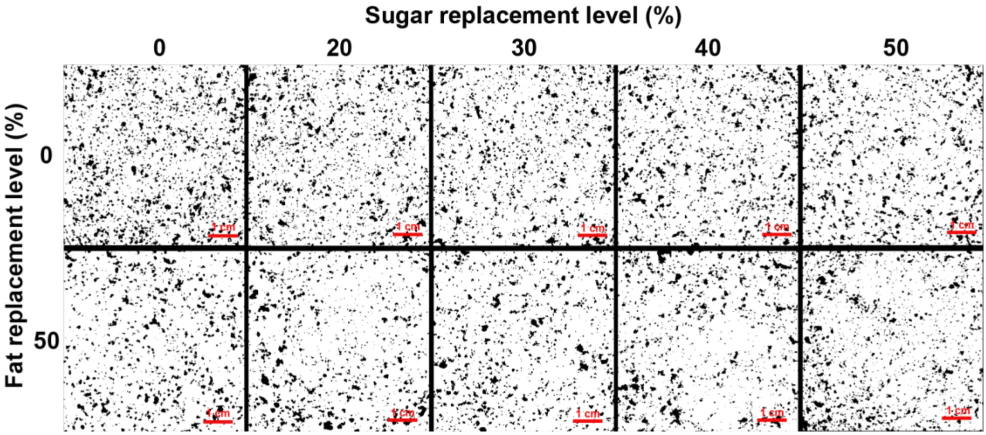

Figure 3. Cellular structure of the crumb. Binarised images of scanned crumbs

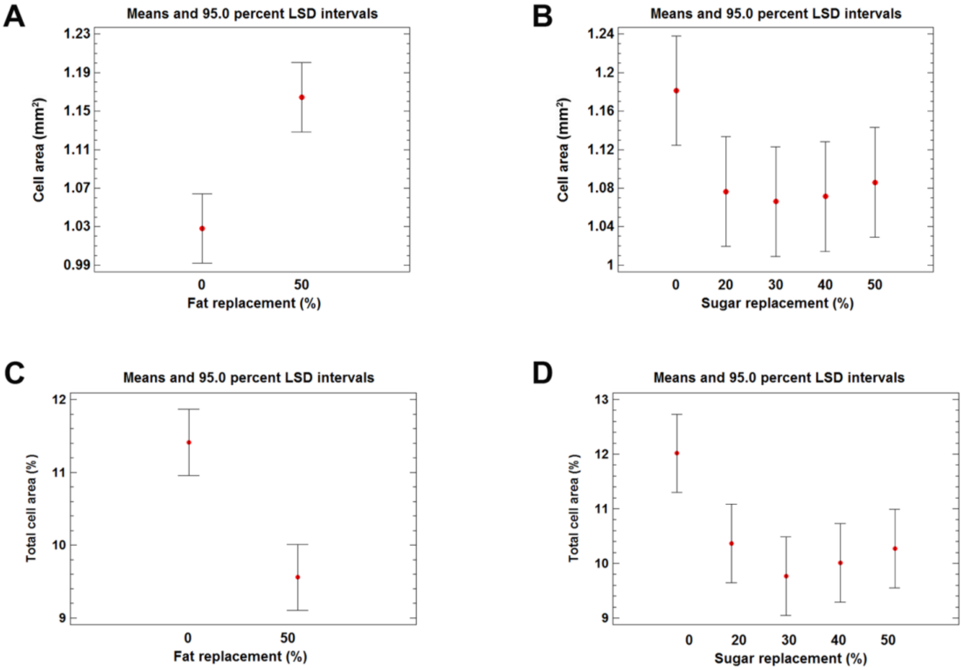

Figure 4. Mean plots with LSD intervals. A, B: mean values for the cell area according to the fat and sugar replacement, respectively. C, D: mean values for the total cell area according to the fat and sugar replacement, respectively

3.6. Texture profile analysis

The influence of fat and sugar replacement on cake hardness, chewiness, springiness and cohesiveness is depicted in Figure 5. Fat replacement did not show interactions with sugar replacement for hardness; the mean plots with LSD intervals are shown in Figure 5A, B. No significant differences ($P > 0.05$) were observed in hardness when 50% of fat was replaced. Nevertheless, as sugar replacement increased, hardness decreased significantly ($P < 0.05$), as other authors observed when replacing sugar by a sucralose/polydextrose mixture in muffins (Martínez-Cervera et al. 2012b), by rebaudioside A in combination with several fibres in muffins (Zahn et al. 2013) and by polyols and oligosaccharides in sponge cakes (Ronda et al. 2005). In contrast, sugar-replaced cakes had less height, as was reported before. There are some hypotheses that seek to clarify the explanation of these results. On the one hand, an appropriate air cell expansion is achieved in cakes with sugar because it increases the starch gelatinization and protein denaturation temperatures. The decrease in expansion in sugar-reduced cakes seems to have two main causes: decrease in batter stability during the heating stage –related to batter viscosity decrease and foam bubble size increase– and changes in the thermosetting mechanism (Ronda et al. 2005). The lack of diffusion pathways when sugar is replaced may be associated with the early thermosetting, resulting in a low-volume cake with a crumb characterized by small cells with almost no interconnectivity. The narrow cell size distribution of small cells and their homogeneous distribution around the crumb as individual pores could provide a less hard crumb. On the other hand, in conventional sugar cakes, the egg protein denaturation and starch gelatinization occur in the same temperature range. When the protein sets and the resulting cake is cooled, the starch component of the matrix gels, contributing to the strength of the cake crumb (Pateras and Rosenthal 1992). In sugar-reduced cakes, the change in the thermosetting mechanism could imply a decrease in the strength of the crumb, which could be one of the causes of the softer crumb texture.

The chewiness values followed the same trend as the hardness values (Figure 5C, D). When sugar replacement increased, chewiness decreased significantly ($P < 0.05$); thus, sugar-reduced cakes were easy to chew.

C50-0 showed the highest springiness value (Figure 5E). Similar results were found in a previous work (Rodríguez-García et al. 2012) and by Zahn et al. (2010). In general, sugar replacement decreased significantly the springiness values ($P < 0.05$). The decrease in springiness has been previously associated to a decrease in the number of crumb cells and the existence of a denser matrix (Martínez-Cervera et al. 2012b; Sanz et al. 2009).

In general, 50% of fat replacement gave cakes with significantly higher cohesiveness ($P < 0.05$) than cakes with 0% of fat replacement (Figure 5F). However, sugar replacement decreased crumb cohesiveness. The lower cohesiveness would indicate that less energy was required for the second compression (Baixauli et al. 2008a). An evident effect was that these cakes were very easily crumbled during hand manipulation.

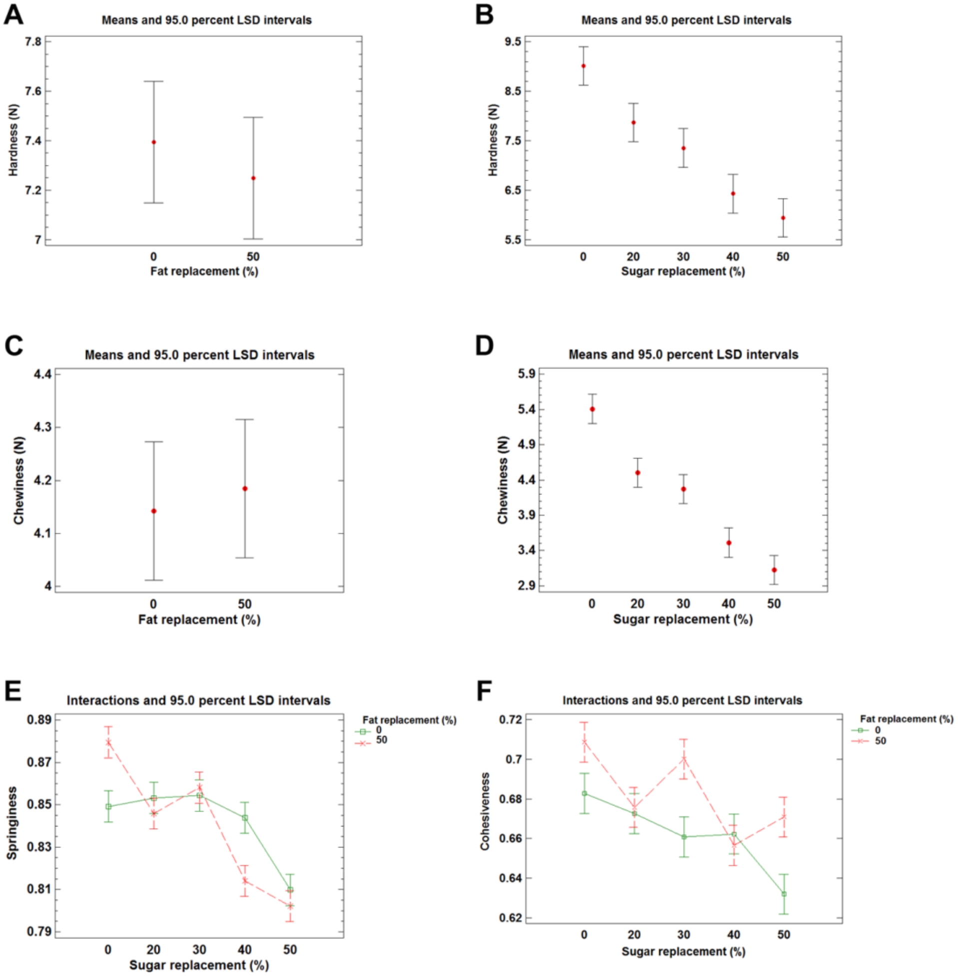

Figure 5. Interactions and mean plots with LSD intervals. A, B: mean values for hardness according to the fat and sugar replacement, respectively. C, D: mean values for chewiness according to the fat and sugar replacement, respectively. E, F: interaction between sugar and fat replacement levels for springiness and cohesiveness, respectively

3.7. Sensory acceptance

Consumer acceptability of the cakes is shown in Table 2. Fat replacement by inulin at 50% (C50-0) and sugar replacement by oligofructose at 30% (C0-30) did not show significant differences ($P > 0.05$) with the control cake. Samples with the highest sugar and fat replacement (C50-50) obtained the lowest value when this attribute was scored. Texture and taste acceptability followed a similar trend than overall acceptability. In general, differences were not found among samples when colour and appearance acceptability were studied. With respect to the intention to purchase, samples with sugar and fat replacement (C50-30 and C50-50) and the sample with sugar replacement at 50% (C0-50) obtained a lower percentage, coinciding with the results obtained in the acceptability test.

The JAR scale is conventionally used to identify along what sensory attributes and to what degree a product 'fails to deliver' its optimum. The implicit assumption is that the JAR scale can be used to 'diagnose' the nature of a sensory problem and the approximate direction and magnitude for its correction (Moskowitz 2004). The JAR results were analysed by penalty analysis relating JAR scales to liking data. In this analysis, an attribute was considered significant when the respondent percentage was higher than 20%. Penalty analysis is illustrated in Figure 6. Sample C0-0 had the two evaluated attributes ('sponginess and sweetness') on the left side of the plot, indicating that this formulation was not necessary to be modified and is accepted as is. When the fat or sugar content was lower, both attributes should be increased to increase the acceptability. In samples, C0-30, C50-0 and C50-30, 35% of consumers considered that these attributes need to be modified with a drop point in acceptability of 0.6-1.1 in 'sponginess' and 'sweetness', respectively. On the other hand, samples C0-50 and C50-50 had the attributes on the upper right-hand corner (more than 50% consumers), representing the worst punctuated ones with a drop point in acceptability of 1.2-1.8 in 'sponginess' and 'sweetness', respectively.

Table 2. Consumer acceptability of the cake samples

Cake	Overall acceptability	Appearance	Colour	Texture	Taste	Intention to purchase (%)
C0-0	7.15^{a}(1.41)	7.40^{a}(1.39)	7.23^{a}(1.51)	7.28^{a}(1.45)	7.01^{a}(1.65)	59.09
C0-30	6.70^{ab}(1.75)	7.10^{a}(1.36)	7.08^{a}(1.39)	7.18^{a}(1.47)	6.61^{ab}(1.89)	50.90
C0-50	6.31^{b}(1.90)	6.89^{ab}(1.55)	6.99^{a}(1.57)	6.39^{bc}(2.07)	6.09^{b}(2.15)	41.68
C50-0	6.75^{ab}(1.50)	7.16^{a}(1.34)	6.82^{a}(1.64)	7.02^{ab}(1.51)	6.52^{ab}(1.80)	48.63
C50-30	6.40^{b}(1.66)	6.95^{a}(1.28)	6.78^{a}(1.56)	6.32^{c}(1.72)	6.21^{b}(1.71)	39.45
C50-50	5.40^{c}(1.92)	6.40^{b}(1.66)	6.66^{a}(1.69)	5.60^{d}(1.95)	5.04^{c}(2.14)	17.28

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test

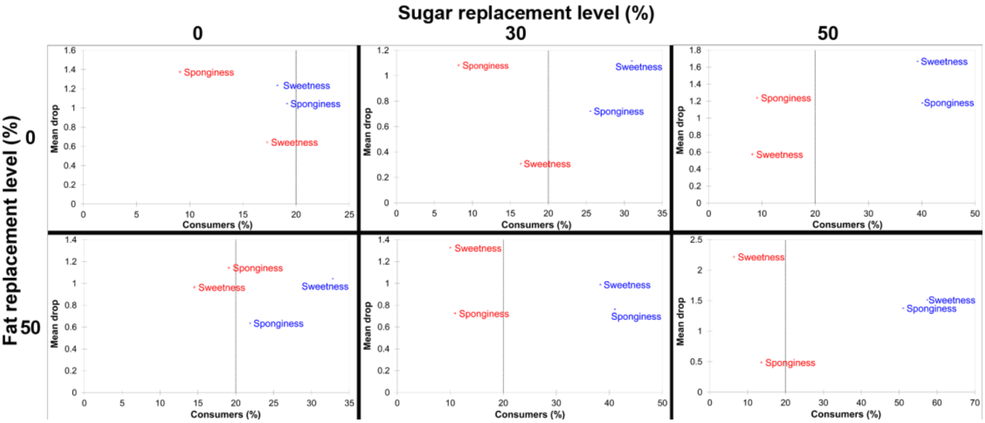

Figure 6. Representation of drops on overall liking by a proportion of panellists

4. Conclusion

Fat and sugar replacement by inulin decreased batter apparent viscosity values. The simulated microbaking under the light microscope showed that when fat and sugar were replaced, a higher number of small bubbles were occluded, a marked change in bubbles expansion was observed, and a wide bubble size distribution was achieved at the end of baking. The weight loss during baking decreased as inulin addition increased, showing an important effect on the water holding capacity of the formulation. In general, sugar- and fat-replaced cakes had fewer cells, had lower height, and were softer and easily crumbled. Sensory evaluation showed that cakes with 30% of sugar replacement (C0-30) and cakes with 50% of fat replacement (C50-0) were similar to the control. Cakes with both replacements simultaneously (C50-30) gave also good results in the overall acceptability, so a good quality cake with both replacements can be achieved and can be labelled as a ‘good source of fibre’ according to the EU regulations (European Union 2006).

Further work to improve sponginess and sweetness in the final product testing different combinations of bulking agents, thickening agents and sweeteners should be carried out.

5. Acknowledgments

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Capítulo 3

Escalado y optimización del proceso de elaboración de bizcochos

Optimising mixing during the sponge cake manufacturing process

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Abstract

Sponge cakes are manufactured using multi-stage mixing methods to enhance the potential foam formation of the eggs. However, single-stage methods, or all-in mixing procedures, are superseding multi-stage methods of large-scale batter preparation to reduce the costs and time of production. In this work, these two mixing procedures and three mixing times were tested in sponge cakes to optimise a mixing method for pilot scale research. No significant differences were demonstrated between the mixing procedures in batters and the physical properties of the cakes, including the relative density, elastic moduli, volume, total cell area, and hardness, among others. The batters mixed over short times generated well-aerated cakes with high volume and low hardness; these batters also exhibited lower relative density values. In contrast, after longer mixing times, low relative densities and high viscosity values were achieved; these cakes did not expand properly, generating cakes with a low volume and high hardness values. Longer mixing times provided a better-developed gluten network, stiffening the batter and inhibiting bubble expansion during mixing to create a denser crumb. Therefore, all-in mixing over short mixing periods might be suitable when scaling up sponge cake manufacture.

1. Introduction

The quality of a cake depends on several factors: the selection of ingredients and the knowledge of their function, as well as a balanced formula with precise measurements of the ingredients and optimal mixing and baking procedures (Conforti 2006a). The mixing procedure differs when performing a laboratory study, a pilot experiment or full-scale production. The major parameters include the batch volume, the equipment, and the mixing method.

The three main goals of mixing cake batters are 1) to combine all the ingredients into a smooth, uniform batter, 2) to form and incorporate air cells into the batter, and 3) to develop the proper texture in the finished product (Lai and Lin 2007). The complete dispersion of the ingredients is a fundamental requirement when producing a good cake, and the presence and dispersion of air bubbles are also essential because these bubbles may be regarded as the nuclei for cake expansion (Conforti 2006a).

Multi-stage methods are based on separating particular ingredients to prevent the formation of gluten or to enhance the potential foam formation with the eggs (Cauvain and Young 2007). Mixing methods for foam-style cakes, such as sponge cakes, depend on the occlusion of air and stable foam formation provided by the eggs in conjunction with the other ingredients, such as sugar and acid. To achieve the maximum batter volume, the whites and yolks are beaten separately (Conforti 2006a). Egg whites are the first ingredient to be whipped, thereby forming a foam. Second, sugar is added to stabilise the egg-white foam and form a meringue. Next, an acid is added to the egg whites during the foamy stage, thereby lowering the pH and further stabilising the whites (Conforti 2006b). Afterward, the yolk is incorporated; the presence of proteins and lecithin endows the yolk with unusual extensibility that is conducive to foam formation (Conforti 2006a). Whipped-type cakes prepared using wire whips require smaller batches (Conforti 2006a).

However during large-scale cake manufacturing, ingredient incorporation and mixing procedures must be optimised to reduce costs and time. The single-stage, or all-in, mixing method has become increasingly common, and in a significant number of cases, this method supersedes the multi-stage methods of batter preparation (Cauvain and Young 2007). In many modern bakeries, all-in methods are used where batter preparation is completed in one stage, particularly with the introduction of high-speed mixers (Bennion and Bamford 1997). When this mixing method is used, emulsifiers containing mono- and diglycerides are important because they help to trap air in the mixture and promote a finer texture (Lai and Lin 2007).

When preparing cake batter, the movement of the mixing tool pushes the material aside, creating a void behind the trailing edge. As the batter flows into the void, small pockets of gas (air) are entrained. The continuing movement of the mixing tool through the batter continues to trap air, decreasing the density of the batter. The length of the mixing time profoundly affects the density of cake batter: the longer the mixing time, the lower the batter density will be until the minimum density is reached (Cauvain and Young 2007). A low batter density is associated with good bubble retention during beating. However, air and leavening gas retention during baking is also affected by the batter viscosity (Bath et al. 1992).Therefore, the ability to occlude air and the bulk properties of the batter are important for air bubble retention and the creation of a sponge structure in the oven that does not collapse after baking (Sahi and Alava 2003).

This work addresses the aspects of cake production related to the mixing procedure; two different mixing methods are studied, that is, multi-stage mixing and all-in mixing, over three mixing times (3, 5 and 15 min). The objective of this research is to confirm whether the characteristics of the cakes significantly differ depending on the mixing procedure and time. Moreover, this work will help to know whether replacing the multi-stage mixing method with an all-in procedure is possible in cakes elaborated without emulsifier.

2. Materials and methods

2.1. Ingredients

The ingredients used to prepare the cake batters included the following (percentages based on flour): golden dawn plain white flour (100%) (ADM Milling Ltd., Brentwood CM14 4HG, UK; composition: 13.9% moisture, 9.7% protein); pasteurised liquid egg yolks (27%) and whites (54%) (Framptons Ltd., Shepton Mallet Somerset BA4 5PD, UK); white granulated sugar (100%) (British Sugar plc, Sugar Way, Peterborough PE2

9AY, UK); skimmed long-life milk 50% (Tesco, UK); sunflower oil (46%) (Olympics Oils Ltd., Liverpool, L24 9GS, UK); sodium bicarbonate (4%) (Brunner Mond, Cheshire, CW8 4DT, UK), citric acid (3%) (VWR International Ltd.; Poole, BH15 1TD, UK), and salt (1.5%).

2.2. Batter and cake preparation

The ingredients were weighed and allowed to reach approximately 20 ºC. Two different mixing procedures were studied: multi-stage and all-in mixing processes. Moreover, the last portion of the mixing was performed at the highest speed over three different durations: 3, 5 and 15 min.

The multi-stage mixing process was carried out according to a previously reported method (Baixauli et al. 2008) with slight modifications. The egg white was whipped in a Hobart N50 mixer (Hobart Manufacturing Company Ltd., London, UK) for 2 min at speed 3 (255 rpm). Next, the sugar was added and mixed for 30 s at speed 3. The egg yolk, citric acid and half of the milk were added and mixed at speed 1 (60 rpm) for 1 min. Afterward, the wheat flour was added and mixed at speed 1 for 1 min. The sodium bicarbonate and salt were added and mixed at speed 1 for 2 min. The oil and the rest of the milk were added and mixed at speed 3; at this speed three different times were tested: 3, 5 and 15 min.

The all-in mixing procedure was performed according to the Campden BRI method (Sahi and Alava 2003), with some modifications. The liquid egg and milk were placed in a Hobart N50 mixer (Hobart Manufacturing Company Ltd., London, UK). The dry ingredients were sieved and added to the liquids, and the oil was finally place on top. The mixing proceeded with a whipping accessory at speed 1 (60 rpm) for 30 s, followed by 1 min at speeds 2 (124 rpm) and varying intervals at speed 3 (255 rpm); at this speed, three different times were utilised: 3, 5 and 15 min. Two replicates of the same formulation were prepared in different days. Batter samples were collected for measurements of relative density and rheology.

Batters were scaled at 300 g in paper cases, placed in 400 g bread tins (145 mm x 75 mm at the base), and baked in a Bone Reel oven (Frederick Bone & Co. Ltd., Purley, UK) for 45 min, at 180 ºC. Two replicates with the same formulation were baked on different days; therefore, two sets of cakes were obtained with each formulation. The baked products were cooled at room temperature and analysed within 24 hours; weight, volume, crumb cellular structure, and texture were studied.

2.3. Relative density of the batter

The relative density (RD) of the batter was measured with a calibrated density cup of known volume. After mixing, the cup was filled with batter up to the brim and was weighed. The same procedure was performed using water. The RD was determined gravimetrically by dividing the weight of the known volume of batter by the weight of an equal volume of water. First, the RD was measured once per minute during the mixing procedure to study its evolution. Second, the RD was also measured after the mixing procedure (after 3, 5, and 15 min at the highest speed). The measurements were performed in duplicate.

2.4. Rheological properties of the batter

A rheological study of the batters was carried out with a rheometer (Rheometrics ARES model, TA Instruments Ltd., Crawley, UK). The measurements were obtained with a 50 mm diameter parallel plate immediately after mixing. The strain was selected by carrying out a strain sweep at 1 Hz, and a strain of 0.1 was chosen, corresponding to the region in which the batter displayed linear behaviour. Dynamic oscillatory frequency tests were performed in duplicate with a frequency sweep from 0.1 to 20 Hz. The viscoelastic functions were monitored (Rheometric scientific software; version V6.4.3.) including the elastic (G') and viscous (G'') moduli, as well as the complex viscosity (η^*). The measurements were performed in triplicate.

2.5. Weight loss during baking

The weight lost (WL) during baking was calculated using the following equation (Sumnu et al. 2005):

$$\text{WL}\ (\%) = (W_{\text{batter}} - W_{\text{cake}} / W_{\text{batter}}) \times 100$$

Where W denotes the weight (g).

The measurements were performed in triplicate.

2.6. Cake volume

The cake volume was measured with a BVM-L 370 (Tex-Vol Instruments AB, Perten Instruments, Sweden), and the measurements were analysed and stored in a database (Bread Calcu Version 7.2.4d_contin.). The measurements were performed in triplicate.

2.7. Crumb cell structure

The cakes were cut into four vertical slices (15 mm thick) with a Graef slicing machine (Gebr. GRAEF GmbH & Co. KG, Arnsberg, Germany). Two slices were scanned in a C-Cell (CCFRA Technology Ltd., Chipping Campden, UK) using the standard method for collecting images. The scanned images were analysed using the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). First, the image was split in colour channels, the contrast was enhanced, and finally the image was binarised using a greyscale threshold. The total cell area within the crumb (%), cell area (mm^2) and cell circularity were calculated. Three cakes for each formulation were used during the measurements. Because all the formulations were baked twice, producing a second set of cakes, the data were obtained by measuring the cells in twelve different images for each formulation.

2.8. Cake texture

A texture profile analysis (TPA) was carried out with a TA-TXT plus Texture Analyser (Stable Micro System, Godalming, UK) using the Texture Exponent 32 software (version 4.0.8.0, Stable Microsystems, Godalming, UK). The measurements utilised three cakes from each batch, and all the formulations were repeated with a second batch of batter. Three cakes from each formulation were selected, and; four slices (15 mm thick) were cut from the central region with a Graef slicing machine (Gebr. GRAEF GmbH & Co. KG, Arnsberg, Germany). The texture profile was analysed at 5 mm s^{-1} with a strain 25% of the original height and a 1 s interval between the two compression cycles. A trigger force of 5 g was selected. The double compression test was performed with a disc-shaped steel probe (45 mm diameter). The parameters obtained from the curves were hardness, springiness, and cohesiveness.

2.9. Statistical analysis

A categorical multifactorial experimental design with two factors was used: mixing procedure and mixing time at highest speed. An analysis of variance (ANOVA) was performed on the data using the Statgraphics Centurion XVI version 16.1.11 software package (Statistical graph Co., Rockville, Md, USA). A least Significant Difference (LSD) Fisher's test was used to evaluate the differences between the mean values ($P < 0.05$).

3. Results and discussion

3.1. Relative density of the batter

The changes in the relative density (RD) during the last step of the mixing procedure are shown in Figure 1. The relative density increased during the first 2 min of the multi-stage mixing and the first 4 min of the all-in mixing because at this time, the ingredients were not fully mixed.

No significant interactions ($P > 0.05$) were observed between the mixing procedure and mixing time when the RD of the batter was studied. The mean plots with their LSD intervals are shown when significant differences in RD are present for one of the factors; significant differences ($P < 0.05$) in RD were observed with different mixing times (Figure 2A). As the mixing time increased, the RD significantly ($P < 0.05$) decreased (Figure 2A).

After 12 min of mixing, the minimum RD of the batter was reached (Figure 1). During the mixing process, the amount of entrained air eventually equals the amount of air released; this equilibrium coincides with the minimum batter density and is unique for each recipe and type of mixer (Cauvain and Young 2007). Afterwards, when the mixing continues beyond 15 min, the multi-stage mixed batters showed an increase in the RD, while the all-in mixed batters showed a plateau. The air is disentrainment upon continued mixing, allowing some gas bubbles to escape with the air and any carbon dioxide present. Cauvain and Young (Cauvain and Young 2007) showed that after about ten minutes of mixing, the combined loss of carbon dioxide and air disentrainment result in an increase in the batter density. Moreover, if the bubble-stabilising mechanism in the batter begins to deteriorate during continued mixing, the air disentrainment and batter density will rise.

The all-in mixing procedure was more efficient at reducing the RD of the batter since the mixing began (Figure 1). However, when the RD values at the three times studied were evaluated, no significant (P > 0.05) differences between the procedures were observed (data not shown).

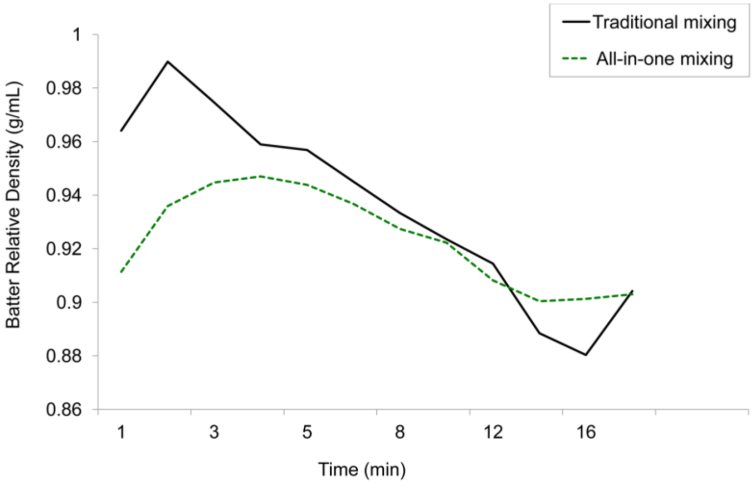

Figure 1. Relative density (RD) evolution during mixing

3.2. Batter rheology properties

No significant interactions (P > 0.05) were found between the mixing procedures or times when the elastic modulus (G') was studied. The mean plots with their LSD intervals are shown when there were significant differences (P < 0.05) in G' for one of the factors; Figure 2B shows the mean plots of the G' values relative to the mixing time. The interaction plots with their LSD intervals for the viscous moduli (G") and the complex viscosity (η*), where significant interactions (P < 0.05) between factors were observed, are shown in Figure 2C and D.

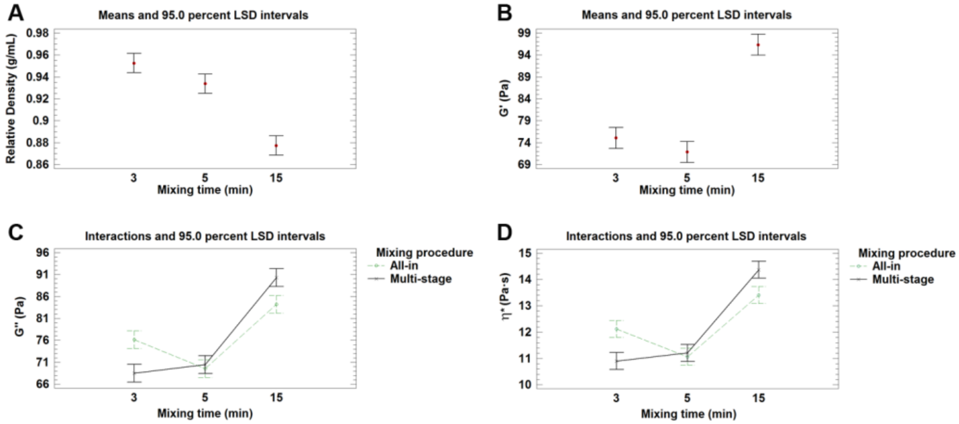

Figure 2. Mean and interactions plots with LSD intervals. A: mean values for the batter relative density according to the mixing time. B: mean values for the elastic moduli (G') according to the mixing time. C: interactions between the mixing time and the mixing procedure for the viscous moduli (G''). D: interactions between the mixing time and the mixing procedure for the complex viscosity (η^*)

Mixing over 3 and 5 min provided similar ($P > 0.05$) G' values. When batters underwent 15 min of mixing, G', G'', and η^* were significantly higher ($P < 0.05$) than for the shorter mixing times for both procedures due to the better developed gluten network that formed after longer mixing times, generating a stiffer batter. Loewe (1993) observed that during batter mixing at ambient or refrigerated temperatures, the viscosity increases due to the development of gluten.

The mixing procedure significantly affected ($P < 0.05$) the G'' and η^* values, although the effects varied relative to the mixing time. When multi-stage mixing was used no significant differences ($P > 0.05$) between G'' and η^* at 3 min or 5 min were observed. However, when the all-in mixing procedure was used, G'' and η^* were significantly higher ($P < 0.05$) after 3 min of mixing than after 5 min. After the longest mixing times (15 min), the all-in method provided batters with lower G'' and η^* than multi-stage mixing.

3.3. Weight loss during baking

No significant interactions (P > 0.05) were observed between the mixing procedure and the mixing time when the weight loss during baking (WL) was studied. The mean plots with their LSD intervals are shown when significant differences in WL were present for one of the factors. There were no significant differences (P < 0.05) in WL relative to the mixing times. Significant differences (P < 0.05) were observed in the WL between the two mixing procedures (Figure 3A). Cakes obtained after the all-in mixing showed significantly (P < 0.05) less WL than those from the multi-stage mixing procedure. The method applied to incorporate the ingredients for mixing significantly affected the dispersion, dilution and hydration of those ingredients. The weight lost from cakes reflects the moisture loss (Sumnu et al. 2005); therefore, all-in mixing might enhance the dilution and hydration of the ingredients, thereby alleviating moisture loss during baking.

3.4. Cake volume and crumb cell structure

The mixing procedure did not interact significantly (P > 0.05) with the mixing time regarding the volume, total cell area, and cell area. The mean plots with their LSD intervals are shown when significant differences were present in the studied parameters for one of the factors. The mean plots with their LSD intervals are shown in Figure 3B, C, D and E for volume, total cell area, and cell area, respectively. The interaction plots with LSD intervals for cell circularity exhibited significant interactions (P < 0.05) between factors and are shown in Figure 3F.

3 min of mixing provided cakes with significantly (P < 0.05) higher volumes and total cell areas. When the mixing time was increased, the cake volume, total cell area, and cell area decreased significantly (P < 0.05) (Figure 3B, C and D).

Although the batters mixed for 15 min had lower relative densities and higher complex viscosities, cakes with lower volumes were obtained. Moreover, these cakes had a crumb structure characterised by significantly (P < 0.05) lower total cell areas, smaller cell areas and higher circularities (Figure 3C, E and F) due to the higher batter viscosity values after longer mixing times (15 min), restricting cake expansion during baking, preventing proper bubble expansion during heating, and generating inadequately developed cell crumb structures.

No significant differences ($P > 0.05$) were observed regarding the volume and total cell area when different mixing procedures were used. Cakes obtained after multi-stage mixing showed a more uniform crumb structure, as characterised by small cell areas and high cell circularities (Figure 3E and F).

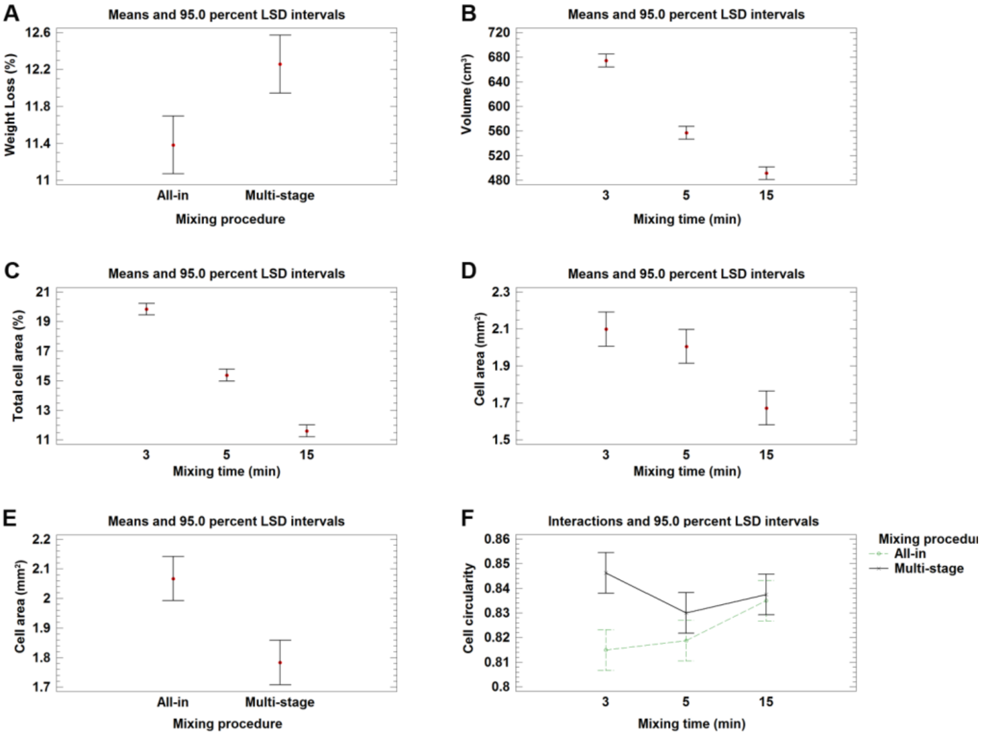

Figure 3. Mean and interactions plots with LSD intervals. A: mean values for the weight loss according to the mixing procedure. B: mean values for the volume according to the mixing time. C: mean values for the total cell area according to the mixing time. D and E: mean values for cell area according to the mixing time and the mixing procedure, respectively. F: interactions between the mixing time and the mixing procedure for cell circularity

3.5. Cake texture

No significant interactions ($P > 0.05$) were observed between the mixing procedure and the mixing time when the cake hardness, springiness and cohesiveness were studied. The mean plots with their LSD intervals are shown when there were significant differences in the hardness, springiness and cohesiveness for one of the factors. Figures 4A, B, C and D show the mean plots with LSD intervals for the hardness, springiness and cohesiveness, respectively.

When the shortest mixing time (3 min) was applied, the cakes exhibited the lowest ($P < 0.05$) hardness values. When the mixing time increased, cakes with significantly ($P < 0.05$) higher hardness values were obtained (Figure 3A). The structure of the cakes depends on air bubble retention, allowing a controlled expansion and maintaining the volume after cooling. Cakes obtained from batters mixed for 3 min had the best qualities (volume, crumb structure and hardness). No significant differences ($P > 0.05$) were observed in hardness when the different mixing procedures were applied.

The cakes obtained from batters mixed for 3 and 5 min had significantly ($P < 0.05$) more springiness than cakes from batters mixed over 15 min (Figure 4B). The decreased springiness was associated with a decrease in the volume and in the total percentage of cells, generating a denser crumb structure. No differences were found for the springiness values between mixing procedures.

The cakes obtained after a 3-min mixing procedure showed the highest cohesiveness values ($P < 0.05$). When the mixing time increased (5 min and 15 min), cakes with lower cohesiveness were obtained (Figure 4C). The all-in mixing procedure provided cakes with significantly ($P < 0.05$) higher cohesiveness than the multi-stage mixing procedure (Figure 4D). The cohesiveness is related to the energy required for the second compression, and provides information related to the density and the energy required to chew the food (Sanz et al. 2009). High cohesiveness values indicate that more energy was required for the second compression.

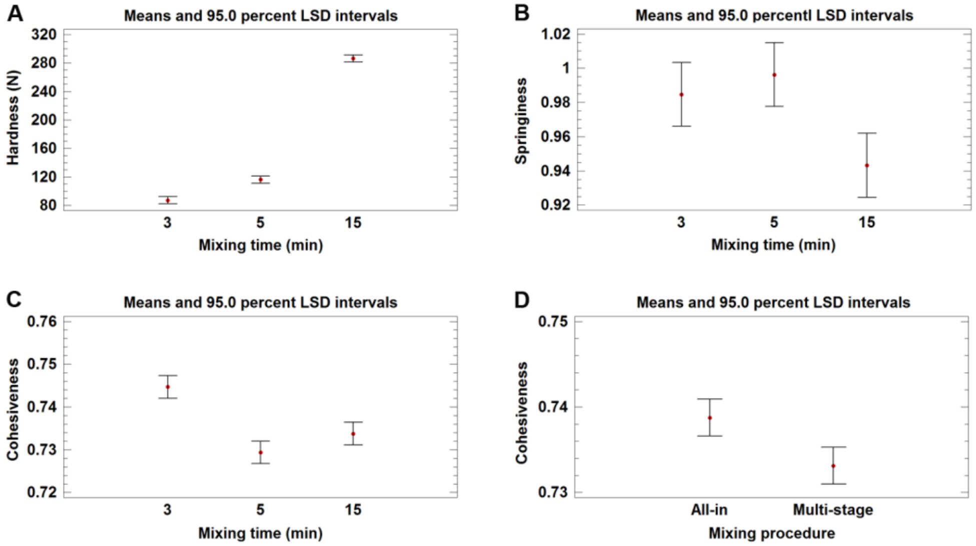

Figure 4. Mean plots with LSD intervals. A: mean values for the hardness according to the mixing time. B: mean values for the springiness according to the mixing time. C and D: mean values for the cohesiveness according to the mixing time and the mixing procedure, respectively

4. Conclusions

A 3-min mixing time is suitable for obtaining high-quality cakes. The cakes obtained after 3 min of mixing showed the highest volume and total cell area, as well as the lowest hardness. When the mixing time was increased, higher bubble occlusion was obtained, but inadequate expansion was observed due to the higher viscosity of these batters. In general, there were no significant differences between the mixing procedures for most of the parameters, including the relative density, volume, total cell area and hardness. Therefore, an all-in mixing procedure can supersede a multi-stage mixing procedure, thereby improving and scaling up the cake manufacturing process with satisfactory results regarding cake quality; even when the cake recipe does not incorporate emulsifiers. The most suitable mixing procedure at a pilot scale should be an all-in mixing procedure with a 3-min duration. This mixing method enables to optimize energy costs and process time.

5. Acknowledgments

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Capítulo 4

Mejora de la formulación de bizcochos con contenido reducido de grasa

Functionality of lipase and emulsifiers in low-fat cakes with inulin

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Abstract

The functional effects of lipase (0.003 and 0.006 g/100 g of flour) and emulsifier (0.5 and 1 g/100 g of flour) on fat-replaced (0%, 50% and 70%) batters and cakes with inulin (0, 7.5 and 10 g/100 g of flour, respectively) were studied. Emulsifier addition significantly lowered the relative density of the batter. Emulsifier incorporation increased the viscoelastic properties of the batter. In contrast, lipase incorporation decreased the degree of system structuring. The evolution of the dynamic moduli and complex viscosity with rising temperatures were studied. Batters with 1 g/100 g emulsifier displayed a significantly lower complex viscosity during heating, resulting in collapsed cakes. Differential scanning calorimetry results revealed that the thermal setting in the control cakes occurred at higher temperatures, and accordingly, greater cake expansion was observed. Cakes with 0.003 g/100 g lipase or 0.5 g/100 g emulsifier displayed volume and crumb cell structure that were similar to those of control cakes. Higher concentrations of both improvers gave rise to cakes with lower volume, higher hardness and lower springiness. During storage time, cakes with lipase displayed lower hardness. Both improvers, at low concentrations, could improve certain physical characteristics, such as crumb structure, of fat-replaced cakes with inulin.

Keywords: cake, emulsifier, fat replacement, inulin, lipase

1. Introduction

Consumers are increasingly balancing health concerns with pleasurable eating, which has given rise to a healthier menu movement in quick- and full-service restaurants. Approximately one-third of the best-selling new foods and beverages introduced in 2010-11 carried a natural claim; one-quarter claimed added nutrients/nutrition, high fibre/whole grain, reduced calories or low-fat/fat-free content (Sloan 2012). A promising way for the food industry to provide advantageous food is to replace fat with dietary fibre (Zahn et al. 2010).

Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of β-(2-1) fructosyl-fructose links (Roberfroid and Delzenne 1998). Inulin-type fructans are plant carbohydrates that, because of the β-(2-1) configuration of the fructosyl-fructose glycosidic linkages, resist digestion in the upper gastrointestinal tract, but they are quantitatively fermented in the colon. Therefore, they undoubtedly form part of the dietary fibre complex and must be labelled as dietary fibre on consumer food products (Roberfroid 2007). Prebiotics are a category of nutritional compounds that are grouped together by their ability to promote the growth of specific beneficial (probiotic) gut bacteria; many dietary fibres, especially soluble fibres, exhibit some prebiotic activity, such as inulin, oligofructose and frutooligosaccharides (Kelly 2008). Inulin appears to modulate a variety of body functions that are associated with health and well-being. Indeed, it has the potential to reduce the risk of developing osteoporosis, acute and chronic inflammation of the bowel, colorectal cancer and some metabolic disorders associated with obesity (Gibson and Delzenne 2008).

From a technical point of view, inulin is easy to use, and it also contributes to the desired taste and texture of baked goods and cereals (Niness 1999). Inulin has been used in several studies to replace fat or improve dietary fibre content in cereal products (Aravind et al. 2012; Devereux et al. 2003; Juszczak et al. 2012; Morris and Morris 2012; Rodríguez-García et al. 2012; Rodríguez-García et al. 2013; Zahn et al. 2010; Ziobro et al. 2013).

Cake batters are complex emulsions of fat or oil in an aqueous phase containing flour, eggs, sugar and minor ingredients (Sahi 1999). The fat in cake batter not only helps the incorporation of air, but it also produces emulsifying properties and holds considerable amounts of liquid to increase and extend cake softness and "shortens", that is, it interrupts the protein particles to break gluten continuity to tenderise the crumbs (Bennion and Bamford 1997). Therefore, fat reduction implies major changes, such as reduced volume and increased crumb firmness.

Emulsifiers are widely used within the cake baking industry to help suspend ingredients, incorporate air and provide stability to batter; their use has simplified cake making methods and enables a greater variety of ingredients to be used (Alava et al. 1999). Several studies conducted on the addition of emulsifier to cakes are found in the literature. As the addition of a lipid-like emulsifier to cake systems can affect both the interfacial and bulk properties of batter, Sahi and Alava (2003) worked to improve the understanding of the influence of emulsifiers on both batter and cake properties to optimise recipe formulation and product quality. Khalil (1998) evaluated the effect of fat replacement by carbohydrate-based fat replacers alone and in combination with an emulsifier on batter, cake characteristics and sensory properties. Kim and Walker (1992) determined the effects of additional emulsifiers and other ingredients, such as various starches and sugar, on batter and cake quality. Moreover, the emulsifier effect has been studied in cake systems where fat and/or sugar were partially replaced. Kumari et al. (2011) conducted a study to establish the effect of replacing hydrogenated fat with sunflower oil, coconut oil, emulsifiers, and hydrocolloids on the rheological, fatty acid profile and quality characteristics of pound cakes.

Other studies have been conducted to develop and apply other improvers to enhance the manufacture and quality characteristics of bakery goods. The lipase enzyme has been used to improve some characteristics of breads, such as volume, texture and shelf-life (Katina et al. 2006; Moayedallaie et al. 2010; Stojceska and Ainsworth 2008). However, few works in the literature report on the use of enzymes in cake making. Lipase has been applied to improve the performance of cakes using the surfactants produced by lipase to replace emulsifier additives. A commercial lipase enzyme was added in a high-ratio cake (Guy and Sahi 2006); the lipase enzyme reduced surface tension and surface viscosity at the air/water interface of the batter. Moreover, the surfactants stabilised the bubbles to produce greater overall expansion, cake-specific volume and fine crumb texture. The replacement of emulsifiers with enzymes will be beneficial for those companies preferring to reduce additives (E-numbers) in their products.

The objective of this work was to improve cake quality by incorporating a commercial emulsifier mix and a lipase into cake batters in which fat was replaced with inulin. A number of rheological properties were studied.

2. Materials and Methods

2.1. Ingredients

The ingredients used in the preparation of the cake batters were golden dawn plain white flour (ADM Milling Ltd., Brentwood CM14 4HG, UK; composition: 13.9 g/100 g moisture and 9.7 g/100 g proteins), pasteurised liquid egg yolk and egg white (Framptons Ltd., Shepton Mallet Somerset BA4 5PD, UK), white granulated sugar (British Sugar plc, Sugar Way, Peterborough PE2 9AY, UK), skimmed long life milk (Tesco, UK), sunflower oil (Olympics Oils Ltd., Liverpool, L24 9GS, UK) and Frutafit® HD (a highly dispersible native inulin, Sensus, Roosendaal, The Netherlands; specifications provided by the supplier: average chain length 8-13, 2 kcal/g, sweetness of 10% compared to sugar (100%)). To achieve the proper dispersion of inulin to act as a fat mimetic, the appropriate amounts of water (from skimmed milk) were added on the basis of manufacturer suggestions, for an inulin-to-water ratio of 1 to 2. The lipase enzyme (TS-E 1367) was supplied by Danisco A/S (Denmark). A commercial emulsifier widely used in current products was chosen: Colco (Aromatic AB, Hertfordshire, SG5 3JH, UK), which is a vegetable all-round alpha-gel emulsifier composed of glycerol monostearate (GMS; E471) and polyglycerol esters of fatty acids (PGE; E475). Sodium bicarbonate (Brunner Mond, Cheshire, CW8 4DT, UK), citric acid (VWR International Ltd.; Poole, BH15 1TD, UK), and salt were also used.

2.2. Batter and cake preparation

The batters were prepared with the following ingredients (quantities given on flour basis: g/100 g of flour): wheat flour 100 g, egg yolk 27 g/100 g, egg white 54 g/100 g, sugar 100 g/100 g, sodium bicarbonate 4 g/100 g, citric acid 3 g/100 g, and salt 1.5 g/100 g. Skimmed milk, sunflower oil and inulin (Frutafit® HD) were also added. Moreover, the effect of lipase addition of 0.003 g/100 g (designated L1) and 0.006 g/100 g (designated L2) and emulsifier addition of 0.5 g/100 g (designated E1) and 1 g/100 g (designated E2) were studied on formulations where fat was replaced at three levels, 0%, 50% and 70% (Table 1). A batter without improvers was also studied (designated 0).

The ingredients were weighed out and allowed to reach a temperature of approximately 20 ºC. The liquid egg and milk were placed in a Hobart N50 mixer (Hobart Manufacturing Company Ltd., London, UK). The enzyme powder with the flour and the other dry ingredients were blended, sieved and added into the mixer. Finally, the oil was placed on the top, and the mixing was performed with a whipping accessory at speed 1 (60 rpm) for 30 s, followed by 60 s at speed 2 (124 rpm) and continued at speed 3 (255 rpm) for 180 s. A number of measurements were conducted on the batters, including relative density, batter rheology, and differential scanning calorimetry analyses. Two replicates of the same formulation were prepared on different days.

The batter was scaled at 300 g and baked in paper cases placed in 400 g bread tins (145 mm x 75 mm at the base) in a Bone Reel oven (Frederick Bone & Co. Ltd., Purley, UK) for 45 min, at a set oven temperature of 180 ºC. Eleven sponge cakes were baked for each formulation in duplicate. The baked products were cooled at room temperature and sealed in polypropylene bags. After 24 hours, the sponge cakes were analysed for their weight, volume, crumb cellular structure, texture, moisture content, and water activity. The last three tests were conducted within 24 h after baking and repeated after 7 days and 14 days of storage at 20 ºC and a relative humidity 65% to study ageing phenomena.

2.3. Relative density of the batter

The relative density (RD) of the batter was measured with a calibrated density cup of known volume. After mixing, the cup was filled with batter just up to brim level, and then the filled cup was weighed.

Table 1. Formulations of the different batters and cakes (quantities given in flour basis: g/100g of flour)

Fat replacement-Improver*	0-0	0-L1	0-L2	0-E1	0-E2	50-0	50-L1	50-L2	50-E1	50-E2	70-0	70-L1	70-L2	70-E1	70-E2
Skimmed milk	50.00	50.00	50.00	50.00	50.00	66.50	66.50	66.50	66.50	66.50	72.00	72.00	72.00	72.00	72.00
Sunflower oil	46.00	46.00	46.00	46.00	46.00	22.00	22.00	22.00	22.00	22.00	14.00	14.00	14.00	14.00	14.00
Frutafit® HD	0.00	0.00	0.00	0.00	0.00	7.50	7.50	7.50	7.50	7.50	10.00	10.00	10.00	10.00	10.00
Lipase	0.00	0.003	0.006	0.00	0.00	0.00	0.003	0.006	0.00	0.00	0.00	0.003	0.006	0.00	0.00
Emulsifier	0.00	0.00	0.00	0.50	1.00	0.00	0.00	0.00	0.50	1.00	0.00	0.00	0.00	0.50	1.00

*The first digit corresponds to the fat replacement level (%): 0 (0%), 50 (50%) or 70 (70%), and the second digit corresponds to the improver incorporation (g/100g flour):0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

The same procedure was conducted with water as the filler. The RD was determined gravimetrically by dividing the weight of this known volume of batter by the weight of an equal volume of water. Measurements were performed in duplicate.

2.4. Rheological properties

The rheological properties of the batters were measured using a rheometer (Rheometrics ARES model, TA Instruments Ltd., Crawley, UK). The measurements were taken straight after mixing, using a 50 mm diameter parallel plate. The strain was selected by performing a strain sweep at a frequency of 1 Hz, and a strain of 0.1 was chosen as corresponding to the region in which the batter displayed linear viscoelastic behaviour. Dynamic oscillatory frequency tests were performed in duplicate by performing a frequency sweep at a frequency range from 0.1 to 20 Hz. The viscoelastic functions monitored (Rheometric scientific software; version V6.4.3.) were the elastic (G‘) and viscous (G”) moduli. Measurements were performed in duplicate.

To simulate the effect of heating on the batter structure, temperature sweeps were performed from 30 ºC to 100 ºC at a heating rate of 5 ºC/min and a strain amplitude of 0.1. Two replicates from different batches of each formula were measured. The viscoelastic functions monitored were the elastic (G‘) and viscous (G”) moduli and the batter complex viscosity (η^*). Measurements were performed in duplicate.

2.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermographs were produced using a Perkin-Elmer DSC Pyris 1 (Perkin Elmer, Norwak, Connecticut, USA). Approximately 40 mg of each batter mix was weighed into stainless-steel pans capable of remaining intact up to medium pressures. An empty sealed pan was used as a reference. The samples were scanned from 25 ºC to 130 ºC, and the heating rate was 10 ºC/min. DSC data were evaluated from the thermograms to calculate the transition temperatures, onset (T_o), peak (T_p) and conclusion (T_c), and enthalpy (ΔH) using Perkin Elmer Pyris Version 3.81 software. Measurements were performed in triplicate.

2.6. Cake volume

Cake volume was measured with a BVM-L 370 (Tex-Vol Instruments AB, Perten Instruments, Sweden), and the measurements were analysed and stored in a database. Three cakes from each batter were measured, and all the formulations were repeated with a second set of batters.

2.7. Crumb cell structure

Cakes were cut vertically in 4 slices (15 mm thick) using a Graef slicing machine (Gebr. GRAEF GmbH & Co. KG, Arnsberg, Germany). Two slices were scanned in a C-Cell (CCFRA Technology Ltd., Chipping Campden, UK) using the standard method for collecting images. The scanned images were analysed using the software ImageJ (National Institutes of Health, Bethesda, Maryland, USA). First, the image was split in colour channels, and then the contrast was enhanced. Finally, the image was binarised after applying a grey-scale threshold. The total cell area within the crumb (%), cell area (mm2) and cell circularity were analysed; the total cell area within the crumb was calculated as (total area of cells / total crumb area) x 100. Three cakes from each batter were measured, and all the formulations were repeated with a second set of batters. Thus, the data were obtained by measuring cells in twelve different images for each formulation.

2.8. Cake texture

Texture profile analysis (TPA) was performed using a TA-TXT plus Texture Analyser (Stable Micro System, Godalming, UK) using the Texture Exponent 32 software (version 4.0.8.0, Stable Microsystems, Godalming, UK). Measurements were performed on three cakes from each batch, and all the formulations were repeated with a second set of batters. The cakes were cut to obtain four slices (15 mm thick) from the central regions of each cake with a Graef slicing machine (Gebr. GRAEF GmbH & Co. KG, Arnsberg, Germany). Texture profile analysis was performed using a test speed of 5 mm s-1 with a strain of 25% of the original height and a 1 s interval between the two compression cycles. A trigger force of 5 g was used. The double compression test was performed with a steel disc probe (45 mm diameter). The parameters obtained from the curves were hardness, chewiness, springiness, and cohesiveness.

2.9. Moisture and water activity

Crumb moisture was measured using an oven method (Bs oven 250 Suze 1 Gallenkamp, Weiss Technik UK, Leicestershire, UK) at 103 ºC, from 12 to 20 h, using samples (5 g) cut from the centre of 3 cakes. Water activity (a_w) was determined using an AquaLAB 4TE (Decagon Devices Inc., Pullman, USA). Measurements were performed in triplicate, and all the formulations were repeated with a second set of batters.

2.10. Statistical data analysis

A categorical multifactorial experimental design with two factors, improver incorporation and level of fat replacement, was used for the samples analysed 24 h after baking. For the study of the effects of storage time on cakes, a design with three factors was used: improver incorporation, level of fat replacement and time of storage. Analysis of variance (ANOVA) was performed on the data using Statgraphics Centurion XVI version 16.1.11 software package (Statistical graph Co., Rockville, MD, USA). The Least Significant Difference (LSD) Fisher's test was used to evaluate the mean values differences ($P < 0.05$).

3. Results and Discussion

3.1. Relative density of the batter

The relative density (RD) of batter is a very important physical property as it is related to the number of air bubbles incorporated into cake batter (Lee et al. 2005). No significant interactions ($P > 0.05$) were observed between improver incorporation and the fat replacement level for the RD of the batter. The mean plots with LSD intervals are shown in Figure 1A and B. L1 and L2 incorporation did not significantly affect ($P > 0.05$) the RD of the batter as compared with batter with no improver incorporated (0). This suggests that the enzyme was either not active, or enzyme action did not create a sufficient amount of surface-active material at that stage; thus, the lipase was not able to influence the batter's air occlusion and air-holding capacity. In contrast, as emulsifier incorporation increased, E1 and E2, the RD of the batter significantly decreased ($P < 0.05$). Low RD is associated with good batter aeration. Therefore, as expected, the emulsifier promoted the incorporation of air into batter. These results are in agreement with Khalil (1998), who found that improvement in specific density might be due to the effect of emulsifier on enhancing the aeration capacity of batters. Fat replacement did not significantly affect the RD of the batter ($P > 0.05$). Therefore, reduced oil content does not significantly influence air occlusion during mixing, unlike emulsifier addition.

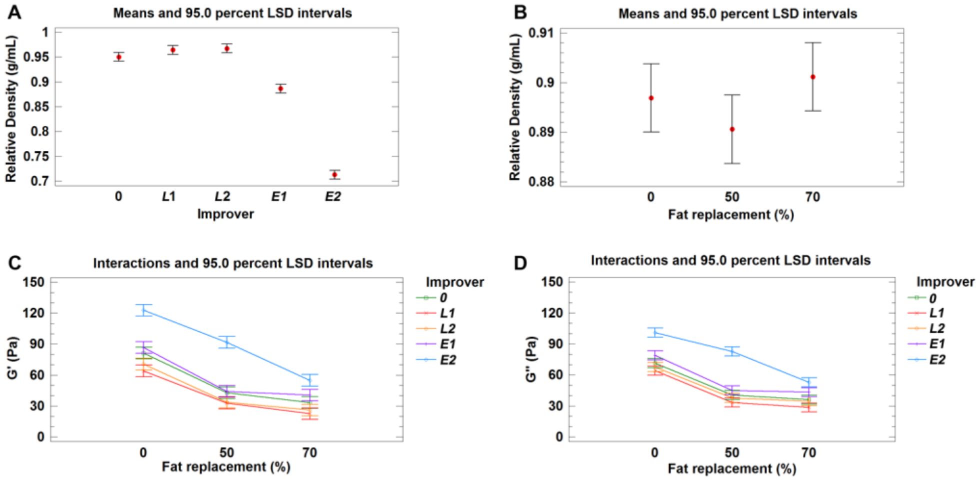

Figure 1. Mean and interactions plots with LSD intervals. A and B: mean values for the batter relative density according to the improver incorporation and the fat replacement level, respectively; C and D: interactions between the fat replacement level and the improver incorporation for batter's G' and G", respectively. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

3.2. Rheological properties

The interaction plots with the LSD intervals for the elastic (G') and viscous (G") moduli, where significant interactions ($P < 0.05$) between factors were observed, are shown in Figure 1C and D. These figures show the G' and G" values at 1 Hz and 25 ºC.

L1 and L2 displayed a slight decrease in the G' and G" values, as compared with batters with no improver incorporated (0). These results indicate a lower degree of system structuring with lipase incorporation, suggesting that batter becomes more fluid. Regarding emulsifier addition, a general trend was observed; G' and G" increased when the emulsifier was incorporated, and this increase was significant ($P < 0.05$) when E2 was incorporated, suggesting that batter was stiffer. There are a number of reasons for such increases. Emulsifiers are known to bind water. Increased water binding may reduce the availability of free recipe water and promote increased batter consistency. Batter consistency may also be influenced by stronger, or a greater number, of interactions between the emulsifier and other recipe components (Sahi 1999). Moreover, changes in batter rheology can be related to food aeration (Campbell and Mougeot 1999). This suggests that a great incorporation of air into the batter not only lowers its density but may also help to reduce the fluidity of the batter (Sahi 1999). Other researchers have reported similar results when incorporating an emulsifier into a batter formulation (Alava et al. 1999; Sahi 1999; Sahi and Alava 2003).

Fat replacement by inulin resulted in a significant decrease ($P < 0.05$) in G' and G". When fat was replaced, inulin and an extra aqueous component were added, which could influence G' and G" in fat-replaced batters because of a dilution effect. In their review of dynamic rheological properties of wheat proteins and flour dough, Song and Zheng (2007) reported that water plays an important role in determining the viscoelastic properties of dough; both G' and G" lower as water content increases.

Rheological changes occurring during heating were also studied. The evolution of the elastic moduli (G'), the viscous moduli (G"), and complex viscosity (η^*) with increasing temperatures is shown in Figure 2 for the different batters. Initially, within the 30-45 ºC temperature range, both viscoelastic moduli and complex viscosity generally remained virtually unchanged. In contrast, batters with E2 had higher G', G" and η^* values at the beginning of the test. As of 50 ºC, all the batters had higher G', G" and η^*. The curves displayed a maximum, and then G' and G" dropped, and η^* began to also lower to 80 ºC. This trend might be attributed to CO_2 formation in batter, diffusion into occluded air cells, and expansion, which reduced the RD of the batter. From the beginning of the slope (T = 50 ºC), G' presented slightly higher values than G" for all the batters, indicating a more elastic behaviour. Batters with no improver added (0) displayed higher G', G" and η^* values, followed by the batters with L1 and E1. High batter complex viscosity is important because it slows down the rate of gas diffusion and allows gas retention during the early baking stage (Gómez et al. 2007). When E2 was added, the three parameters –G', G" and η^*– decreased with increasing temperature; when the batter complex viscosity is too low, bubbles rise to the surface and are lost to the atmosphere (Matsakidou et al. 2010).

A dramatic increase in complex viscosity and both moduli was observed at around 80-90 ºC in all the batters. This change in the viscoelastic properties can be attributed to starch gelatinisation and protein denaturation, as confirmed by DSC (T_o of around 86-91.8 ºC). Wilderjans et al. (2008) also reported a rapid increase in viscosity from the combination of starch gelatinisation (temperature range 80-95 ºC) and egg protein denaturation (temperature range 70-100 ºC) in the pound cake system.

3.3. Differential Scanning Calorimetry

Significant interactions ($P < 0.05$) were found between the incorporation of an improver and the fat replacement level when the batter's thermal properties were studied in terms of onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and the enthalpy process (ΔH) (Figure 3A, B, C, and D).

The control batter (0-0) displayed the highest thermal parameters values (T_o: 90.35 ºC, T_p: 96.2 ºC, and T_c: 104 ºC). In line with these results, a dramatic increase in the elastic modulus was observed at approximately 80-90 ºC (Figure 2); this increase could be attributed to starch gelatinisation. During this process, starch granules take up water and swell progressively, which, in turn, leads to increased system rigidity, as manifested by an increased storage modulus (G') (Lee et al. 2005). These results also agree with other studies (Wilderjans et al. 2010) where the temperature ranges for starch gelatinisation and egg protein denaturation during baking were determined at 85

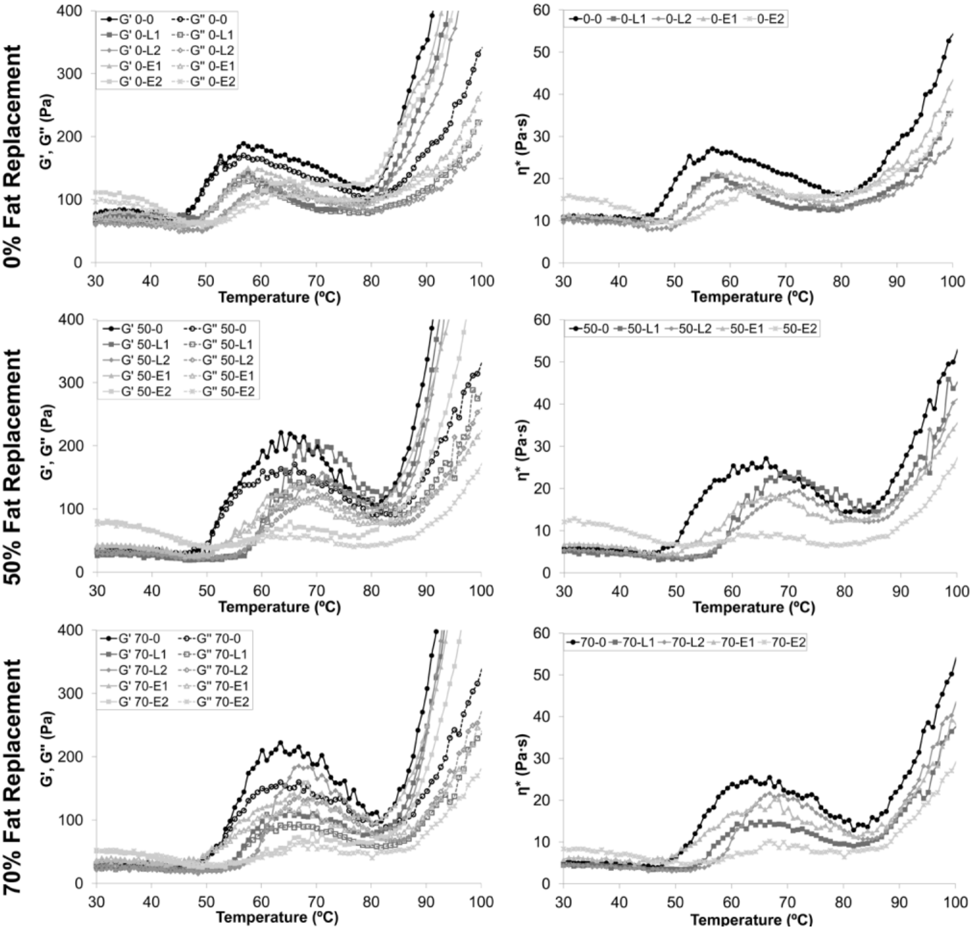

Figure 2. Evolution of the viscoelastic functions with temperature. 1st column: evolution of the elastic moduli (G', solid symbol and continues line) and the viscous moduli (G", open symbol and discontinue line) according to the improver incorporation. 2nd column: evolution of the complex viscosity (η*) according to the improver incorporation. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g).

to 97 ºC with DSC for simplified pound cake recipes. Lipase incorporation (L1 and L2) in batters with 0% of fat replacement significantly decreased ($P < 0.05$) the thermal parameters (T_o, T_p, and T_c) as compared with batters with no improver (0). However, when 50% and 70% of fat was replaced in batters with L1 and L2, similar thermal parameters values to the control batter were observed (0).

Emulsifier incorporation and fat replacement were the most influential factors on the batter's thermal properties. Emulsifier addition at both levels, E1 and E2, had the same effect on batter T_o, T_p, and T_c, at all the fat replacement levels studied. When 50% of fat was replaced with inulin, the T_o, T_p, and T_c of the batters with emulsifier were shifted to higher values than in all the other batters. These results could be related with the fact that emulsifier addition is known to delay the transport of water into the starch granule because of the formation of complexes between the lipid and amylose during baking (Sakiyan et al. 2004). However, for fat replacement at 70%, no significant differences ($P > 0.05$) were found among the batters.

When fat was replaced, the thermal parameters (T_o, T_p, and T_c) decreased significantly ($P < 0.05$). During heating, oil melted and coated starch granules, as observed previously by Rodríguez-García et al. (2012). When fat was replaced, fewer interactions took place between the oil and the starch granules surfaces. Thus, the starch granules could be easier hydrated because they were not surrounded by oil. Moreover, the increase in the aqueous component, due to fat replacement by inulin, allowed starch to swell at lower temperatures.

The DSC enthalpy (ΔH) represents the net sum of all the endothermic processes that take place during heating (Ratnayake et al. 2009). The control batter (0-0) had a low ΔH value, which rose significantly ($P < 0.05$) with increasing fat replacement. The batters with an emulsifier, E1 and E2, displayed low ΔH values in fat-replaced batters, mainly when 50% of fat was replaced.

3.4. Cake volume and crumb cell structure

Significant interactions ($P < 0.05$) were found between improver incorporation and the fat replacement level for the cake volume, total cell area and cell circularity (Figure 4A, B, and E). No significant interactions ($P < 0.05$) were observed for cell area when different types of improvers and fat replacement levels were used (Figure 4C and D).

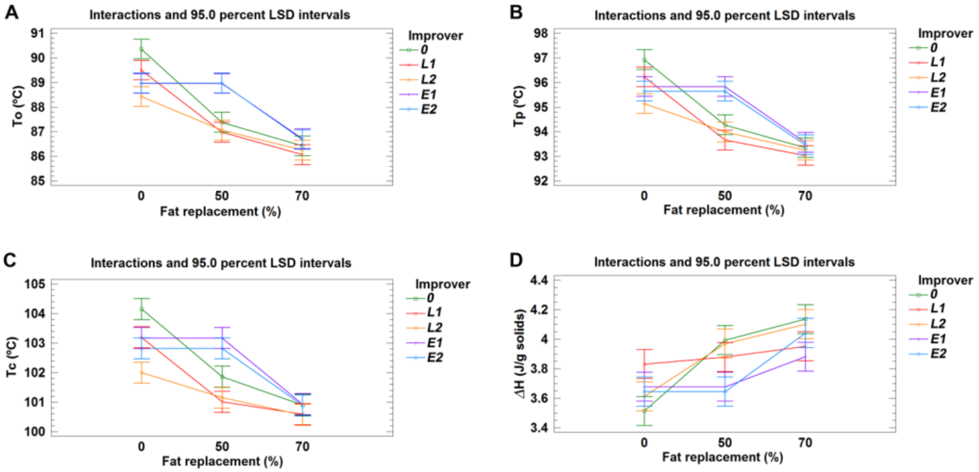

Figure 3. Interactions plots with LSD intervals. A, B, C, and D: interactions between the fat replacement level and the improver incorporation for the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and ΔH of batters, respectively. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

Cakes with no improvers (0) displayed high volume values, and a crumb structure was characterised by an even distribution of small cells and by the presence of continuous air channels (Figure 5). High viscosities have been found to confer batters a greater capacity to retain the expanding air nuclei during heating and to resist the settling of starch granules, thereby improving both cake volume and crumb grain (Baixauli et al. 2008). Moreover, the formation of continuous air channels has proven to be essential in maintaining cake volume on cooling once removed from the oven (Matsakidou et al. 2010).

The addition of L1 resulted in cake volumes similar to cakes with no added improver. The cell crumb structure was also similar to that of the cake with no improvers, but there were fewer continuous channels (Figure 5). These results may be related to the presence of an extra stabiliser at the bubble interface, which could prevent the collision and coalescence of neighbouring bubbles. Nevertheless, cakes with L2 had a significantly ($P < 0.05$) reduced cake volume; similar results have been provided by Guy and Sahi (2006) in high-ratio layer cakes. A crumb structure characterised by higher total cell area (Figure 4B), cells with bigger areas (Figure 4C), and lower circularity values (Figure 4E) were observed.

Cakes with E1 displayed similar volume and crumb structure as cakes with L1 and control cakes. In sponge cake batters, the air incorporated into the aqueous phase is stabilised by surface-active components, predominantly by proteins and perhaps by some contribution from polar lipids that are naturally present in the egg and the flour. When a sufficient concentration of emulsifier is present to completely replace the protein molecules, the bubbles decrease in size and have a more uniform appearance (Sahi and Alava 2003). Therefore, the crumb structure displayed an improvement when E1 was incorporated.

However, the addition of E2 led to a dramatic decrease in cake volume (Figure 4A and 6). Although it is feasible to expect that a lower RD of batter might result in greater cake volume, the opposite result was obtained. This behaviour indicates that the final cake volume depends not only on the quantity of air that has been occluded in the batter during mixing but also on the amount of air that can be retained in batter during baking. Initially, a large amount of air was incorporated into the batter, but, during heating, the batter rheology was characterised by a marked decrease in G', G'' and η^*; low batter viscosities during the heating process enhanced bubble migration and loss of air cells before the batter set. As a result, low-volume cakes with a more compact crumb, low total cell area and small cell areas (Figure 4B and C) were obtained.

Fat replacement at both levels (50% and 70%) caused cake volume to significantly ($P < 0.05$) decrease (Figure 4A). When 50% of fat was replaced, the crumb structure was similar to that of full-fat cakes (0%) in terms of total cell area (Figure 4B) and individual cell areas (Figure 4D). In contrast, when 70% of fat was replaced, the total cell area and cell area values significantly ($P < 0.05$) increased (Figure 4B and D). These results may be related to inulin and water incorporation, which lowered the viscoelastic properties values and may also modify the setting mechanism.

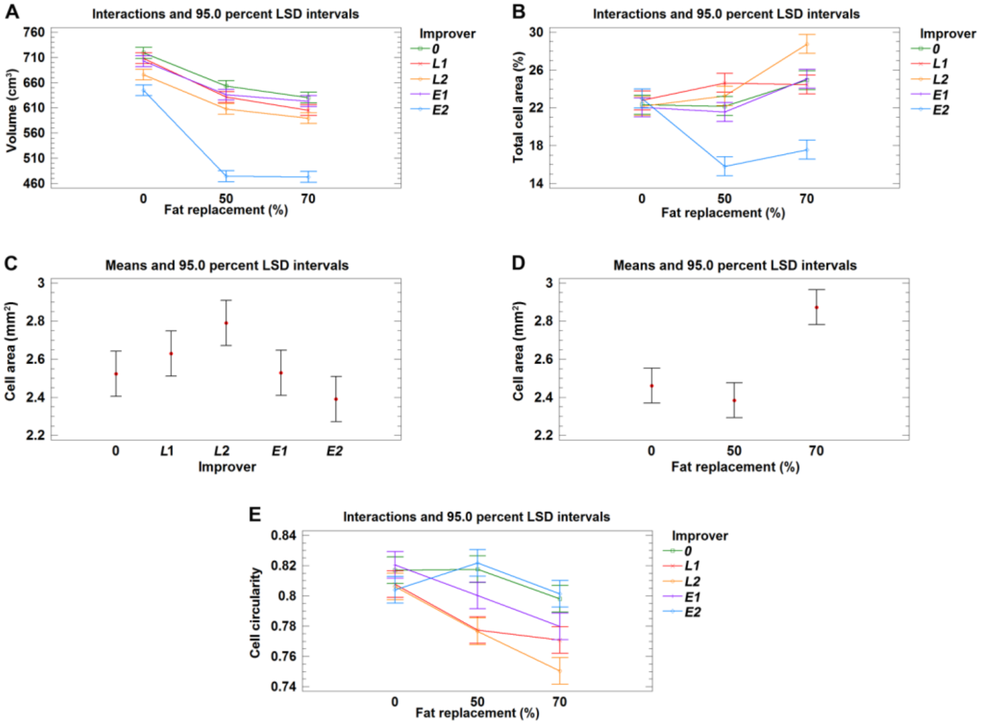

Figure 4. Interactions and mean plots with LSD intervals. A: interactions between the fat replacement level and the improver incorporation for cake volume. B: interactions between the fat replacement level and the improver incorporation for total cell area within the crumb. C and D: mean values for the cell area according to the improver incorporation and the fat replacement level, respectively. E: interactions between the fat replacement level and the improver incorporation for the circularity of cells. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

3.5. Texture profile analysis

The texture results for hardness, chewiness, springiness and cohesiveness are shown in Figure 6A, B C, D, and E. In general, cakes lipase addition (L1 and L2) and E1 incorporation resulted in similar hardness values to the control cake (0). However, in cakes with E2, when 50% and 70% of fat was replaced, hardness increased significantly ($P < 0.05$) (Figure 6A). As expected, cake hardness was highly sensitive to the volume and total cell area within the crumb. A drop in both parameters characterised the macrostructure of these cakes. Chewiness displayed a similar trend as hardness (Figure 6B).

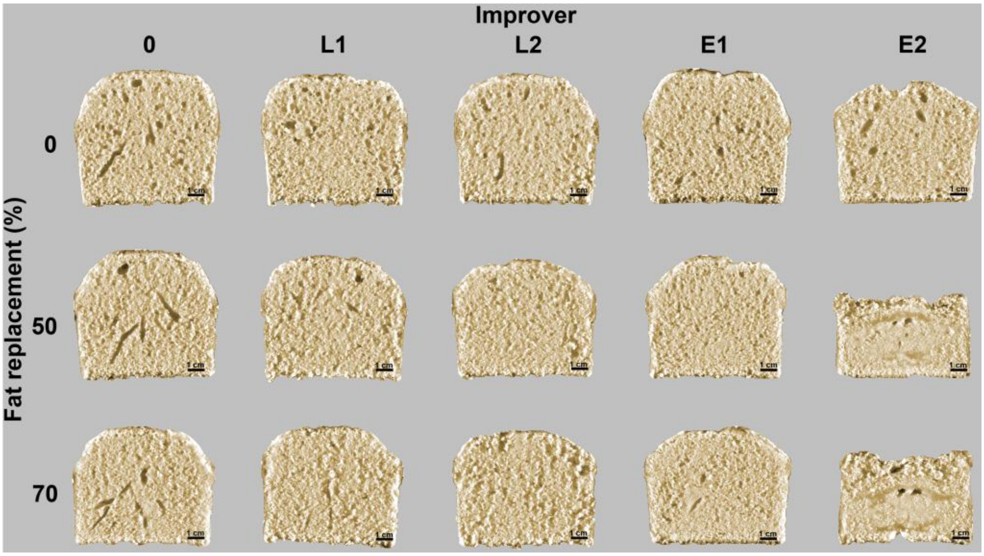

Figure 5 Scanned cake crumbs. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

Figure 6C and D show the mean plots with the LSD intervals for springiness, in which no significant interactions ($P < 0.05$) were observed between improver addition and the fat replacement level. Springiness decreased significantly ($P < 0.05$) as lipase was added (L1 and L2) as compared with control cake springiness (0). When E2 was incorporated, springiness decreased markedly as compared with cakes with no improver and those with E1. Springiness is a measure of the ability of a cake to recover after compression. Other authors have also observed a decrease in springiness with emulsifier addition (Sahi and Alava 2003); the use of emulsifiers is known to reduce the strength of interactions between starch and protein fractions of flour in bakery systems and can lead to a rather crumbly product if applied in excess. Moreover, the decrease in springiness was associated with a decrease in the volume and in the total percentage of cells and, therefore, with a denser crumb structure. Springiness was not significantly affected ($P > 0.05$) by fat replacement.

The interaction plots with the LSD intervals for cohesiveness, where significant interactions ($P < 0.05$) between factors were observed, are shown in Figure 6E. In general, improver addition, L1, L2 and E1, diminish cohesiveness as compared with cakes with no improver (0). However, when adding E2 in cakes with fat replacements of 50% and 70%, the cohesiveness increased significantly ($P < 0.05$). Moreover, the greater the level of fat replacement was, the higher cohesiveness was. These results indicate that greater energy is required during the second compression.

3.6. Influence of storage time on hardness, moisture, and a_w

The influence of storage time on cake properties was also evaluated (data not shown). The addition of E2 was not analysed because of the increased hardness observed after 24 h.

Hardness increased significantly ($P < 0.05$) with storage time. The samples with lipase displayed the lowest hardness values after 7 and 14 days of storage. Stojceska and Ainsworth (2008) reported that, on one hand, lipases modify the interaction between the flour lipids and gluten. On the other hand, the increased level of monoglycerides that forms the amylose-lipid complex plays an important role in retarding the starch retrogradation process and staling of breads. Cakes with E1 displayed higher hardness than control cake at the end of the storage time. Fat-replaced cakes had higher hardness during storage than full fat cakes.

Moisture and aw decreased significantly ($P < 0.05$) during storage time. Those cakes with lipase displayed significantly ($P< 0.05$) higher moisture and aw values throughout the storage time than the control cakes and the cake with an emulsifier. The cakes with E1 displayed a similar aw and moisture as the control cake. Fat-replaced cakes displayed higher hardness, moisture and aw after storage than full-fat cakes. On the one hand, the presence of oil shortened the gluten structure, and thus, its substitution resulted in harder crumbs. On the other hand, these results can be related to the high affinity of fibre to water. These observations are related to the presence of moulds on day 14 in cakes with fat replacement and lipase addition.

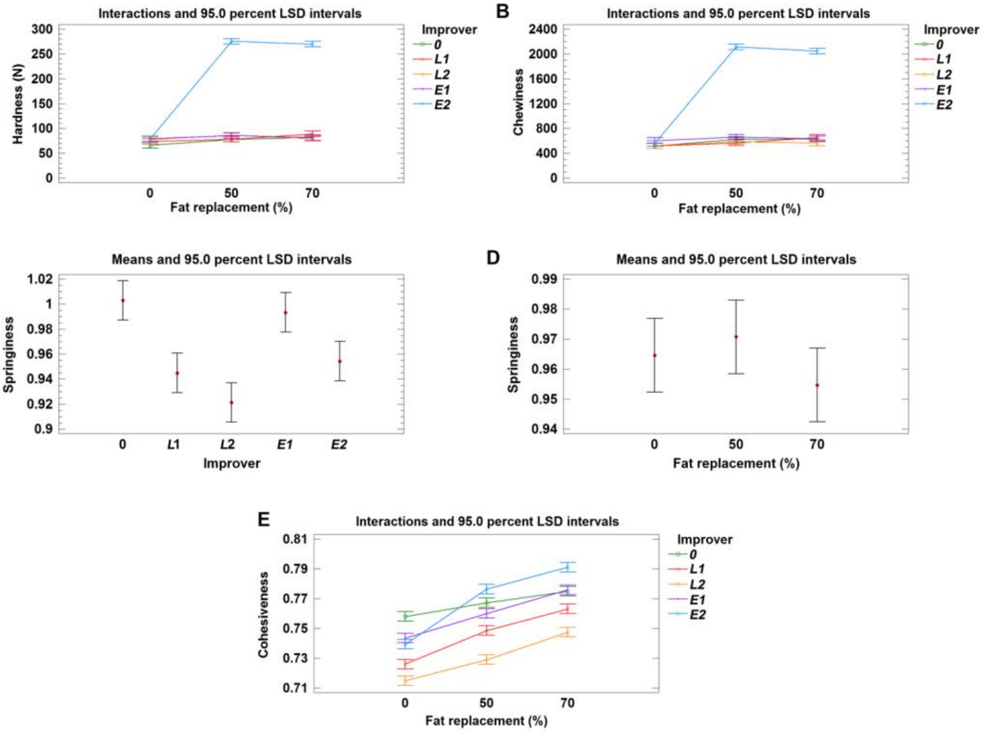

Figure 6. Interactions and mean plots with LSD intervals. A and B: interactions between the fat replacement level and the improver incorporation for hardness and chewiness, respectively. C and D: mean values for springiness according to the improver incorporation and the fat replacement level, respectively. E: interactions between the fat replacement level and the improver incorporation for cohesiveness. Improver incorporation (g/100 g of flour): 0 (none), L1 (lipase 0.003 g/100 g), L2 (lipase 0.006 g/100 g), E1 (emulsifier 0.5 g/100 g), E2 (emulsifier 1 g/100 g)

4. Conclusions

The evaluation of the utility of lipase and emulsifier as improvers for fat-replaced cakes has focused on the physical effects on cakes before, during and after baking compared with those of control cakes. The viscoelastic properties measurements indicate that lipase incorporation reduces the degree of system structuring, whereas emulsifier addition increases batter consistency. However, during heating, batters with the lowest concentrations of both improvers presented similar trends as control batter with high complex viscosity values; the observations made of the cakes baked with these batters indicate that surfactant-stabilised bubbles confer greater overall expansion and even cell distribution within the crumb. As a result, fat-replaced cakes with 0.003 g/100 g of lipase and 0.5 g/100 g of emulsifier provide improved cake crumb with a more homogeneous cell structure. Moreover, 0.5 g/100 g emulsifier addition resulted in cakes similar to the control cakes as far as physical properties such as volume and texture. Those cakes with 0.003 g/100 g of lipase displayed a better texture profile during storage. Therefore, good-quality cakes with 50% and 70% fat replacement can be obtained using lipase or emulsifier at low levels.

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Capítulo 5

Reemplazo de grasa por inulina en galletas

Effect of fat replacement by inulin on textural and structural properties of short dough biscuits

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Abstract

The aim of this study was to evaluate the effects of inulin as fat replacer on short dough biscuits and their corresponding doughs. A control formulation, with no replacement, and four formulations in which 10%, 20%, 30%, and 40% of shortening was replaced by inulin were studied. In the dough, shortening was observed surrounding flour components. At higher fat replacement levels, flour was more available for hydration leading to significant ($P < 0.05$) harder doughs: from 2.76 (0.12) N in 10% fat-replaced biscuits to 5.81 (1.56) N in 30% fat-replaced ones. Biscuit structure was more continuous than dough structure. A continuous fat layer coated the matrix surface, where starch granules were embedded. In general, weight loss during baking and water activity decreased significantly ($P < 0.05$) as fat replacement increased. Biscuit dimensions and aeration decreased when fat replacement increased, e.g., width gain was +1.20 mm in 10% fat-replaced biscuits and only +0.32 mm in 40% fat-replaced ones. Panellist found biscuits with 20% of fat replacement slightly harder than control biscuits. It can be concluded that shortening may be partially replaced, up to 20%, with inulin. These low fat biscuits are similar than the control biscuits, and they can have additional health benefits derived from inulin presence.

Keywords: short dough biscuit, shortening, inulin, microstructure, texture

1. Introduction

Biscuits are one of the most popular bakery items consumed nearly by all levels of society; this is mainly due to its ready to eat nature, good nutritional quality, and availability in different varieties and affordable cost (Sudha et al. 2007b). Among cereal-based products, biscuits are characterized by their enrichment with two major ingredients, sugars and fats, and by their low final water content (1-5%) (Chevallier et al. 2000). Fat functionality is very versatile in baked products; its major functions are giving shortening, richness, and tenderness to improve flavour and mouthfeel. Shortening induces a number of desirable functions in bakery food; these are as follows: tenderness and texture, mouthfeel, structural integrity, lubrication, incorporation of air, heat transfer, and extended shelf life (Ghotra et al. 2002). However, high fat intake is associated with increased risk for obesity and some types of cancer, and saturated fat intake is associated with high blood cholesterol and coronary disease (Akoh 1998). Consumers have become increasingly aware of the link between diet and health; hence, food manufacturers have responded to consumer demands by introducing low-fat foods into the market (Noronha et al. 2008). However, it is not possible to eliminate fats and oils without drastically altering the product's original integrity, although it is feasible to partly replace fat with other ingredients such as dietary fibre (Grigelmo-Miguel et al. 2001).

Inulin is a natural dietary fibre derived from chicory root, garlic, wheat, bananas, and artichokes, and so it has always been part of the human diet (Niness 1999). Chemically, inulin is a carbohydrate built up from β(2,1)-linked fructosyl residues mostly ending with a glucose residue (Meyer et al. 2011). Inulin possesses several nutritional and functional properties that may be used to formulate innovative healthy foods for today's consumers (Akalin and Erisir 2008). Not only the dietary fibre properties of inulin are important but also the prebiotic properties, which are linked to a variety of beneficial physiological effects as improving bowel habits, increasing calcium absorption, lowering of serum lipids, a positive effect on feeling of satiety, and stimulating the immune system (Meyer et al. 2011). Furthermore, it affords other interesting technological properties such as acting as a low-calorie sweetener, fat substitute or texture agent (Bayarri et al. 2011).

At present, inulin has been used as a fat replacer in a wide variety of products. Franck

(2002) described inulin uses in several products as table spread, butter-like products, dairy spreads, cream cheeses and processes cheeses, frozen desserts, meat products, sauces, and soups. Mendoza et al. (2001) achieved a fat replacement of 50-60% in dry sausages by adding inulin. The influence of inulin with different average chain lengths in perceived texture of low-fat dairy desserts was evaluated by Bayarri et al. (2011). Moreover, several studies dealing with the addition of inulin or other dietary fibres as fat replacers have been conducted to evaluate physical, chemical, and sensory properties of biscuits. Zoulias et al. (2002) used four types of fat mimetics – polydextrose, maltodextrine, inulin, and a blend of microparticulate whey proteins and emulsifiers– to partially replace fat in sugar free cookies. A fat replacement up to 50% was achieved using the inulin fat mimetic, the maltodextrine, or the blend of whey proteins with emulsifiers. However, the resulting cookies were hard, brittle, and did not expand properly after baking. Sudha et al. (2007a) studied the effect of fat replacement with either maltodextrine or polydextrose on rheology of biscuit dough and on the quality of biscuits. They observed that when fat is reduced in a biscuit formulation, the resultant dough became hard, but biscuit texture improved significantly when fat was replaced with maltodextrine.

Different studies have been carried out in order to understand the structure development of biscuits during their elaboration. Burt and Fearn (1983) conducted a simple stereological analysis system in order to obtain quantitative information on the distribution of the major components of a biscuit at different locations within the structure. They employed staining and polarized light techniques to identify each component. Furthermore, several structural studies have been conducted to characterize dough and biscuit structure at a macroscopic level (dimensions, bulk structure) and at microscopic level (starch damage, protein aggregates; Chevallier et al. 2000). Moreover, Chevallier et al. (2002) determined the changes that occur during baking of dough components (starch, protein, sugar, and water) by monitoring with a video camera the variations in their sizes and measuring moisture content, insoluble protein fraction, starch damage, and oligosaccharides. Moreover, changes occurring in the structure of doughs and biscuits during the manufacture were studied using a fixation protocol, microtome sectioning, staining techniques, and microscopical examination under bright field and polarized light (Flint et al. 1970).

However, little research has been aimed to study microstructural changes when fat is reduced or replaced. Pareyt et al. (2009) reported on the macro- and microscopic changes occurring when sugar and fat levels of a sugar-snap cookie recipe were altered. They studied the macroscopic changes (dimensions of the baked cookie), texture, and microscopic changes (porosity, mean cell size, and others with X-ray microfocus computed tomography (μCT), but they did not show visualization of the interaction of components in details. Baltsavias et al. (1999) examined the fat distribution in biscuits by diffusion experiments using a fat-soluble stain sprinkled onto the surface of the biscuits during 7 months.

The objective of the present study is to investigate dough and biscuit microstructure by complementary microscopic techniques (CLSM and cryo-SEM) to study the feasibility of the use of inulin as fat replacer on short dough biscuits. Microstructural, textural, and physical changes are evaluated for a better understanding of the product structure and properties.

2. Materials and methods

2.1. Ingredients

Five formulations were prepared using the same quantity of all the ingredients except shortening and inulin (Table 1). Doughs (D) and biscuits (B) formulations were named depending on the shortening/inulin proportions, as follows: 100:0 (D0/B0), 90:10 (D10/B10), 80:20 (D20/B20), 70:30 (D30/B30), and 60:40 (D40/B40).

The ingredients used in dough preparation were: soft wheat flour suitable for biscuits (Belenguer, S.A., Valencia, Spain; composition data provided by the supplier: 11% protein, 0.6% ash; alveograph parameters P/L00.27, where P is the maximum pressure required and L is the extensibility, and W0134, where W is the baking strength of the dough), shortening (78% fat, St. Auvent, Vandemoortele France), inulin Frutafit® HD (Sensus, The Netherlands, Europe; specifications provided by the supplier: average chain length, 8-13, 2 kcal/g, sweetness of 10% compared to sucrose (100%)), sugar (Azucarera Ebro, Madrid, Spain), milk powder (Central Lechera Asturiana, Peñasanta, Spain), salt, sodium bicarbonate (A. Martínez, Cheste, Spain), ammonium hydrogen carbonate (Panreac Quimica, Barcelona, Spain), and tap water.

Table 1. Formulations of the different dough (D) and biscuits (B) (percentage given in dough basis)

	D0/B0	D10/B10	D20/B20	D30/B30	D40/B40
Flour	50.00	50.00	50.00	50.00	50.00
Shortening	30.00	27.00	24.00	21.00	18.00
Frutafit® HD	0.00	3.00	6.00	9.00	12.00
Sugar	15.00	15.00	15.00	15.00	15.00
Milk powder	0.30	0.30	0.30	0.30	0.30
Salt	0.10	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Ammonium bicarbonate	0.10	0.10	0.10	0.10	0.10
Water	4.50	4.50	4.50	4.50	4.50

2.2. Dough and biscuit preparation

Doughs and biscuits were prepared according to Laguna et al. (2011). The shortening/inulin mixture was creamed in a mixer (Kenwood Major Classic, UK) for 4 min at minimum speed (60 rpm) to obtain a homogenous cream. After this, the sugar was added and mixed in for 2 min at speed 4 (255 rpm). The milk powder, previously dissolved in the water, was added and mixed in for 2 min at the minimum speed (60 rpm). Finally, the flour, salt, sodium bicarbonate, and ammonium hydrogen carbonate were mixed in together at minimum speed (60 rpm) for 2 min. The dough was then sheeted using a rolling mill (Parber, Vizcaya, Spain) to a thickness up to 15 mm. The sheeted dough was cut into rectangular pieces measuring 80×30×15 mm (length×width×height). The dough pieces were evaluated on the following 24 h.

Twenty-five pieces were placed on a perforated tray. The biscuits were baked in a conventional oven for 4 min at 175 ºC and then the trays were turned 180º and baked for a further 4.5 min at the same temperature. After reaching ambient temperature, the biscuits were packed and stored in heat-sealed metalized polypropylene bags (Sealboy 320, Audion Elektro). The biscuit samples were evaluated on the following.

Dough characteristics

2.3. Confocal laser scanning microscopy (CLSM)

A Nikon confocal microscope C1 unit that was fitted on a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) was used. An Ar laser line (488 nm) was employed as light source to excite fluorescent dyes rhodamine B and nile red. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{ex\ max}$ 488 nm and $\lambda_{em\ max}$ 580 nm was solubilised in distilled water at 0.2%. This dye was used to stain proteins and carbohydrates. Nile red (Fluka, Sigma-Aldrich, Missouri, USA) with $\lambda_{ex\ max}$ 0488 nm and $\lambda_{em\ max}$ 515 nm was solubilised in PEG 200 at 0.1 g/L. A 60×/1.40NA/Oil/ Plan Apo VC (Nikon, Tokyo, Japan) objective lens was used. This dye was used to stain fat.

For sample visualization, a microscopy slide was elaborated with two razor blades (platinum-coated double edge blades with 0.1 mm thickness) stuck to a glass (Alava et al. 1999; Sahi and Alava 2003). 20 µL of the sample was placed in the microscope slide; within the central gap of the blades, rhodamine B solution and nile red solution were added, and the cover slide was carefully positioned to exclude air pockets. By this mean, it was possible to visualize the sample without deforming native sample structure when putting the cover. Observations were performed 10 min after diffusion of the dyes into the sample. Images were observed and stored with 1024×1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

2.4. Cryo scanning electron microscopy (Cryo-SEM)

A Cryostage CT-1500C (Oxford Instruments Ltd., Witney, UK) was used coupled to a JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan). The sample was immersed in slush N_2 (-210 ºC) and then quickly transferred to the Cryostage at 1 kPa where fracture of the sample took place. Sublimation (etching) was carried out at -90 ºC, during 17 min. The sample was coated with gold at 0.2 Pa, with an ionization current of 2 mA. Observation in the scanning electron microscope was carried out at 15 kV at a working distance of 15 mm and temperature ≤-130 ºC. Samples were taken from the centre of the dough piece.

2.5. Texture analysis

The sheeted dough (15 mm thick) from the different formulations was analysed. A TA-TXT plus Texture Analyser equipped with the Texture Exponent software (version 2.0.7.0. Stable Microsystems, Godalming, UK) was used. A test speed of 1 mm s^{-1} and a trigger force of 0.049 N were used in all the tests. Each test was conducted on six replicates of each formulation.

2.5.1. Wire cutting measurements

Rectangles of biscuit dough measuring 80×30×15 (length×width×height) were sheared transversally, through the middle, to a depth of 10 mm, with a wire cutter probe (Butter cutter A/BC). The area of the force/time curves was calculated and defined as dough hardness index.

2.5.2. Sphere penetration measurements

Dough discs with a diameter of 45 mm and a thickness of 15 mm were penetrated to a depth of 10 mm with a 5 mm diameter spherical stainless steel probe (P/0.5). The maximum force (N) attained during penetration was measured.

Biscuit characteristics

2.6. Cryo scanning electron microscopy (Cryo-SEM)

The microscopic observation was carried out as described in dough cryo-SEM section, with the following changes: sublimation (etching) was carried out at -90 ºC, during 5 min. The sample was coated three times with gold (0.2 Pa and 2 mA).

2.7. Weight loss during baking and water activity

The weight loss (WL) during baking was calculated by using the following equation:

$WL\ (\%) = (W_{dough} - W_{biscuit} / W_{dough}) \times 100$

Where W denotes weight (g). From each formulation, 12 samples were weighed individually before (W_{dough}) and after ($W_{biscuit}$) baking. It was determined in duplicate.

Water activity (aw) was determined in two duplicates of each formulation, using a Decagon AquaLab meter (Pullman, WA, USA) calibrated with a saturated potassium acetate solution (aw = 0.22).

2.8. Dimensions of the biscuits

The biscuit length and width were measured by placing 10 biscuits edge-to-edge (both vertically and horizontally). The biscuit thickness was measured by stacking 10 biscuits. Measurements were expressed in millimetres as the mean value/10 of two different trials. Changes in the dimensions were expressed as gains (+) in comparison with the initial dimensions of the dough biscuits 80×30×15 (length×width×height).

Several biscuits were cut vertically. The cut side was photographed with a digital camera (E-510 Olympus, Hamburg, Germany) with a vari-focal lens (14-24 mm ED, Zuiko Digital). The images were stored in a format of 3648×2736 pixels. The images were processed using the software ImageJ (National Institutes of Health, Bethesda, Bethesda, Maryland, USA). The image was cropped in a 100×120 mm section. First, the image was split in colour channels, then the contrast was enhanced, and finally, the image was binarised after grey scale threshold.

2.9. Texture analysis

The texture of the biscuits was measured using the texture analyzer described above. A test speed of 1 mm/s was used for all tests. Ten replicates of each formulation were conducted.

2.9.1. Breaking strength

Biscuits were broken using the three point bending rig probe (A/3PB). The experimental conditions were as follows: supports 50 mm apart, a 20 mm probe travel distance, and a trigger force of 0.196 N. The force at break (N) was measured.

2.9.2. Bite test

Penetration test was conducted with the upper Volodkevich Bite Jaw (VB), penetrating the sample (whole biscuit) to 10 mm; a trigger force of 0.196 N was set. Two 'bites' were made in each biscuit (one third in from each end), so a total of 20 values were registered for each formulation. The maximum force at penetration (N) was measured.

2.10. Sensory analysis

Fifty panellists evaluated the biscuits noting differences between two samples following a triangle test. A triangle test is a type of difference test to determine if there is a sensory difference between two products. Three coded samples (two samples are equal and the other different) were presented to each panellist, and each panellist was asked to pick out which sample they felt was different from the other two. Panellists were also asked about the differences between samples. Results were analyzed using the corresponding table following a unilateral hypothesis (Roessler et al. 1978).

2.11. Statistical analysis

Analysis of variance (one-way ANOVA) was performed on the data; least significance differences were calculated by the Tukey test, and the significance at $P < 0.05$ was determined. These analyses were performed using XL-STAT 2009.4.03 (Addinsolf, Barcelona, Spain).

3. Results and discussion

Dough characteristics

3.1. Confocal laser scanning microscopy (CLSM)

CLSM is a very useful tool to investigate the distribution of the different ingredients within the dough, as it is a technique that is based on differential staining. Figure 1 shows CLSM images of doughs stained with rhodamine B and nile red. Ingredients were identified as follows: protein and dispersed carbohydrate in red, fat in green, and starch granules in black. Micrographs show a continuous matrix composed mainly by

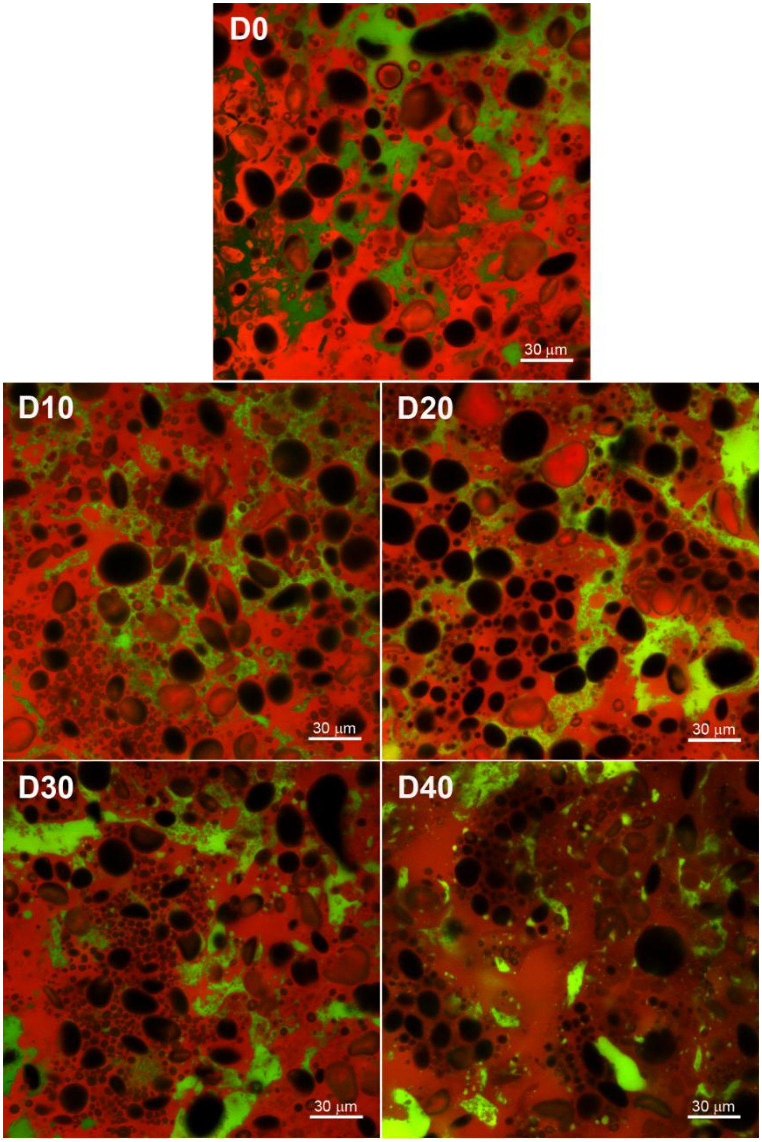

Figure 1. Confocal laser scanner microscopy (CLSM). Images of stained doughs with rhodamine B and nile red (proteins and carbohydrates in red, fat in green, and starch in black) (60×). D0, D10, D20, D30, and D40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

sugar, inulin, and proteins, all of which are stained in red. Starch granules, in black, were not evenly distributed in the matrix, and fat, in green, was located surrounding the starch granules. This description is in accordance with Chevallier et al. (2000), who presented dough structure as a suspension of proteins, starch-protein associations, and isolated starch granules in a liquid continuous phase based on an emulsion of lipids in a sugar solution.

In D0, D10, and D20, which were doughs with high fat content, fat was distributed homogeneously as a continuous phase. In contrast, in D30 and D40, where fat content was lower than in the previous formulations, fat was dispersed in the matrix by forming isolated particles, with no connection between them. These observations in short doughs are in agreement with Baltsavias et al. (1997), who concluded that short doughs are bicontinuous systems, but when the fat content is reduced, fat-dispersed systems are obtained.

3.2. Cryo scanning electron microscopy (Cryo-SEM)

Cryo-SEM micrographs of the inner part of the doughs are shown in Figure 2. Doughs are observed as agglomerates of protein and starch; these agglomerates are partially covered with fat depending on the content of fat of the dough. In this context, high fat content doughs –D0, D10, and D20– showed some areas where a smooth surface can be observed, corresponding to fat surrounding protein-starch aggregates. However, in fat replaced doughs –D30 and D40– smooth surfaces were not observed, and starch granules could be easily identified.

Therefore, in D0, D10, and D20, when a higher amount of fat was present, it surrounded the proteins and the starch granules, isolated them, thereby breaking the continuity of the protein and starch structure and affecting the properties of the dough as observed previously by Pareyt and Delcour (2008).

3.3. Dough texture

The wire cutting test involves pushing a wire through the specimen from an initial indentation to a steady-state cutting stage. The force-displacement relationship obtained for the wire-cutting test depends on a combination of fracture, plastic/viscous deformation, and surface friction effects (Laguna et al. 2011).

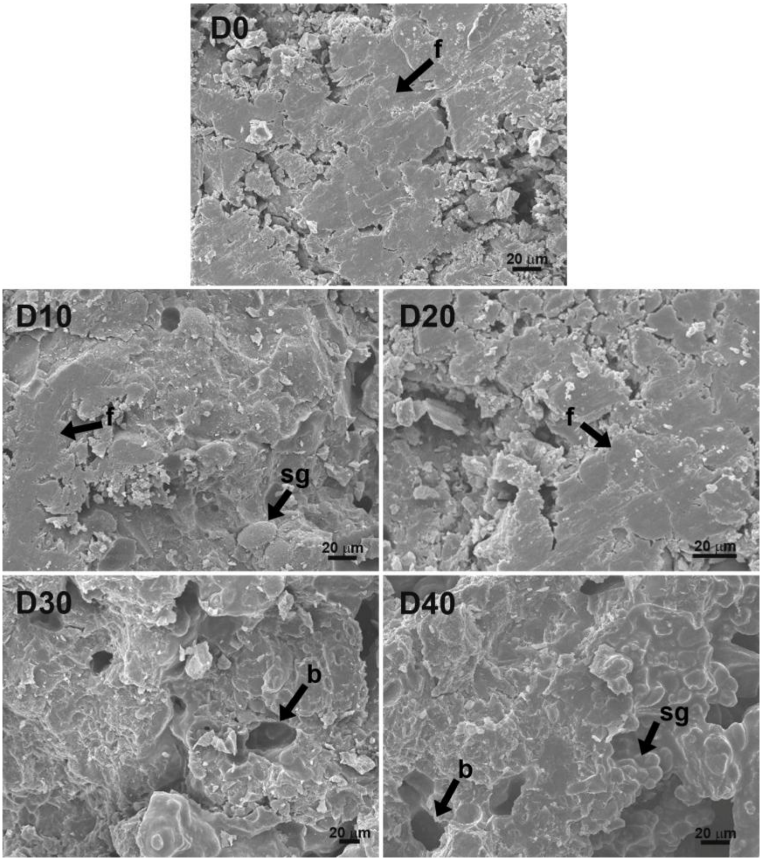

Figure 2. Cryo-SEM micrographs of the inner part of the biscuit doughs. f: smooth fat surface, sg: starch granules, b: air bubbles. D0, D10, D20, D30, and D40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

Typical force-displacement curves were obtained for the four formulations (Figure 3) with two differentiated phases: In the first, the wire indents into the dough; in the second, the material starts to separate and a steady-state cutting phase proceeds to form a plateau (Goh et al. 2005). Two types of behaviour in the wire cutter curves can be distinguished; D0, D10, and D20 had shorter indentation cutting phases and lower plateau force values than D30 and D40, which had longer indentation periods and higher plateau force values with a decrease in force values at the end of this last

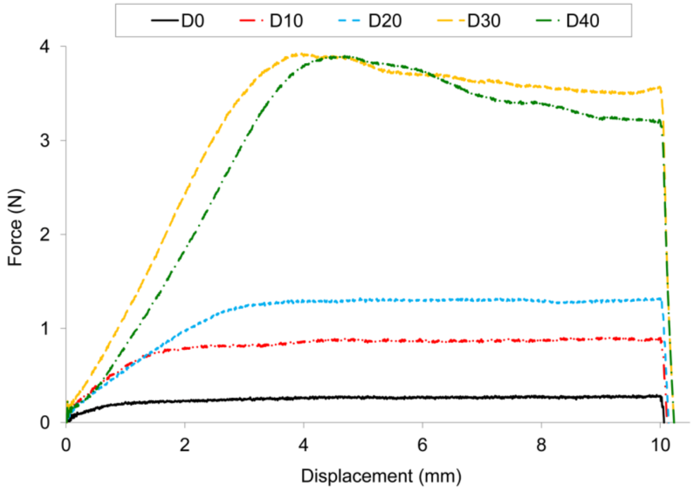

Figure 3. Wire cutting representative curve profiles of biscuit doughs with inulin as a fat replacer. D0, D10, D20, D30, and D40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

cutting phase. The difference in force in the cutting path depends on the cohesive forces between the dough constituents. Doughs with higher fat levels –D0, D10, and D20– showed short indentation phase, which meant that these doughs were easily penetrated by the wire cutter due to its soft texture. In addition, D0, D10, and D20 showed low plateau force values, which were related to its low hardness. Values shown in Table 2 (first column) corroborate this behaviour. The area of the force/time curves was an index of dough hardness. It can be seen that this parameter increased significantly ($P < 0.05$) with fat replacement by inulin. These observations can be related to the microstructure of these doughs; when high fat content is present in the dough formulation, fat was distributed surrounding flour particles, thus limiting its hydration and hindering amilo-protein interactions. These results are in agreement with data obtained by O'Brien et al. (2003), who found that the high free fat content in formulations containing microencapsulated high fat powders disrupted the gluten network development resulting in softer doughs. In contrast, D30 and D40 samples, with lower levels of fat, showed steeper slope of the cutting force curves and rose to higher force values in the indentation phase, which could be attributed to higher difficulty to penetrate the dough. In addition, D30 and D40 plateau force values were higher than D0, D10, and D20 ones and also showed a slightly decrease at the end of the plateau phase, which could be attributed to secondary sample microcracking. During mixing, the fat component and the aqueous phase compete for the surface of the flour particles (O'Brien et al. 2003). As it has been observed in the micrographs above, doughs with lower fat contents showed the fat dispersed, with no continuity and difficulties to lubricate the entire matrix. Therefore, if there is not enough fat to surround flour particles, as in D30 and D40, flour is hydrated leading to hard dough. This idea is supported by previous studies (Ghotra et al. 2002), which stated that in baked product without shortening, gluten and starch particles adhere to each other and give the sensation of hardness and toughness when chewed.

Table 2. Dough textural characteristics obtained with wire cutter and sphere penetration tests for the five formulations

Dough	Area (N·s) (wire cutter)	Max. force (N) (sphere penetration)
D0	2.53^{a} (0.36)	0.80^{a}(0.08)
D10	7.70^{b} (0.29)	2.76^{b} (0.12)
D20	11.14^{c} (1.10)	2.69^{b} (0.20)
D30	24.39^{d} $(4.38)^{a}$	5.81^{c} (1.56)
D40	31.94^{e} (2.83)	2.83^{b} (1.56)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different (P < 0.05) according to the LSD multiple range Test.

The results for sphere penetration showed a significant (P < 0.05) increase in dough hardness when shortening was replaced by inulin, except for D40 (Table 2, 2nd column).The value of the force at the end of the test, corresponding to a penetration of 10 mm, is not representative of any true textural value and is simply a consequence of the increase in friction between the plunger and the cohesive mass of the sample.

Consequently, it was appropriate to record the value of the first relative maximum force as the 'hardness' of this kind of sample. Confocal laser scanning micrographs (Figure 1) showed that as fat decreased and inulin increased in the formulation, flour particles were less surrounded by fat and thus more available for hydration, leading harder and compact doughs. However, in D40, the aqueous component was not enough to hydrate flour particles and the increased amount of inulin added. Moreover, fat phase in D40 was not enough neither to lubricate the matrix. Therefore, D4 texture was not only harder but also less cohesive and fragile; the dough broke easily into pieces.

D0, D10, and D20 penetration curves followed the same behaviour (Figure 4). The value of the penetration force increased slightly until the conclusion of the test, i.e., to a depth of 10 mm. These results could be due to the functionality of fat to lubricate the matrix components. The microstructure analysis showed that when there is enough fat content, it acts linking the matrix component, giving place to structural integrity and lubrication. The shape of the curves of D30 and D40 samples was different, producing a shoulder or inflexion, which indicated a rather weak force at breaking after a brief period of compression (at a displacement of 4 mm). As observed in CLSM (Figure 1) and cryo-SEM micrographs (Figure 2), in these doughs, fat was dispersed in the matrix, filling it, but there was not enough fat to lubricate the matrix. Thus, the ingredients could be more hydrated but less linked in the matrix, giving place to this textural behaviour –harder doughs that, once the structure is disrupted, break into pieces due to the lack of lubrication. These results were in accordance with Sudha et al. (2007a), who studied changes in the rheological properties of dough due to fat replacement by maltodextrine or polydextrose. These authors measured the consistency of the dough using the research water absorption meter and observed that the biscuit dough became hard when fat content in the formulation was reduced.

Biscuit characteristics

3.4. Cryo Scanning Electron Microscopy

Figure 5 shows the cryo-SEM micrographs of the inner part of the short dough biscuits. The starch granules are immersed in a continuous matrix mainly formed by denatured proteins. During baking, the fat melted and coated the surface giving place to a smoothened appearance. Other microscopy results (Flint et al. 1970) showed that in short dough biscuits, no continuous protein network was present; the structure consisted of a mixture of protein and starch, and fat form large interconnected masses between the starch-protein masses. However, biscuits seemed to be more continuous systems than doughs as a consequence of fat melting (compare Figures 2 and 5). Similar results were observed by Doescher et al. (1987) in scanning electron micrographs of cookie doughs and baked cookies.

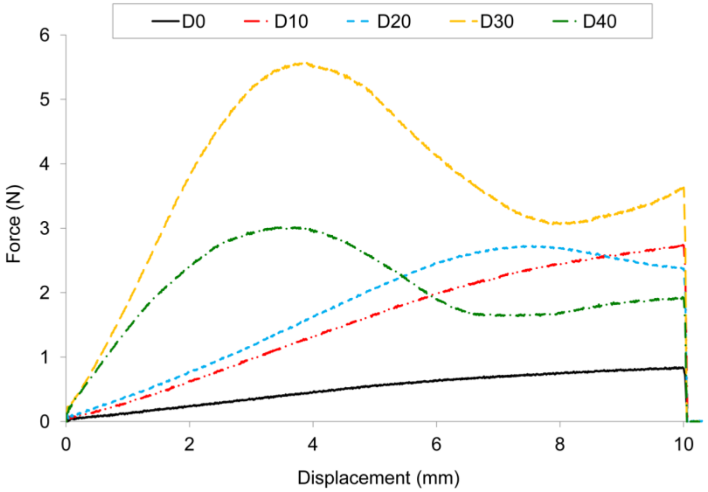

Figure 4. Sphere penetration representative curve profiles of biscuit doughs with inulin as a fat replacer. D0, D10, D20, D30, and D40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30 and 60:40, respectively

There was a continuous fat layer coating the matrix surface in B0, B10, and B20; as stated before, the melted fat gave place to smooth surfaces. As the fat replacement level increased –B30 and B40– a rougher surface was observed. Starch granules were more easily identified in B30 and B40. In these samples, fat lubricated the matrix, but it was not enough to form a coating layer all along the surface Starch granules were probably not gelatinized due to low water content in the formulations and water competition between protein, sugar, inulin, and starch. Other authors also had reported that due to water limitation (Chevallier et al. 2002) and high levels of sugar (Pareyt and Delcour 2008), most of the starch granules are not gelatinized after short dough baking.

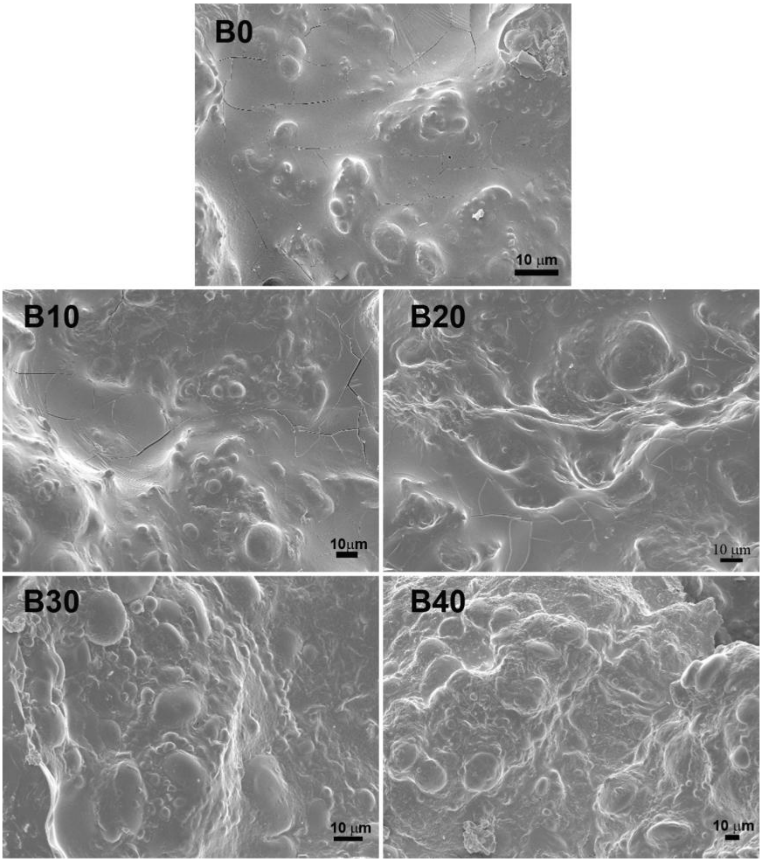

Figure 5. Cryo-SEM micrographs of the biscuits inner part. B0, B10, B20, B30, and B40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

3.5. Weight Loss during Baking and Water Activity

The main proportion of the weight loss during baking was attributed to the evaporated water (Table 3). Weight loss (WL) values decreased significantly ($P < 0.05$) when fat replacement increased, except for B40, which had the highest ($P < 0.05$) weight loss. In addition, water activity decreased significantly ($P < 0.05$) when fat replacement by inulin increased. The lower weight loss and water activity in formulations where fat was replaced by inulin can be attributed to the higher accessibility of the water to flour components and inulin. Therefore, water can be absorbed by the sample, less water can be evaporated during baking, and less free water would remain in the biscuits. However, the higher weight loss during baking in B40 could be related to its structure where water can be easily evaporated. As explained before, although gluten, starch, and inulin were more accessible to water in B40, fat was not enough to bind all the components. Therefore, B40 texture was hard, but not as much as B30, and easily broken into pieces due to the lack of a structural linkage. In conclusion, the high weight loss during baking could be related to the fact that there was not a developed structure that would retain its shape and its components to any external change –as baking or biting. Therefore, the lack of structural integrity in B40 could allow easier water evaporation during baking, even if water was already hydrating the matrix.

Table 3. Mean values of weight loss during baking, water activity and physical characteristics of biscuits

Biscuit	Weight Loss (%)	a_w	Width gain (mm)	Height gain (mm)
B0	8.82^a (0.77)	0.62^a (0.02)	+1,21	+0,82
B10	8.93^a (0.20)	0.59^{ab} (0.00)	+1,20	+0,88
B20	7.38^b (0.33)	0.58^{ab} (0.01)	+0,73	+0,90
B30	7.04^c (0.23)	0.57^b (0.01)	+0,66	+0,87
B40	9.35^d (0.74)	0.51^c (0.04)	+0,32	+0,52

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different. ($P < 0.05$) according to the LSD multiple range Test.

3.6. Dimensions of the Biscuits

Table 3 shows that biscuit size decreased as fat replacement increased. This can be due to the fat that melts during baking, and so the dough can flow more easily under the force of gravity (Pareyt and Delcour 2008). Similar results were observed by Pareyt et al. (2009) in biscuits, where an increase in fat level was generally associated with higher spread rate due to the increased mobility in the system when fat melts during baking. Moreover, as stated before, lubrication is the major function of the fat by coating the matrix. Hence, fat reduction in the formulation made flour components more accessible to the water. As a consequence of a higher gluten hydration, harder doughs were obtained, as observed in D30; this change in the textural properties could influence dough development during baking, giving place to less spread biscuits. Similar results were observed by Sudha et al. (2007a), who showed that biscuit dough increased its elastic properties in the absence of enough fat; this elastic nature of the dough would be responsible for the adverse effect on the spread and thickness of the respective biscuits.

Furthermore, aeration is the second major function of fats in cookie doughs; the results are increased volume, uniform and fine grain, and tender crumb (Lai and Lin 2007). As it can be observed in Figure 6, there were more air cells (10.55-11.30% of total cell area within the crumb) in biscuits with higher fat content –B0, B10, and B20– if compared to B30 and B40, where less air cells were formed (6.45-7.43% of total cell area within the crumb).

3.7. Biscuit texture

The baking process of short dough includes a transformation into a cellular solid, with a characteristic final texture (Pareyt and Delcour 2008). B0, B10, and B20 slopes (Figure 7) are typical of the force-deformation curves described for materials exhibiting brittle fracture, characterized by an essentially elastic response and a small fracture strain. These curves were characterized by a lower pea force values (Table 4), indicating lower break corresponded to B30 (Table 4), indicating more compact and dense structure (Figure 6). As it was stated before, when fat content was reduced in dough formulation flour particles would be more hydrated because flour components were more accessible to the water. As a consequence of a higher gluten hydration, harder doughs were obtained. These harder doughs resulted in harder biscuits –as B30 biscuits– when baked. Dough hardness increased with fat replacement, and so fat replacement in B10, B20, and B30 caused a progressively significant ($P < 0.05$) increased in the biscuits hardness. This effect was also observed by Sudha et al. (2007a) in soft dough biscuits on which fat was replaced by maltodextrine and polydextrose. However, in B40, a fall in hardness value was observed. D40 CLSM micrographs showed the fat is forming isolated particles in the matrix; its content is very low to act as a lubricant or impart integrity to the structure. Moreover, B40 structure was less aerated and had a more compact crumb. Therefore, this biscuit presented a high initial force gradient and a high penetration force due to its hard. However, after this high force at the beginning to penetrate the biscuit, the lack of enough fat to lubricate the matrix and to develop a linked structure resulted in an easily broken into pieces biscuit, showing the force values drop sharply. In conclusion, as explained before, although gluten, starch, and inulin were more accessible to water in B40, fat was not enough to bind all the components obtaining a more crumbly biscuit.

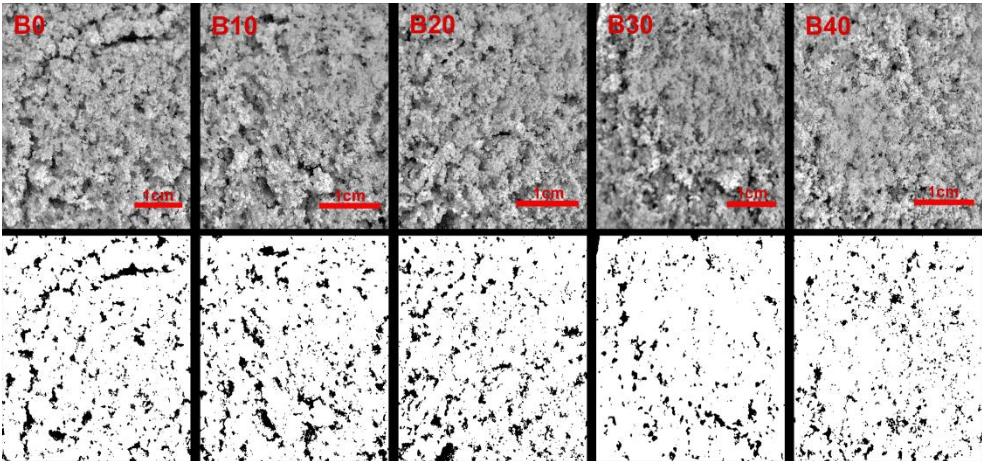

Figure 6. Biscuit crumbs. 1st column grayscale images. 2nd column binarized images. B0, B10, B20, B30, and B40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40

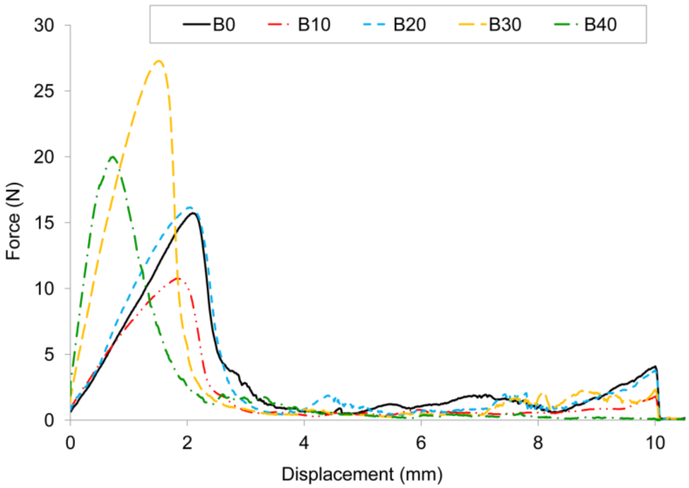

Figure 7. Three point bending representative curve profiles of biscuit doughs with inulin as a fat replacer. B0, B10, B20, B30, and B40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

Table 4. Biscuit texture characteristics obtained with three point bending rig probe and Volodkevich Bite Jaw for the five biscuit formulations

Biscuit	Force at break (N) (3-point break test)	Max. force (N) (bite test)
B0	16,44^{ab} (1,84)	14,81^{a} (1,88)
B10	10,01^{c} (0,92)	11,33^{a} (1,87)
B20	13,72^{b} (1,84)	13,97^{a} (1,73)
B30	24,90^{d} (3,65)	28,56^{b} (5,01)
B40	18,76^{a} (5,93)	53,44^{c} (15,45)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($P < 0.05$) according to the LSD multiple range Test.

Biscuit quality can be summarized in two general terms; the first is the size of the biscuit, both the width and the height; the second is how the biscuit bites (Pareyt and Delcour 2008). Volodkevich Bite Jaws probe imitates the force of biting the biscuit (Figure 8). All the formulations except B40 showed a typical penetration curve with a maximum corresponding to the point when the probe penetrated the sample following of a decrease in force values, indicating relaxation of the stress, and finally, the force rose again as the increasing dimensions of the probe penetrated the sample. In fact, no significant differences ($P > 0.05$) in force values of B0, B10, and B20 were found (Table 4). B30 showed a significant increase ($P < 0.05$) in the force value due to a lower fat content that enhanced hydration of flour components resulting in a higher hardness biscuit. However, B40 had a different penetration curve. This sample presented a higher initial force gradient and a significantly ($P < 0.05$) higher maximum penetration force, but finally, the force values dropped sharply. This behaviour under the force of biting could be related with the microstructure of the sample; micrographs showed that the small amount of fat in D40 was observed in the matrix as isolated particles, without the chance of lubricate and shorten the matrix. Moreover, B40 structure was less aerated and had more compact crumb. This behaviour was related not only to hard structure but also to fragile biscuit structure.

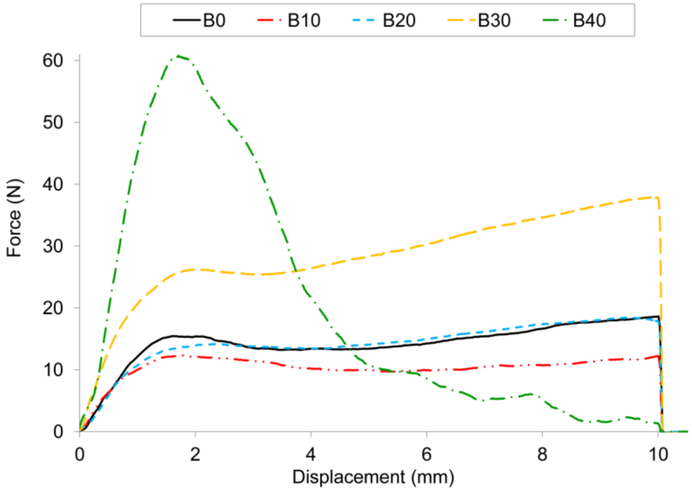

Figure 8. Volodkevitch bite penetration representative curve profiles of biscuit doughs with inulin as a fat replacer. B0, B10, B20, B30, and B40 correspond to shortening/inulin proportions: 100:0, 90:10, 80:20, 70:30, and 60:40, respectively

3.8. Sensory analysis

A triple test was carried out presenting to the panellists B0 and B20 samples. These samples were chosen, as B20 was the sample with higher fat replacement that showed similar structural and textural behaviour than B0. Fifty total triangle test responses would require 23 correct responses to be significant at the 95% confidence level (Roessler et al. 1978). Using the composite data, the proportions of correct responses for the biscuit samples were 33:50. Therefore, the results indicate that panellists were able to differentiate between B0 samples and the B20 samples. However, when panellists were asked about the differences between samples, they described only slight differences: B0 was slightly less hard and less sweet than B20. The higher sweetness of B20 is due to the sweetness of the inulin added to replace the fat (inulin sweetness is 10% if compared to sucrose 100%), and although panellists found slight differences in hardness, no significant differences in penetration force were found between these two samples in instrumental hardness, as it is explained before.

4. Conclusions

Biscuit texture, dimensions, and weight loss during baking were strongly related to microstructure of doughs and biscuits. In high fat content doughs (0, 10, and 20% fat replacement levels) fat was observed surrounding flour components. This ingredient distribution hindered flour hydration and promoted the lubrication of the structure. These effects gave doughs with soft texture. During baking, fat melted and coated the matrix surface. This fact restricted flour hydration and so, water loss during baking increased. Moreover, high fat content improved biscuit expansion and aeration. Biscuit texture was highly influenced by its microstructure; biscuits with high fat content were brittle and soft, in contrast to dough with higher fat replacement (30%) where the absence of fat enables a higher hydration of the component, giving harder biscuits. However, when fat replacement by inulin was 40%, there was not enough fat to lubricate the system; thus, a fragile structure was obtained. Biscuits with 20% of fat replacement had similar structural and textural behaviour than its counterpart, although in the sensory evaluation the panellists described slightly differences between B0 and B20 samples. In conclusion, a fat replacement with inulin up to 20% gave place to a good quality short dough biscuits.

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Resumen de resultados

Bizcochos, funcionalidad de los ingredientes

La masa de bizcocho es una matriz muy compleja debido al elevado número de componentes, la forma en la que se encuentran dispersos y las interacciones entre ellos. A través de diferentes técnicas microscópicas se ha logrado conocer de forma más detallada la distribución de estos ingredientes. La masa se observó como una matriz lipoproteica, formada por proteína y grasa procedentes principalmente de la harina y el huevo. En esta matriz se encontraron dispersos los gránulos de almidón, el aceite en forma de glóbulos y las burbujas de aire. Además, en las micrografías obtenidas por microscopía confocal laser de barrido se observó la presencia de pequeños glóbulos de grasa formando un film alrededor de burbujas de aire. En las masas de bizcochos tipo *foam* el aire se ocluye en la fase acuosa y las burbujas incorporadas quedan estabilizadas por las proteínas de la clara del huevo. En este estudio se observó que parte del aceite se dispone también en la interfase de las burbujas estabilizando parcialmente la fase gas. Hasta el momento la mayoría de investigaciones, sólo habían atribuido a las grasas sólidas la capacidad de estabilización de burbujas de aire en las masas de productos horneados.

La distribución de las burbujas de aire depende en gran medida de la viscosidad y composición de la masa. Las masas sin aceite mostraron una fase gas muy inestable, con baja viscosidad aparente y amplio movimiento y coalescencia de burbujas. Durante el horneado se produjo una mayor difusión y pérdida de la fase gas hacia la superficie y exterior del producto, dando lugar a bizcochos compactos. La incorporación de aceite en las formulaciones de bizcochos incrementó la viscosidad aparente de la masa y la estabilidad de la fase gas, dando lugar a una distribución homogénea de burbujas de tamaño pequeño. Este tipo de masas dieron lugar a bizcochos con una estructura de gran volumen y miga homogénea y aireada. Sin embargo, la ausencia de aceite e incorporación de inulina se tradujo en masas de viscosidad aparente menor y una distribución heterogénea de burbujas de mayor tamaño. Esto se relacionó con una mayor inestabilidad de la fase gas durante el horneado y por consiguiente con la formación de una estructura alveolar más abierta, es decir de alveolos grandes, con interconexiones entre ellos y con bizcochos menos voluminosos.

El agente impulsor ejerció un efecto crucial en la expansión de la estructura durante el horneado. En masas estables, cuyas propiedades físicas permitieron la oclusión de aire y el crecimiento de las burbujas, la adición del agente impulsor incrementó significativamente la expansión de la masa y mejoró la estructura alveolar del producto final.

Reemplazo de grasa y azúcar por inulina y oligofructosa en bizcochos

La grasa se reemplazó por inulina y leche, ya que la adición de leche aportaba componente acuoso extra suficiente para dispersar la inulina. Sin embargo, esto disminuyó la viscosidad aparente de la masa, la estabilidad de la fase gas y los cambios de la estructura durante el horneado. Los resultados obtenidos fueron buenos hasta niveles de sustitución de grasa del 50% y 70%. Estas masas mostraron una viscosidad suficiente para retener las burbujas de aire durante el horneado, dando lugar a bizcochos de estructura alveolar aireada, en la que la formación de canales alveolares contribuyó a mejorar el volumen del producto, en comparación con los resultados obtenidos tras un reemplazo total del aceite. Por otro lado, a través de la microscopía electrónica de barrido a bajas temperaturas se observó cómo en las formulaciones con cantidades suficiente de aceite, éste se distribuía lubricando la matriz tras el horneado, lo cual se relacionó con una textura menos dura y más elástica. El análisis sensorial llevado a cabo mostró que estos bizcochos, con 50 y 70% de reemplazo de grasa, no difirieron del control en la valoración de los atributos sensoriales por los consumidores.

A partir de estos resultados se trabajó con las formulaciones con 50% de sustitución de grasa por inulina, para más adelante aplicar un reemplazo simultaneo de grasa y azúcar en ellas. Durante el horneado de estas masas se observó un aumento de la variedad de tamaños de burbujas y una gran expansión de estas. Se obtuvieron bizcochos con alveolos más grandes y en general de menor altura que los bizcochos control. Sin embargo, presentaron características texturales semejantes a este último.

La sustitución del azúcar por oligofructosa afectó también a las propiedades físicas de la masa y a los cambios e interacciones entre el resto de ingredientes. La viscosidad aparente de la masa también disminuyó significativamente, y el proceso de desproporción de las burbujas se vio favorecido. Sin embargo, la masa solidificó sin mostrar grandes cambios en el tamaño de las burbujas de aire. Los bizcochos con reemplazo de azúcar mostraron una estructura de poca altura y una miga con alveolos pequeños y sin apenas interconexiones. Estas características se vieron perfectamente reflejadas en las propiedades texturales del producto: poca dureza, elasticidad y cohesividad. El azúcar retrasa las temperaturas de gelatinización del almidón y de desnaturalización de las proteínas, ya que limita la cantidad de agua libre para que estos componentes se hidraten. Al disminuir el contenido de azúcar en la matriz estos procesos se adelantan dando lugar a un bizcocho en el que la expansión no ha tenido lugar de manera completa y que por tanto presenta una miga más compacta. Por otra parte, la reducción de azúcar e incorporación de otros compuestos puede dar lugar que estos procesos de gelatinización y desnaturalización tengan lugar de manera escalonada en el tiempo de horneado, alterando así la estructura de la miga final, viéndose reducida su fuerza y cohesividad. En general, la sustitución de azúcar afectó de manera más significativa a la estructura del producto final que el reemplazo de grasa. Así tras el análisis sensorial por parte de los consumidores, se observó que hasta el 30% del azúcar se podría reemplazar por oligofructosa y hasta el 50% de la grasa se podría reemplazar por inulina obteniendo los bizcochos así formulados una valoración semejante al bizcocho control. Además, los bizcochos que presentaron estas sustituciones de forma simultánea también obtuvieron un resultado óptimo en aceptabilidad global. A través de las escalas JAR se identificó que deberían mejorarse los atributos de esponjosidad y dulzor para conseguir una mayor valoración de estos bizcochos. Esto pudo deberse a que no se empleó ningún edulcorante de alta intensidad para alcanzar un dulzor óptimo, que con la oligofructosa no se consiguió. Por otra parte, la adición de fibra alimentaria en forma de inulina, además de los cambios que ejerce en el desarrollo de la estructura, pudo alterar la esponjosidad del bizcocho final.

Escalado y optimización del proceso de elaboración de bizcochos

Tradicionalmente para la elaboración de bizcochos tipo *foam,* el procedimiento de mezclado consta de varias etapas. Sin embargo, a escala industrial es más fácil emplear procedimientos que consten de una sola etapa de mezclado, en este sentido, la adición de emulsionantes mejora la incorporación y estabilización de burbujas de aire. En este trabajo se quiso evaluar los efectos del mezclado en una sola etapa en la formulación control, sin la adición de emulsionante. Los resultados fueron muy satisfactorios ya que aplicando este proceso de solo una etapa se obtuvieron bizcochos semejantes a los tradicionales. Además, se evaluaron diferentes tiempos de mezcla, y el tiempo más corto fue el que ofreció mejor resultados en términos de volumen, estructura alveolar y textura de los bizcochos.

Mejora de la formulación de bizcochos con contenido reducido de grasa

El siguiente objetivo se enfocó en los bizcochos con contenido reducido en grasa (nivel de sustitución de grasa por inulina del 50% y 70%). Para conseguir una estructura alveolar homogénea y mejorar las propiedades texturales se estudiaron los efectos de la incorporación de dos mejorantes: un emulsionante comercial y la enzima lipasa.

Los mejores resultados se consiguieron con un nivel de incorporación de emulsionante del 0.5% y de lipasa del 0.003%. Se obtuvieron bizcochos con una distribución de tamaños de alveolos más homogénea y una dureza semejante al bizcocho control. El emulsionante mostró una gran eficiencia a la hora de reducir la densidad relativa de la masa. Sin embargo, cuando se empleaba emulsionante a concentraciones elevadas, durante el calentamiento, estas masas mostraron valores de viscosidad compleja muy bajos, y se obtuvieron bizcochos colapsados. Esto se relacionó con la incapacidad de la estructura de retener la gran cantidad de fase gas que se había ocluido durante el batido. La lipasa mejoró el perfil de textura de los bizcochos durante un almacenamiento de 14 días. Esta enzima actúa a nivel de los la fracción lipídica de la harina y produce dos efectos; por una parte modifica las interacciones entre los lípidos de la harina y el gluten, y por otra produce monoglicéridos que participan en la formación de complejos amilosa-lípido, los cuales juegan un papel muy importante en retrasar la retrogradación del almidón.

Reemplazo de grasa por inulina en galletas

Una galleta de masa corta se caracteriza por su textura suave, blanda y friable consecuencia, en parte, de que la grasa rodea los componentes de la harina impidiendo una hidratación profunda de estos y la formación de una estructura tridimensional dura y elástica. A través de las diferentes técnicas microscópicas empleadas se observó cómo al reemplazar parte de esta grasa, ésta ya no se distribuía uniformemente lubricando la matriz, sino que se encontraba como partículas aisladas dejando acceso al agua hacia los componentes de la harina. Por ello las masas con sustitución de grasa fueron más duras. Además, la reducción de grasa hizo que expandieran menos durante el horneado y que se obtuvieran galletas con una estructura más compacta. En estos sistemas de baja humedad tan solo con un reemplazo del 20% de la grasa ya se observaron diferencias significativas en el análisis sensorial.

Conclusiones

Las principales conclusiones que se extraen de la presente tesis son:

- El aceite presenta una propiedad funcional hasta ahora atribuida fundamentalmente a las grasas sólidas: se sitúa en la interfase aire- matriz, mejorando la estabilidad de la masa y la retención del aire durante el horneado.
- El reemplazo parcial de aceite por inulina y componente acuoso extra disminuye significativamente la viscosidad aparente de la masa, afecta a la distribución de tamaños de burbujas y disminuye la estabilidad de la fase gas durante el horneado, dando lugar a bizcochos menos aireados y más duros.
- La elaboración de bizcochos con contenido reducido en grasa al reemplazar hasta el 70% del aceite por inulina es factible. Estos bizcochos muestran características físicas y sensoriales semejantes al bizcocho control.
- La sustitución de azúcar por oligofructosa afecta al mecanismo de solidificación de la matriz durante el horneado produciendo disminución del número de alveolos en la miga, de la altura de los bizcochos, así como de la dureza y elasticidad de estos. Los resultados muestran que la estructura no se expande de manera adecuada y suficiente durante el horneado.
- El uso de oligofructosa permite un reemplazar hasta el 30% del azúcar en bizcochos, los cuales muestran un nivel de aceptabilidad similar al bizcocho control.
- Se consigue la sustitución simultánea de grasa y azúcar por inulina y oligofructosa con resultados óptimos hasta niveles de reemplazo del 50% y 30%, respectivamente. Estos bizcochos muestran valores de aceptabilidad general similares al control. Sin embargo, habría que realizar mejoras a nivel de textura y sabor para conseguir bizcochos de mayor calidad.
- La optimización del proceso de mezclado para adecuar la elaboración de los bizcochos a una producción a nivel de escala piloto, no modifica de manera negativa las características del producto final. Se consigue reemplazar un método de mezclado formado por diversas etapas con uno de una sola etapa, en el que se economiza tiempo y energía.

- La adición de 0.003% de lipasa y 0.5% de emulsionante mejora la estructura alveolar de los bizcochos con contenido reducido en grasa; estos bizcochos muestran una presencia menor de canales alveolares y mayor homogeneidad. Además, la adición de lipasa mejora el perfil de dureza de los bizcochos durante el almacenamiento.

- Las galletas con hasta el 20% de sustitución de grasa por inulina muestran una estructura y propiedades texturales similares a la galleta control. Sin embargo, los consumidores reconocen ligeras diferencias, como mayor dureza y dulzor en las muestras con el reemplazo.

- El estudio e interrelación de la micro, macroestructura y propiedades físicas de masas y bizcochos, permite avanzar y mejorar la comprensión de la funcionalidad, distribución e interacciones de los ingredientes. Con ello se consigue mejorar la reformulación de los alimentos para obtener productos de elevada calidad y contenido reducido en grasa y azúcar.

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