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EFECTO DE LA SUSTITUCIÓN DE HARINA DE TRIGO POR HARINAS DE CHÍA (*Salvia hispanica* L.), AMARANTO (*Amaranthus caudatus*) Y QUÍNOA (*Chenopodium quinoa*) EN LAS PROPIEDADES NUTRICIONALES, TECNOLÓGICAS Y SENSORIALES DE UN PRODUCTO PANARIO PRECOCIDO Y CONGELADO

Departamento de Medicina Preventiva y Salud Pública, Ciencias de Alimentación, Toxicología y Medicina Legal

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TESIS DOCTORAL
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INFORMA QUE

La presente Tesis doctoral se enmarca en los proyectos QuiSalhis-Food (AGL2016-75687-C2-1-R) y Food4ImNut (ID2019-107650RB-C21), financiados por el Ministerio de Ciencia, Innovación y Universidades (MICIU) y CYTED, la ValSe-Food (119RT0S67). Además, la doctoranda Karla Carmen Miranda Ramos ha podido disfrutar de una ayuda predoctoral de la Fundación Carolina, en los períodos comprendidos entre 15/10/2017 y 15/07/018 (9 meses), 1/10/2018 y 29/03/2019 (6 meses), 29/01/2020 y 27/07/2020 (6 meses).

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Dr./Dra. Claudia Monika Haros directora de la estudiante de doctorado en CIENCIAS DE LA ALIMENTACIÓN D./Da. Karla Carmen Miranda Ramos, u en relación a la presentación de la Tesis doctoral "**Efecto de la sustitución de harina de trigo por harinas de chía (*Salvia hispanica* L.), amaranto (*Amaranthus caudatus*) y quinoa (*Chenopodium quinoa*) en las propiedades nutricionales, tecnológicas y sensoriales de un producto panario precocido y congelado**" como compendio de publicaciones.

INFORMA

Que les publicaciones presentadas por la doctoranda en la Tesis en modalidad de compendio de publicaciones D./Da. Karla Carmen Miranda Ramos figura como autor/a principal.
La doctoranda ha participado en el 100% de los trabajos publicados, incluido los experimentos, interpretación y análisis de resultados, escritura de manuscritos, búsqueda bibliográfica y respuesta a revisores.
Les publicaciones incluidas en la tesis no se han utilizado en otras tesis y están publicadas con el siguiente factor de impacto.

Publicaciones:

1. *Evaluation of technological and nutritional quality of bread enriched with amaranth flour.* **Miranda-Ramos, K**.; Sanz-Ponce, N.; Haros, C.M. ***LWT - Food Science and Technology***, DOI: doi.org/10.1016/j.lwt.2019.108418, 114, 108418 (2019).
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2. Effect of chia by-products as breadmaking ingredients on nutritional quality, mineral availability and glycaemic index of bread. **Miranda-Ramos, K.**; Millán-Linares, M.C.; Haros, C.M. ***Foods***, DOI: 10.3390/foods9050663, 9, 663 (2020).
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La segunda coautora es investigadora del IG-CSIC, quien ha participado en la determinación del perfil aminoacídico por carecer de esta técnica en el IATA, es una colaboración.

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Tutor

"En el pensamiento científico siempre están presentes elementos de poesía. La ciencia y la música actual exigen de un proceso de pensamiento homogéneo"

Albert Einstein

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RESUMEN

Los hábitos alimentarios de los consumidores han cambiado progresivamente en las últimas décadas, buscando una alternativa de alimentos con alto valor nutricional e impacto en la salud. En ese sentido, los productos panarios son clave en la dieta y poseen potencial para ser adicionados con ingredientes nutritivos y saludables. Sin embargo, esto presenta un desafío tecnológico debido a la inclusión de ingredientes carentes de gluten, además de cumplir con las expectativas de los consumidores en cuanto a características de producto fresco con el mismo valor nutricional/funcional a pesar de no ser elaborado en el día. Para ello, la tecnología de elaboración de productos panarios precocidos y congelados asegura la disponibilidad del producto a cualquier hora del día. En este sentido, las harinas integrales de amaranto (*Amaranthus caudatus*, *Amranthus spinosus y Amaranthus hypochondriacus),* quínoa *(Chenopodium quinoa)* y/o semillas de chía *(Salvia hispanica)* y sus harinas integrales y desengrasadas, tras la extracción del aceite, son las materias primas investigadas en este estudio para ser incorporadas a formulaciones panarias por ser ingredientes con alto valor nutricional. Para ello, se determinó el nivel máximo de sustitución de harina de trigo por los nuevos ingredientes teniendo en cuenta la conservación de la calidad tecnológica, un mayor aporte nutricional y la aceptabilidad por parte de los consumidores. Con esta información se desarrolló una formulación optimizada con la inclusión de harinas integrales de amaranto, quínoa y chía, utilizando un diseño factorial (19% amaranto; 4% quínoa y 10 % chía). Este producto panario, con solo un 33 % de sustitución de harina, presentó mejores características tecnológicas, nutricionales y sensoriales que un producto integral de trigo. El

proceso de precocido/congelado/almacenamiento en congelación no mermó de manera significativa sus características tecnológicas, a excepción de algunos parámetros de textura, conservando su valor nutricional. La ingesta de este producto optimizado con mayor contenido de fibra, proteínas de alto valor biológico, ácidos grasos insaturados, mayor contenido de minerales y menor índice glucémico podría contribuir a la prevención del desarrollo de enfermedades precedidas por el síndrome metabólico. Las harinas sustitutas alternativas propuestas en esta investigación podrían incrementar sustancialmente el valor nutricional y funcional de alimentos a base de cereales a bajos porcentajes de sustitución.

ABSTRACT

Consumers eating habits have progressively changed in recent decades, seeking an alternative food with high nutritional value and impact on health. In this sense, bread products are key in the diet and have the potential to be added with nutritious and healthy ingredients. However, this presents a technological challenge due to the inclusion of gluten-free ingredients, along with meeting consumer expectations regarding fresh product characteristics with the same nutritional/functional value despite not being made on the same day. Therefore, the technology for making pre-cooked and frozen bread products ensures the availability of the product at any time of the day. In this sense, the wholemeals of amaranth (*Amaranthus caudatus*, *Amranthus spinosus* and *Amaranthus hypochondriacus*), quinoa (*Chenopodium quinoa*) and/or chia seeds (*Salvia hispanica*) and their wholemeal and defatted flours, after extracting the oil, are the raw materials investigated in this study to be incorporated into bakery formulations as they are ingredients with high nutritional value. For this, the maximum level of substitution of wheat flour by the new ingredients was determined, taking into account the conservation of technological quality, a greater nutritional contribution and acceptability by consumers. With this information, an optimized formulation was developed with the inclusion of wholemeal amaranth, quinoa and chia flours, using a factorial design (19% amaranth; 4% quinoa and 10% chia). This bread product, with only 33% flour substitution, presented better technological, nutritional and sensory characteristics than a whole wheat product. The pre-cooked/frozen/frozen storage process did not significantly reduce its technological characteristics, with

the exception of some texture parameters, preserving its nutritional value. The intake of this optimized product with a higher fiber content, high biological value proteins, unsaturated fatty acids, higher mineral content and lower glycemic index could contribute to the prevention of the development of diseases preceded by metabolic syndrome. The alternative substitute flours proposed in this research could substantially increase the nutritional and functional value of cereal-based foods at low substitution percentages.

RESUM

Els costums alimentaris dels consumidors han canviat progressivament durant les últimes dècades, tot i cercant aliments alternatius amb un alt valor nutricional i fort impacte sobre la salut. En aquest sentit, els productes de panificació són clau en la dieta i posseeixen potencial per ser addicionats amb ingredients nutritius i saludables. Tanmateix, això presenta un desafiament tecnològic a causa de la inclusió d'ingredients mancats de gluten, a més de complir amb les expectatives dels consumidors en quant a les característiques d’un producte fresc amb el mateix valor nutricional/funcional a pesar de no haver estat elaborat el mateix dia. Per això, la tecnologia d'elaboració de productes de panificació precuinats i congelats assegura la disponibilitat del producte a qualsevol hora del dia. En aquest sentit, les farines integrals d’amarant (*Amaranthus caudatus*, *Amranthus spinosus* i *Amaranthus hypochondriacus*), quinoa (*Chenopodium quinoa*) i/o les llavors de xia (*Salvia hispanica*) i les seves farines integrals i desgreixades, després de l'extracció de l'oli, són les matèries primeres investigades en aquest estudi per ser incorporades a formulacions de pa, ja que són ingredients amb un alt valor nutricional. Amb aquest objectiu, es va determinar el nivell màxim de substitució de la farina de blat pels nous ingredients, tot i considerant la conservació de la qualitat tecnològica, una major aportació nutricional i l'acceptabilitat per part dels consumidors. Amb aquesta informació s'ha desenvolupat una formulació optimitzada amb la inclusió de farines integrals d’amarant, quinoa i xia, mitjançant un disseny factorial (19% amarant; 4% quinoa i 10% xia). Aquest producte de panificació, amb només un 33 % de substitució de farina, va presentar millors característiques tecnològiques, nutricionals i sensorials que un producte integral de blat. El procés de precuinar/congelar/emmagatzemar en congelació no va minvar de manera significativa les seues característiques

tecnològiques, amb l’excepció d'alguns paràmetres de textura, tot i conservant el seu valor nutricional. La ingesta d'aquest producte optimitzat amb un major contingut de fibra, proteïnes d'alt valor biològic, àcids grassos insaturats i minerals, així com un menor índex glucèmic podria contribuir a la prevenció del desenvolupament de malalties precedides per la síndrome metabòlica. Les farines substitutes alternatives propostes en aquesta investigació podrien incrementar substancialment el valor nutricional i funcional dels aliments a base de cereals i baixos percentatges de substitució.

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Índice

I. INTRODUCCIÓN

1 Pan de trigo

1.1 Generalidades

El pan constituye la base de la alimentación desde hace 7000 u 8000 años (Cauvain, 2012). Al principio era una pasta plana, no fermentada, elaborada con una masa de granos machacados groseramente y cocida, muy probablemente sobre piedras planas calientes. Parece que fue en Egipto donde apareció el primer pan fermentado, cuando se observó que la masa elaborada el día anterior producía burbujas de aire y aumentaba su volumen, y que, añadida a la masa de harina nueva, daba un pan más ligero y de mejor gusto. Existen bajorrelieves egipcios (3000 años AC) sobre la fabricación de pan y cerveza, que sugieren que fue en la civilización egipcia donde se utilizaron por primera vez los métodos bioquímicos de elaboración de estos alimentos fermentados (Mesas & Alegre, The bread and its processing , 2002).

La harina de trigo (*Triticum aestivum*), es el principal ingrediente para la elaboración del pan y se puede considerar una harina panificable cuando contiene los siguientes componentes: humedad: 13 - 15%; proteínas: 9 - 14% (85% gluten); almidón: 68 - 72%; cenizas: 0.5 - 0.65%; materias grasas: 1 - 2%; azúcares fermentables: 1 - 2%; materias celulósicas: 3%; enzimas hidrolíticos: amilasas, proteasas, etc.; vitaminas: B y E (Mesas et al., 2002)

1.2 Valor nutricional del pan de trigo

El valor nutricional del pan se debe a la composición de la harina de trigo, que puede variar según la región, las condiciones de cultivo y el año de cosecha. El trigo es un cereal que constituye una buena fuente de hidratos de carbono, cantidades mínimas de lípidos y de aminoácidos esenciales entre ellos la lisina, por otro lado, aporta fibra, vitaminas y minerales, pero no es capaz de cubrir por si solo, las necesidades nutricionales de un individuo.

1.2.1 Proteínas

El contenido de proteína del trigo harinero es en promedio de 11 a 14% (Solah et al., 2016) y puede clasificarse acorde a su solubilidad o a su funcionalidad. La clasificación por solubilidad, fue desarrollada por Osborne (1924) y consiste en una serie de extracciones consecutivas con: agua, solución de salina diluida, solución de alcohol y solución de ácidos o álcalis diluidos. Utilizando esta secuencia de separación, las proteínas se pueden clasificar en albúminas, globulinas, gliadinas y gluteninas, respectivamente.

Desde el punto de vista de la tecnofuncionalidad, se pueden distinguir dos grupos de proteínas de trigo: proteínas pertenecientes al gluten, con un desempeño muy importante en la elaboración del pan y proteínas no pertenecientes al gluten, con un desempeño secundario en la elaboración del pan (Vega, 2009). Cabe indicar que el desempeño de la harina de trigo en la panificación se relaciona principalmente con las propiedades de las proteínas gliadina y glutenina. Además, influyen en la textura, el color, contribuyen en reacciones enzimáticas y asociación con lípidos. La reacción de Maillard ocurre simultaneamente con la caramelización y contribuyen a la formación del color de la corteza y al aroma del pan (Prost, et al., 2012). Por otro lado, el contenido de aminoácidos esenciales como la lisina y la treonina es reducido en comparación al contenido de los mismos en el pan integral (Dewettinck et al., 2008).

Considerando el consumo recomendado de pan de 250 g por día (Martínez et al., 2018), para un individuo adulto con un peso de 70 Kg, la contribución a la ingesta adecuada (IA) de proteína recomendada por EFSA (2012) es del 50% y 67% en pan de trigo y pan de trigo integral, respectivamente; demostrando que el pan integral tiene un mayor aporte en proteínas.

1.2.2 Lípidos

Los lípidos están presentes en los granos de trigo en una proporción de 2-4%, encontrándose en mayor proporción en el germen (aproximadamente 15%). En la harina están presentes en un valor cercano a 2%, siendo superior en la harina integral (Varela et al., 1995). En términos generales, los lípidos del trigo se clasifican en dos grupos: lípidos no polares y polares. A pesar de encontrarse en pequeñas cantidades, tienen un efecto significativo en la textura y en consecuencia en la calidad del producto final, por mejora de la habilidad de la masa para retener el dióxido de carbono proveniente de la fermentación (Goel, et al., 2021).

1.2.3 Carbohidratos

Los granos de cereales almacenan energía en forma de almidón. El almidón es el hidrato de carbono mayoritario en el grano de trigo maduro, representando entre el 65-70% de la harina de trigo (Cornell, 2012). La amilopectina suele constituir más del 75% del gránulo de almidón y la amilosa menos del 25%. Cuando la amilosa es superior a 28%, el cereal se describe como “alto en amilosa”, aunque el compuesto mayoritario sigue siendo la amilopectina.

Los gránulos de almidón, debido a su estado nativo parcialmente cristalino, cuando son sometidos a tratamiento térmico, se produce la gelatinización y posteriormente, la retrogradación. Es por ello que, durante la cocción de alimentos a base de cereales, se observa absorción de agua e hinchamiento del producto, por la gelatinización del almidón con la formación de gel que determina la apariencia y la textura del producto cocido. Cuanto mayor sea el contenido de amilopectina, más se hinchará el almidón y más suave será el gel formado. Por otro lado, el proceso de retrogradación implica el reordenamiento de las moléculas de amilosa o amilopectina tras la gelatinización y, finalmente, se observa un aumento de la cristalinidad (Cornell, 2012).

El fenómeno de envejecimiento del pan se debe en parte, a la retrogradación de la amilopectina, y esta a la relación amilosa/amilopectina, sus pesos moleculares y el contenido de lípidos (Roulet et al., 1990). Desde un punto de vista nutricional, todos los alimentos ricos en hidratos de carbono influyen de la misma manera en los niveles de glucosa e insulina posprandiales. Se ha propuesto que las dietas con índice glucémico (IG) alto promueven una ganancia de peso considerable

(Augustin et al., 2015). Entre los productos con IG alto (>70%) se encuentran los panes de trigo blanco e integral, patatas al horno y hervidas, mermeladas, galletas y dulces. A su vez, en los grupos de productos alimenticios con IG moderado (55-70%) y bajo (<55%), se encuentran principalmente panes elaborados con masas madre, legumbres, verduras y frutas. Cabe indicar que el consumo de alimentos con alto contenido de fibra, suelen tener IG más bajo con la consiguiente prevención del desarrollo de diabetes tipo 2 (Borczak et al., 2018).

1.2.4 Minerales y Vitaminas

El pan integral contiene numerosos minerales (Fe, Mg, Ca, P, Zn, Na, K), aunque su proporción puede verse sensiblemente reducida en los productos elaborados con harinas refinadas, debido a que la harina de trigo integral concentra altas cantidades de minerales en la fracción del salvado y germen (Whitney et al., 2017). El pan de trigo integral también contiene vitamina B1, niacina, pequeñas cantidades de vitaminas B6, E, C y ácido fólico y, carece, como en otros alimentos de origen vegetal, de vitaminas B12, D y retinol, excepto cuando al pan se le añade algún ingrediente de origen animal que las aporte (Varela et al., 1995)

La biodisponibilidad de los minerales en productos integrales está condicionada al contenido de fitatos. El fósforo presente en las harinas se encuentra principalmente como ácido fítico (hexakisfosfato de *mio*-inositol) o sus sales, los fitatos. En general, el ácido fítico se encuentra presente como fitatos de K, Mg, Zn, Fe y Ca. Los niveles de fitatos son mucho más elevados en las harinas integrales que en la harina. La mayor parte está presente como sal de calcio y magnesio llamada fitina. Sin embargo, la fitina puede unirse a otros elementos como el zinc y el hierro y causar deficiencias en dietas desequilibradas (**Figura 1**).

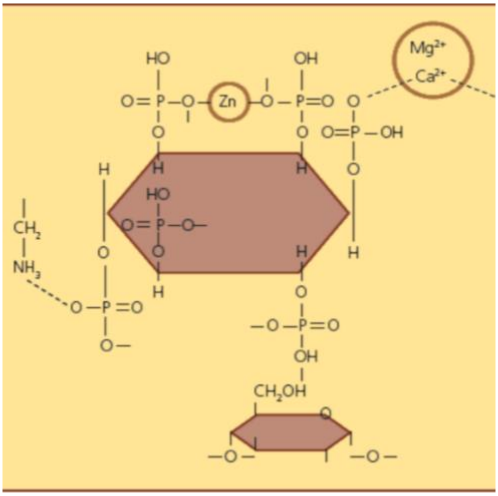

Figura 1. Complejo formado por una molécula de fitato con los cationes Zn, Mg y Ca (Adaptado de Kies, 1989).

1.2.5 Fibra dietética

La fibra dietética corresponde a los polisacáridos y lignina resistentes a la digestión enzimática gastrointestinal. Según su solubilidad puede clasificarse como: insoluble (FDI), constituida por celulosa, hemicelulosa y lignina, presente en mayor proporción en cereales y leguminosas, relacionada con el mejoramiento del tránsito intestinal; y soluble (FDS) conformada por pectina, gomas y mucílagos, presente principalmente en frutas y asociada con la reducción de colesterol y glucosa (Morales et al., 2012). El pan es deficiente en fibra y en otros nutrientes en contraste con el pan integral. En este sentido, el pan sustituido al 25 % con harina de trigo integral, aumenta el contenido de proteína (de 9,4% a 12,0%),

2 Incorporación de Ingredientes funcionales en productos panarios

2.1 Generalidades

El pan y los productos de panadería son alimentos presentes en nuestra vida y representan un componente esencial de la dieta humana a nivel mundial. Representan una matriz alimentaria apropiada para incluir ingredientes funcionales, debido a la alta compatibilidad de estos productos con una amplia gama de ingredientes funcionales, de alta demanda y consumo, y de costo relativamente bajo en comparación con otras categorías de alimentos.

El consumo de cereales integrales o grano entero se ha relacionado con beneficios para la salud, como la disminución del riesgo de padecer enfermedades crónicas precedidas por el síndrome metabólico, como se ha mencionado anteriormente (Jacobs et al., 2011).

Menos del 7% de la población de Estados Unidos consume el mínimo de la ración recomedada de 3 porciones diarias de granos integrales, mientras que el consumo de alimentos elaborados con harinas refinadas es 5 veces mayor que con harinas integrales (Ahluwalia et al., 2019). Ante este desequilibrio el Comité Asesor de Pautas Alimentarias (DGAC, 2015) recomendó que los estadounidenses deberían incluir en su dieta un mayor consumo de granos enteros (Millen et al., 2016).

Como resultado, la industria panaria ha incluido el uso de fibras, harinas integrales, leguminosas, oleaginosas, cereales y pseudocereales para aumentar el contenido de nutrientes y micronutrientes con el consiguiente aumento de la concentración de ácido fítico (InsP_6), el cual forma compuestos insolubles con minerales di y trivalentes en el tracto digestivo inhibiendo su biodisponibilidad. Existen estrategias que pueden incrementar la biodisponibilidad de los minerales como son: el uso masa madre (Baye et al., 2013), inclusión de fitasas exógenas, o iniciadores de la fermentación que producen fitasas (Rosell et al., 2009).

No obstante, al incorporar las harinas integrales como ingredientes funcionales en la elaboración de pan, no solo se presentan cambios a nivel nutricional sino también tecnológicos y sensoriales, que pueden o no ser aceptados o no por el consumidor final. Por este motivo, se ha intentado equilibrar la formulación adecuada de harinas integrales y el efecto de su inclusión en parámetros tecnológicos y sensoriales, los cuales condicionarán la intención de compra de un producto panario como son el volumen de la pieza panaria, la textura de la miga textura, el color de la corteza y miga y la masticabilidad del pan, entre otros aspectos.

2.2 Valor nutricional de panes elaborados con ingredientes funcionales

Dentro de la gama de productos panarios se encuentran galletas, panes, bizcochos y tartas que tienen como principal materia prima la harina de trigo, la cual brinda propiedades de viscoelasticidad idóneas para la preparación de la masa por la presencia de las proteínas que conforman el gluten, otorgándole características deseadas al producto en cuanto al volumen, textura, color, entre otros. Sin embago, la harina es deficiente en aminoácidos esenciales, especialmente lisina, triptófano y treonina (Codină, 2022). La utilización de ingredientes tales como las harinas integrales de cereales, legumbres, tubérculos, pseudocereales, oleaginosas, insectos y frutas en la elaboración del pan incrementa el contenido de lípidos, minerales y contenido de proteína con mejor perfil aminoacídico y fibra total (**Tabla 1**). Por lo tanto, la inclusión de estos nuevos ingredientes presenta un desafío tecnológico por lo que se requiere optimizar los procesos de elaboración de las nuevas formulaciones panarias en cuanto a la temperatura y tiempo de horneado, tipo de levadura, tiempo de fermentación, el uso o no del vapor, tipo de proceso, entre otros factores (Bredariol & Vanin, 2022).

La inclusión de ingredientes saludables en formulaciones panarias tienen una alta aceptación por parte del consumidor, que además de buscar alimentos nutritivos prefiere alimentos con valor añadido en cuanto a que la ingesta de los mismos ayude a la prevención de desarrollar enfermedades (Martinez-Saez et al., 2017).

2.2.1 Cereales

Entre los cereales/pseudocereales utilizados en la industria panadera, además del trigo, se encuentran el maíz, la avena, el centeno, el arroz, el sorgo, la cebada y el trigo sarraceno. Estudios realizados con la harina integral de cebada, sorgo y avena, aportaron productos con altas cantidades de fibra dietética, lípidos, proteínas, con mayor contenido de aminoácidos esenciales (lisina y metionina) en el caso particular de la cebada. En cuanto a la calidad de los productos panarios, el incremento del porcentaje de sustitución de la harina, incrementó la firmeza de la miga, redujo el volumen de la pieza panaria, y su aceptabilidad del producto entre los panelistas. Las formulaciones más acogidas fueron aquellas con un 10% de harinas integrales, seguidas por las formulaciones sustituidas hasta el 30% con harina de sorgo, cebada y avena (Alu'datt et al., 2012; Peymanpour et al., 2012). El pan multigrano compuesto con avena, trigo sarraceno, centeno y trigo (20%,20%,20%,40%, respectivamente), cuadruplicó el contenido de fibra total en comparación al pan de trigo (Collar, 2016). La ingesta de fibra dietetica en cantidad adecuada, produce una respuesta fisiológica más efectiva cuando presenta una ratio de fibra soluble/insoluble de 1:2 (Jaime et al., 2002). En este sentido, formulaciones sustituidas parcialmente por harinas integrales de cereales/pseudoceresles/oelaginosas/legumbres traerían un gran beneficio, especialmente en la dieta de personas que no logran una ingesta adecuada de fibra dietética total, con efectos saludables como son la reducción el colesterol en sangre, menores IG, prevención de estreñimiento, lo que conllevaría a la disminución del riesgo de desarrollar diabetes de tipo 2 o problemas cardiovasculares, entre otras (Nohra & Bochicchio, 2015; Slavin, 2013).

2.2.2 Legumbres

El efecto de la adición de harinas de legumbres (altramuz, garbanzo, judía, guisante, haba, lenteja, algarroba, entre otras) en productos panarios ha sido ampliamente estudiado, ya sean germinadas o sin germinar, las cuales afectan la calidad del producto final. Doxastakis et al. (2002), observaron que los panes elaborados con altarmuz tuvieron menor volumen por el efecto de la dilución del gluten, y el color de la corteza más oscura. Sin embargo, los análisis sensoriales indicaron que los panes enriquecidos con 5 y 10% de harina de altramuz presentan textura, sabor y color altamente aceptable (Dervas et al., 1999). Según lo informado por Turfani et al. (2017), la mezcla de harina de trigo con harina de semilla de lenteja disminuyó la tenacidad y la fuerza de la masa, mientras que la inclusión de harina de algarroba presentó el efecto contrario.

Tabla 1. Propiedades nutricionales de productos panarios parcialmente sustituidos por harinas integrales de otros cereales, pseudocereales, legumbres u oleginosas

Panes elaborados con ingredientes funcionales	Condiciones de panificación	Formulación	Proteinas (%)	Lípidos (%)	Fibra total (%)	Fibra soluble(FS)/Fibra Insoluble (FI) (%)	Minerales (mg/100g)	Referencia
Cereales								
Pan de avena,trigo sarraceno, centeno y trigo	N.I.	20%,20%,20%,40%	9,43	1,01	7,29	FS: 1,6 FI: 5,33	Ca: 21,4 mg Zn: 0,97 mg Fe: 1,4 mg	Collar (2016)
Pan de Sorgo y trigo	Temperatura (160-170ºC) por 30-60 min.	80%/20%	44,4	6,1	2,4	N.I.	N.I.	Mallasy, El Tinay, and Ahmed (2002)
Legumbres								
Pan de garbanzo+trigo	Temperatura (220ºC) por 45 min.	30%/70%	16,9	3,4	6,3	N.I.	N.I.	Man, Paucean, Muste, and Pop (2015)
Pan de lenteja+trigo	Temperatura (180ºC) por 62 min.	24%/76%	25,52	0,80	19,4	F.S: 2,9 F.I: 16,5	N.I.	Celik and Ilyasoglu (2022); Turfani, Narducci, Durazzo, Galli, and Carcea (2017)
Pseudocereales								
Pan de Quínoa+trigo	N.I.	25%/75%	13,2	2,2	8,51	FS: 2,13 FI: 6,38	Ca: 21,7 mg Zn: 1,1 mg Fe: 0,69 mg	Ballester-Sánchez, Millan-Linares, Fernandez-Espinar, and Haros (2019)

Tabla 1 (continuación)

Panes elaborados con ingredientes funcionales	**Condiciones de panificación**	**Formulación**	**Proteinas (%)**	**Lípidos (%)**	**Fibra total (%)**	**Fibra soluble(FS)/Fibra Insoluble (FI) (%)**	**Minerales (mg/100g)**	**Referencia**
Pan de Amaranto+trigo	Temperatura (225ºC) por 20 min	40%/60%	16,30	1,75	5,90	FS: 1,73 FI: 4,17	Ca: 0,99 mg Zn: 24,91 mg Fe: 43,74 mg	Sanz-Penella, Wronkowska, Soral-Smietana, and Haros (2013)
Oleaginosa								
Pan de semilla de Chía+trigo	Temperatura entre 190 °C/18 min and 170 °C/23 min	5%/95%	16,9	2,11	6,82	N.I.	N.I.	Iglesias-Puig and Haros (2013)
Pan de semilla de lino+trigo	Temperatura 230°C / 30 min	15%/85%	16,30	0,95	12,97	FS: 6,38 FI: 6,59	N.I.	Wirkijowska et al. (2020)

FS. Fibra Soluble; FI: Fibra Insoluble; N.I: No informado

La mezcla con harina de garbanzo, muestra una reducción en el volumen y aumento de la firmeza lo que resultó en una menor aceptabilidad del consumidor si la sustitución era mayor a 10%. La mezcla de las harinas de legumbres incrementa el contenido de minerales, proteína, fibra total, compuestos fenólicos, lisina y lignanos (Man et al., 2015; Turfani et al., 2017). Por otro lado, la incorporación de la harina de garbanzo y altramuz en la dieta diaria pueden contribuir a un retardo en la hidrólisis del almidón y por ende una diminución del IG, así como el incremento de la saciedad (Amoah et al., 2022; Collar, 2016).

2.2.3 Pseudocereales

Las harinas de amaranto, quínoa y trigo sarraceno, poseen un alto valor nutricional destacándose por su contenido de fibra total, ácido linoleico, ácido linolénico, lisina, así como de minerales (Martínez-Villaluenga et al., 2020).

Los panes reemplazados al 8% con harina integral de amaranto, no presentaron modificaciones en sus parametros tecnológicos, pero si un aumento en el contenido proteico. Sin embargo, cuando los panes tenían mayor porcentaje de sustitución (10, 15 y 20%) además de incrementarse el contenido proteico, aumentó significativamente el contenido de minerales tales como el hierro, además del contenido de fibra dietética y escualeno, pero disminuyó en un 33% el volumen específico de la pieza panaria, con el consiguiente incremento de la dureza de la miga de menor elasticidad (Bodroza-Solarov et al., 2008). Los panes sustituidos con harina de quínoa en diferentes proporciones (85:15, 70:30, 55:45, 40:60, 25:75, y 10:90) también fueron afectados negativamente en cuanto a su volumen, dureza y masticabilidad en comparación a la muestra sin sustituir (Wang et al., 2015). Sin embargo, estos productos presentaron alta aceptabilidad por los consumidores. Las formulaciones con harina integral de quínoa incrementaron el valor nutricional en terminos de fibra, minerals y lípidos (Calderelli et al., 2010; Iglesias-Puig et al., 2015; Stikic et al., 2012).

Los pseudocereales, en general, mejoran el contenido de Fe, K, Mg, Mn, and Zn pero también incrementan significativamente el contenido de fitatos (Bilgicli & Ibanoglu, 2015; Iglesias-Puig et al., 2015, Haros & Schoenlechner, 2017). La inclusión de harinas integrales de amaranto y quínoa incrementan el valor nutricional de los alimentos que los contienen y puede ayudar a prevenir el desarrollo de enfermedades precedidas por el síndrome metabólico, combatir la malnutrición en los países de orgen, e influir en la disminución del IG (Martínez-Villaluenga et al., 2020).

2.2.4 Oleaginosas

Las oleaginosas más utilizadas en productos de panadería son el lino, nueces, girasol, olivas, sésamo, cacahuete, y más recientemente la chía, entre otras. La inclusión al 10% de semillas de lino en formulaciones de galletas, aporta ácidos grasos insaturados, principalmente omega-6 y omega-3, además de incrementar el nivel de lignanos hasta 30 y 170 veces más, en comparación con las galletas elaboradas con harina integral y harina, respectivamente (Čukelj et al., 2017).

Las semillas de chía y su harina integral se incluyeron en formulaciones panarias con a un nivel de sustitución de 5%, obteniendo productos con mayor contenido de proteínas, lípidos con altas concentraciones de ácidos grasos omega y fibra dietética (Iglesias-Puig and Haros, 2013). La adición de chía no alteró las propiedades de la masa panaria, a excepción de una mayor absorción de agua, principalmente debido a la presencia de mucílago. Desde un punto de vista tecnológico, no se encontraron diferencias significativas de las muestras adicionadas con chía en comparación a la muestra control, a excepción del volumen de la pieza panaria, parámetros de color de la corteza y el retardo del envejecimiento. Cabe señalar que las formulaciones con semillas o harina integral de chía mostraron mayor aceptación por parte del consumidor comparada con la muestra control (Iglesias-Puig et al, 2013; Coelho & Salas-Mellado, 2015).

La adición de harina de chía o semillas a 7.8 g/100g y 11.0 g/100g mostraron ventajas nutricionales en cuanto al contenido y la ratio de ácidos grasos poliinsaturados (PUFAs) y ácidos grasos saturados (SFAs) con valores de 3,1 y 3,9, respectivamente, en comparación a 1,01 para el pan control de trigo (Coelho & Salas-Mellado, 2015), entre los que destaca el ácido alfa-linolénico. La inclusión de semillas de chía origina productos enriquecidos con fibra dietética, vitaminas, antioxidantes y minerales, entre los que se encuentran el potasio, magnesio, calcio y manganeso.

2.2.5 Frutas y vegetales

En productos de panadería podrían ser suplementados por frutas, verduras o algunas de sus partes como son la piel, tallos, hojas, extractos, entre otros. Un ejemplo de ello es la incorporación de piel de la cebolla o tallo de alcahofa en polvo confiriendo propiedades antioxidantes y con una alta concentración de flavonoides, lo que los convierte en ingredientes con potencial funcional para la modulación parcial del metabolismo de la glucosa (Colantuono et al., 2018; Piechowiak et al., 2020). Estudios realizados por Vergara-Valencia et al. (2007) tuvieron como objetivo caracterizar un concentrado de fibra dietética de mango, que presentó actividad antioxidante. La fibra dietética de mango presentó un alto contenido de almidón y un bajo contenido de lípidos, además de niveles equilibrados de fibra dietética insoluble y soluble. El análisis de digestibilidad del almidón *in vitro* de los productos obtenidos mostró un IG bajo, lo que hace que la fibra dietética de mango se convierta en una opción para obtener productos con niveles equitativos de fibra dietética e IG más favorables en productos panarios (Vergara-Valencia et al., 2007).

Por otro lado, la inclusión de orujo de grosella negra, con alto contenido de fibras, mostró algunos efectos negativos en las propiedades tecnológicas de la masa, en términos de textura, viscosidad y estructura del producto. Muestras de harina suplementadas con harinas de cáscara de plátano y tuna presentaron un mayor contenido de fibra, fenoles totales y flavonoides en comparación con la muestra control, lo que demuestra el potencial de estos ingredientes alternativos para ser utilizadas como ingredientes con posibles efectos funcionales (Mahloko et al., 2019).

3 Tendencia moderna en panificación

Los hábitos alimenticios de los consumidores han cambiado progresivamente hace 60 décadas atrás, buscando una alternativa de alimentos funcionales con alto valor nutricional. En ese sentido y considerando que el pan es un alimento cotidianamente consumido, se espera que sea fresco y con buenas propiedades nutricionales al momento de su consumo. Por lo tanto, se han desarrollado estrategias tecnológicas convencionales y no convencionales enfocadas a extender la vida útil del pan, retardando el envejecimiento del mismo y evitando las alteraciones de origen microbiológico, debido a la migración del agua entre los componentes de la pieza panaria (Amigo et al., 2019).

3.1 Mejora de la vida útil del pan utilizando técnicas convencionales

Los preservantes químicos utilizados tradicionalmente son el benzoato de sodio (E211), ácido sórbico (E200), ácido propiónico (E280), ácido benzoico (E210), propionato cálcico (E282) y sorbato de potasio (E202), entre otros, siendo el principal objetivo de su inclusión extender la vida útil del pan e inhibir el crecimiento de mohos (Guynot et al., 2005; Samapundo et al., 2017).

Otros aditivos como el ácido ascórbico (E300), acetato cálcico (E263), propionato sódico (E281) y los emusilficantes como las lecitinas (E322), monoglicéridos (E471) y diglicéridos (E471) de ácidos grasos han tenido siempre un importante papel en las propiedades tecnológicas del pan, aumentando la elasticidad de la masa, mejorando la suavidad de la miga y la textura del producto. Así también, los hidrocoloides que actúan como agentes gelificantes, modificando el proceso de gelatinización del almidón (Chakraborty et al., 2019; Schoenlechner et al., 2013).

El uso de masa madre y/o bacterias del ácido lácticas (LAB) es una tecnología muy difundida para la mejora de las características físicas y sensoriales del pan, especialmente el sabor, pero también para la mejora de la vida útil (Sol et al., 2020). Los resultados de diversos estudios demuestran que la mezcla de *Lactobacillus sanfranciscensis* y *Candida milleri*, mejoran las propiedades reológicas, el aroma e incrementa la vida útil del pan. Resultados similares fueron observados con el uso de la masa madre obtenida a partir de cepas seleccionadas de bacterias ácido lácticas productoras de exopolisacáridos (Scarnato et al., 2017; Torrieri et al., 2014).

Por otra parte, los productos congelados tienen como objetivo, detener el deterioro microbiano, sin embargo, se ha encontrado que las temperaturas de congelamiento afectan el aspecto del producto final. Tal como fue observado por Meziani et al. (2011), las bajas temperaturas de congelación en muestras de masa dulce, afectaron las propiedades reológicas y sufrieron una pérdida de elasticidad de 8,6 % y 12% a temperaturas de -30 °C y -40 °C, respectivamente.

La técnica de envasado en atmósfera de nitrógeno y la adición de extracto de planta de romero fue ensayada en palillos de pan. Se observó que las muestras adicionadas con el extracto de planta incrementaron su vida útil hasta un 42%, significativamente superior a las muestras almacenadas en atmósfera de nitrógeno. Usando ambos métodos se consiguió extender la vida útil de los palillos de pan hasta un 84% a 25°C (Alamprese et al., 2017).

3.2 Mejora de la vida útil del pan utilizando técnicas innovadoras

Los tratamientos de ultra-altapresión (UHP) son ampliamente usados puesto que ejercen influencia en las características funcionales del producto y existen estudios que confirman la eliminación de microorganismos debido a la utilización de empaquetamiento aséptico. Por otro lado, la aplicación de pulsos eléctricos, mostró que a altos voltajes se incrementa la habilidad del gluten para retener y absorber agua (más que los propios gránulos de almidón) por lo que se podría reducir la migración de humedad retrasando el envejecimiento (Esaki et al., 1996). El tratamiento de radiofrecuencia empleado por Jeong, Baik, and Kang (2017), en pan blanco a 60 °C permitió controlar el crecimiento de moho durante 10 días de almacenamiento sin reducción del contenido de tiamina.

La disponibilidad de pan fresco a cualquier hora del día y la existencia de una gran variedad de productos con diversidad de aromas, formas y tamaños son algunas de las exigencias de los consumidores. En este sentido, la aplicación de la tecnología del precocido o parcialmente horneado asegura la disponibilidad del producto a cualquier hora del día y con una buena aceptación. Se entiende por pan precocido el pan cocido en dos tiempos; el tiempo de precocción oscila entre 10 y 15 minutos, posteriormente se utiliza refrigeración o congelación y, por último, envasado en atmósfera modificada 100% N_2 y 30% CO_2 (Kurek et al., 2019). Diversos estudios se han centrado en determinar el efecto de la fermentación, el horneado parcial y el efecto de la congelación (Bárcenas et al., 2002). En la **Figura 2** se muestra el proceso de elaboración de pan precocido comparado con el proceso convencional de panificación.

Otros investigadores han estudiado el efecto del precocido, almacenado en congelación y horneado final, con respecto a las propiedades tecnológicas con alta aceptación por parte del consumidor.

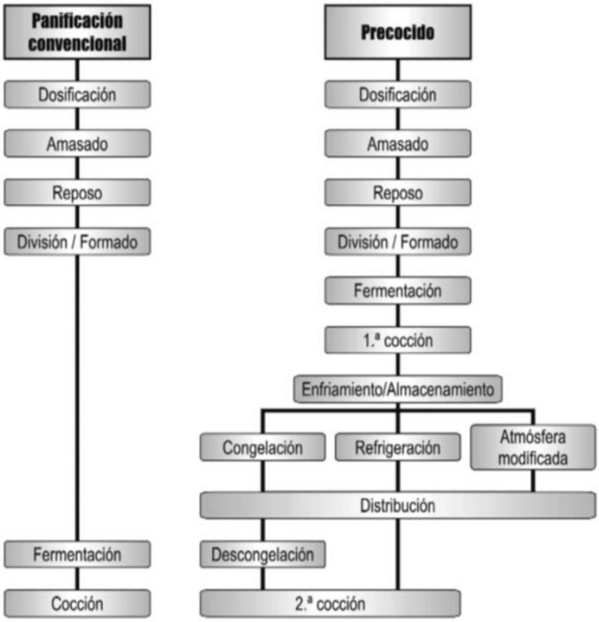

Figura 2. Proceso de panificación de pan precocido comparado con el proceso convencional. Adaptado de Ribotta & Tadini (2009).

Gujral, Singh, and Rosell (2008) elaboraron panes con harina integral, precocidos y almacenados a -18°C, observando que la extensibilidad de la masa fue superior a la de la masa horneada convencionalmente. Los efectos de la metodología del precocido y congelado en panificación no han sido abordados en cuanto a la conservación de la calidad nutricional de los productos, importante por el creciente interés en la inclusión de ingredientes saludables, lo que constituye un reto para la innovación en este sector industrial.

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II. OBJETIVOS

El objetivo principal de esta investigación fue desarrollar nuevas formulaciones de masas panificables precocidas y almacenadas en congelación, empleando harinas de amaranto, quínoa y/o chía con alto valor nutricional y funcional que contribuyan a una dieta saludable.

Para la consecución del objetivo principal se plantean los siguientes objetivos particulares:

1. Caracterizar las harinas de trigo, amaranto, quínoa y semillas de chía en cuanto a su calidad tecnológica, nutricional y funcional.
2. Desarrollar productos de panadería mediante sustitución parcial de la harina de trigo con harina de quínoa, amaranto y/o semillas de chía para encontrar la proporción óptima en cuanto a calidad tecnológica, sensorial, nutricional y funcional.
3. Evaluar las propiedades térmicas del almidón durante las etapas de precocción, almacenamiento en congelación y horneado, e interpretación de los mecanismos y su implicación en la textura final del producto terminado.
4. Evaluar el efecto del proceso y la sustitución de harina por harinas especiales sobre la calidad nutricional de las proteínas y perfil lipídico, índice glucémico y biodisponibilidad mineral de los productos desarrollados.

III. RESULTADOS Y DISCUSIÓN

CAPÍTULO 1

Evaluation of technological and nutritional quality of bread enriched with amaranth flour

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ABSTRACT

The objective of this investigation was to develop bread, with high nutritional and technological quality, using whole flour of Amaranthus spinosus and Amaranthus hypochondriacus. Bread quality was analyzed in terms of chemical composition, loaf specific volume, width/height ratio of the central slice, crust/crumb color, crumb structure and firmness, and sensory analysis. Starch thermal properties were studied in terms of enthalpies of starch gelatinization during baking and amylopectin retrogradation during storage. Incorporation of amaranth flour significantly increased protein, lipid, fiber, ash, and myo-inositol phosphate contents. Bread with amaranth flours exhibited soluble/insoluble fiber ratios close to 1:2, which presents the most effective physiological action. Intake of products with high substitution of amaranth could cover the protein requirement in adults, and could contribute substantially to intake of dietary fiber, Fe, and Zn according to daily recommendations. Bread with A. hypochondriacus showed higher acceptability than formulations with A. spinosus. Inclusion of amaranth allowed delaying and decreasing crumb staling in terms of amylopectin retrogradation. The inclusion of amaranth could be limited to a maximum of 25 g/100 g, with considerable nutritional improvement and acceptable sensory and technological quality, even during the staling process.

INTRODUCTION

Wheat bread is a popular food in the category of bakery products. Refined wheat flour is the most used raw material for this product but it has a low level of essential amino acids such as lysine and threonine, and reduced fiber content compared to whole flour bread. In fact, many studies have been aimed at improving wheat flour nutritional characteristics by the incorporation of new functional ingredients and at developing safe, healthy, nutritious products (Dewettinck et al., 2008). An alternative could be bakery products enriched with a high amount of dietary fiber, amino acids and bioactive compounds from whole flours or entire grain of cereal/pseudocereal that help to prevent diseases associated with the metabolic syndrome such as cardiovascular diseases, arteriosclerosis, and colon cancer (Salas-Salvadó, et al., 2006; Motta et al., 2019). The health benefits of pseudocereals are well known, and one of the most important of them is amaranth (Amaranthus spp.), which is a native plant found in South and Central America, with the exception of some species that belong to tropical and subtropical regions of India, such as Amaranthus spinosus (Reguera & Haros, 2017). From a nutritional point of view, pseudocereals are considered better than cereals such as wheat, barley, or rice because of their content/composition of starch, oil, dietary fiber, vitamins (A, K, B6, C, E, and B), and minerals such as calcium, magnesium, phosphorus, iron, potassium, zinc, copper, and manganese (Alvarez-Jubete et al., 2009; Reguera & Haros, 2017). Although many species are considered opportunistic weeds, only three of them are used for human consumption: A. caudatus (or A. paniculatus, kiwicha, o quihuicha), A. cruentus (or A. flavus, or A. leucocarpus), A. hypochondriacus (or A. edulis, or A. mantegacianus) (Gamel et al., 2006; Sangameswan & Jayakar, 2008; Reguera & Haros, 2017). However, studies have established that the seeds or plant parts of A. spinosus possess various biological activities such as analgesic and anti-inflammatory, anti-diabetic, anti-hyperlipidemic and spermatogenic effects (Sangameswaran & Jayakar, 2008; Jhade et al., 2009; Rjeibi et al., 2016). Amaranth has managed to capture growing interest as a functional ingredient because of its high-quality nutritional and technological properties, especially in baking processes, and because it is very versatile for processing and industrialization (Sanz-Penella, et al., 2013). Amaranth flour is being used in tortillas, breads, cakes, muffins, pancakes, cookies, dumplings, crepes, noodles, and crackers (Alvarez-Jubete et al., 2009). The inclusion of A. cruentus whole flour in bread formulations by flour substitution of up to 40 g/100 g increased protein and total dietary fiber content, but this was accompanied by a marked decrease in sensory acceptability (Sanz-Penella et al., 2013). Amaranth usually contains antinutrients such as phytic acid (myo-inositol (1,2,3,4,5,6)-hexakisphosphate, InsP6) or its salts, phenolic compounds, and trypsin inhibitors (D’Amico et al., 2017). Phytic acid has negative health effects in cases of mineral deficiency because it inhibits mineral availability (Sanz-Penella et al., 2013). Phytate content in Amaranthus spp. has been found to range from 4.8 to 21.1 mol/g (Reguera & Haros, 2017). However, several studies have suggested that this compound has favorable effects, such as antioxidant function, prevention of heart diseases, and an anticarcinogenic effect, which it performs through its hydrolysis products (Haros et al., 2009; Kumar et al., 2010). There are previous studies with regard to bread made with replacement of flour by whole A. cruentus flour (Bodroza-Solarov et al., 2008; Sanz-Penella et al., 2013; Garcia-Mantrana et al., 2014). More recently, the investigations with A. hypochondriacus as a bakery ingredient were substituted up to 25 % level (Kamoto et al., 2018) or in specific breads without fermentation (Banerji et al., 2018). However, there is a lack of information about the effect of replacing wheat flour with other amaranth species, such as A. spinosus. Accordingly, the purpose of this research was to develop bread with high nutritional, technological, and sensory quality, replacing wheat flour with a high level (up to 50 %) of whole amaranth flour of A. spinosus from India, and it was compared to formulations with A. hypochondriacus from Mexico.

MATERIALS AND METHODS

Materials

Commercial wheat flour (Triticum aestivum L.), whole A. spinosus grains from India, and whole A. hypochondriacus grains from Mexico were purchased from the local market (Corporación Proteína Americana, SCRL, and Bio Cesta, MTV, Spain, respectively). The characteristics of the wheat flour, whole A. spinosus flour, and whole A. hypochondriacus flour is shown in Table 1.1. Saccharomyces cerevisiae (Levamax, Spain) was used as starter for the breadmaking process.

Table 1.1

Raw material composition

Composition	**Units**	**Wheat flour**	***A. spinosus***	***A. hypochondriacus***
Moisture	%	13.59±0.05	11.07±0.00	10.50±0.01
Proteins	% d.m.	10.2±0.3 (Nx5.7)	14.07±0.01 (Nx5.85)	14.80±0.09 (Nx5.85)
Lipids	% d.m.	1.08±0.11	6.07±0.01	5.94±0.01
Ash	% d.m.	0.09 ±0.03	2.77±08	2.62±0.02
$\mathrm{Ins}P_6$	μmol/g	n.d.	21±2	18±2
Ca	mg 100 g^{-1} d.m.	56.2±0.1	244.3±0.7	270.3±1.0
Fe	mg 100 g^{-1} d.m.	1.2±0.1	8.39±0.04	8.34±0.02
Zn	mg 100 g^{-1} d.m.	0.65±0.01	3.6±0.1	3.81±0.01

[a] Mean±SD. n=3. Values followed by the same letter in the same column are not statistically different at 95% confidence level. Codes: d.m., dry matter; InsP6: myo-inositol hexakisphosphate or phytates; n.d., not detected.

Breadmaking procedure

The bread dough formula expressed on a flour basis consisted of different flour formulations (500 g), compressed yeast (5%), sodium chloride (1.8%), and water up to optimum absorption corresponding to 500 BU (Brabender Units) (between 50% and 60%, depending on the bread dough formulation). Five formulations were studied, on a flour basis: 100% refined wheat flour as Control (C), and 4 formulations in which wheat flour was replaced with 25% of whole A. spinosus flour (WAs-25), 50% of whole A. spinosus flour (WAs-50), 25% of whole A. hypochondriacus flour (WAh-25), and 50% of whole A. hypochondriacus (WAh-50). A two-stage sponge and dough method was used according to the methodology described by Iglesias-Puig et al. (2015). Finally, the samples were baked at 170 °C/20 min for control sample, 165 °C/25 min for WAh-25 and WAs-25, and 165 °C/25 min for WAh-50 and WAs-50. The breads were cooled at room temperature for 2 hours for further analysis (Sanz-Penella et al., 2009).

Composition of raw materials and bread

Moisture was determined by a gravimetric method (AACC 44-15A), ash content was determined in a muffle by incineration at 900 °C (ICC 104/1, 1990), protein determination was carried out by the Kjeldahl technique (AACC 46-13), lipids were determined by extraction with petroleum ether reflux conditions by the Soxhlet technique (AOAC Official Method 945.16), and dietary fiber content was measured by an enzymatic-gravimetric method (AOAC Official Method 991.43).

Agricultural Science (Madrid, Spain). Previously, samples (0.5 g) were placed in a Teflon perfluoroalkoxy (PFA) vessels and treated with 4 mL HNO3 14 M (Merck, Germany) and 1 mL of H2O2 30% (v/v) (Panreac Quimica, Spain). The Teflon PFA vessels were irradiated at 800 W (15 min at 180 °C) in a Microwave Accelerated Reaction System (MARS) from CEM (Vertex, Spain). At the of the digestion program, the digests were placed in polypropylene tubes and made up to final volume with 5% HCl. Measurements were done in triplicate.

Determination of myo-inositol phosphates

InsP6 present in the flours and the remaining InsP6 and lower myo-inositol phosphates generated during the breadmaking process (InsP5, InsP4, and InsP3) were purified by ion-exchange chromatography and measured by the HPLC method described by Türk and Sandberg (1992), later modified by Sanz-Penella, Collar, & Haros (2008).

Technological characteristics of bread

The technological parameters analyzed were as follow: loaf specific volume (cm3/g) by the measured of the volume (cm3) by seed displacement (volume-meter, Chopin, France) and the weight (g), width/height ratio of the central slice (cm/cm), and color parameters (Chromameter CR-400, Konika Minolta Sensing, Japan).

Crumb texture was determined by texture profile analysis using the TA-XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). A bread slice of 2 cm thickness was compressed twice by using a stainless steel 1.0 cm diameter plunger, moving at 1.0 mm/s to a penetration distance of 50%, with an interval of 50 s between compressions. The following parameters were evaluated: relative firmness, springiness, cohesiveness, chewiness, and resilience (Haros et al., 2002).

Digital image analysis was used to measure bread crumb structure. Images were previously squared at 240 pixels per cm with a flatbed scanner (HP ScanJet 4400C, Hewlett-Packard, USA) supporting by HP PrecisianScan Pro 3.1 Software. A single 10 mm × 10 mm square field of view of two central slices (10 mm thick) of each of two loaves was used, thereby yielding 4 digital images per treatment. Data was processed using Sigma Scan Pro Image Analysis Software (version 5.0.0, SPSS Inc., USA). The crumb grain features chosen were Cell Area/Total Area, cm2/cm2; Wall Area/Total Area, cm2/cm2; number of cells per cm2; and Mean Cell Area, µm2 (Sanz-Penella et al., 2009).

Preliminary sensory analysis of the fresh breads was performed by a panel of 25 untrained tasters who usually consume wheat bread, using a nine-point hedonic scale of global acceptance (Iglesias-Puig et al., 2015).

Differential scanning calorimetry (DSC) analysis

The thermal properties of starch flour during baking of the fermented dough (gelatinization) and changes induced during the bread storage (amylopectin retrogradation) were measured on a calorimeter (DSC-7, PerkinElmer, USA) according the methodology described by León et al. (1997), later modified by Sanz-Penella et al. (2009).

Statistical analysis

Multiple sample comparison of means and Fisher's least significant difference (LSD) test were applied to establish statistically significant differences between treatments. All statistical analyses were carried out with Statgraphics Plus 7.1 software (Bitstream, Cambridge, MN), and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Bread Performance

The incorporation of increasing percentage of amaranth flour in the dough formulations progressively and significantly increased protein, lipid, and ash contents with regard to the control sample (Table 1.2). The higher levels of these nutrients registered in the raw amaranth flours in comparison with the wheat flour directly affected these parameters, as expected. These results agree with other studies on breads incorporating a different species of amaranth (Sanz-Penella et al., 2013). Those authors also found a higher moisture content when they increased the wheat flour substitution from 38.8 % (control sample) to 40.5 % (bread with A. cruentus at 40% substitution level). However, in this study only the substitution with A. hypochondriacus at 50% showed a significant difference in this parameter with regard to the control bread (Table 1.2). Despite the higher water absorption of flour blends measured by the farinograph, from 55.0% for wheat flour to 57.5%–60.5% for the wheat/amaranth combinations, with a higher water retention capacity of the doughs when the whole amaranth flour in the formulation was increased, in general, amaranth did not significantly modify the moisture of the fresh bread, with the exception of WAh-50 (Sanz-Penella et al., 2008). However, it has been suggested that a higher amount of total dietary fiber is related with an increase of moisture, mainly caused by the greater number of hydroxyl groups in the fiber structure, which allow more water interaction through hydrogen bonding than in refined flour (Sudha et al., 2007). In the current study, it was probably because of the use of a strong wheat flour in formulations with a high protein content that the water not only bound to starch and fibers but also to the gluten network, both in the control sample and in the samples with amaranth (Salas-Mellado & Haros, 2016). As was expected, the incorporation of whole amaranth flours in the formulation progressively and significantly increased the total dietary fiber (Table 1.2). In general, previous studies have shown that pseudocereals are a good source of dietary fiber (Alvarez-Jubete et al., 2009; Iglesias-Puig et al., 2015; Reguera & Haros, 2017). In the current investigation, the amount of insoluble dietary fiber increased significantly with the inclusion of amaranth flours, from 3.9 to 7.9 g/100 g with regard to the control bread. These values are higher than the results obtained in bread with whole quinoa flour at 25 and 50%, in which the amount of insoluble fiber was 3.7 and 4.4 g/100 g, respectively (Iglesias-Puig et al., 2015). It is noteworthy that a similar trend was observed in bread formulations with up to 40% replacement with A. cruentus flour (Sanz-Penella et al., 2013). In general, cereals and pseudocereals have more insoluble fiber, mainly consisting of lignin and cellulose. However, in amaranth there is more total dietary fiber than in common cereals, and a higher concentration of soluble fiber (Repo-Carrasco-Valencia et al., 2017). Dietary fiber exhibits the most effective physiological action at a soluble/insoluble ratio of 1:2 (Jaime et al., 2001), but the diet contains a variety of foods, such as wholegrain cereals, nuts, and vegetables, with a ratio of 1:3 (Salas-Salvadó et al., 2006). In the current investigation, an increase in the whole amaranth flour content resulted in breads with soluble/insoluble fiber ratios closer to 1:2 than 1:3, with the exception of WAs-50, which was close to the latter ratio (Table 1.2). On the other hand, in the formulation with 50% of A. spinosus the amount of 11.7 g/100 g of total dietary fiber (Table 1.2) is close to the minimum percentage required daily (25 grams per day according to the WHO/FAO, 2004), assuming an intake of 250 g of bread per day. The substitution of 50% of wheat flour by whole A. spinosus or A. hypochondriacus flour contributed to an increase in the intake of total dietary fiber, reaching 54/53% of the AI for adult men and 83/80% for adult women, respectively (Food and Nutrition Board, Institute of Medicine, 2005). Therefore, the compositional and structural characteristics of dietary fiber from pseudocereals such as amaranth, buckwheat, or quinoa suggest a good potential for exerting a favorable function by regulation of intestinal transit, and reduction of the risk of diabetes, hypertension, coronary heart disease, cardiovascular disease, and colon cancer (Salas-Salvadó et al., 2006).

The mineral content increased significantly as a result of the replacement of wheat flour with up to 50% of amaranth flour, with ash content increasing from 1.7% to 3.5% and 4.0% in WAs-50 and WAh-50, respectively, suggesting the presence of major minerals such as calcium, magnesium, potassium, phosphorus, iron, and zinc (Reguera & Haros, 2017). However, their bioavailability would depend on the content of antinutritional factors, such as phytic acid, which forms insoluble complexes (Sanz-Penella et al., 2009) (Table 1.2). Furthermore, the amaranth breads increased the protein amount to values ranging from 17.6 to 19.0 g/100 g, compared to 16.5 g/100 g in the control bread. The balanced amino acid composition of amaranth proteins, as reported, is close to the optimum protein reference pattern of the human diet according to FAO/WHO requirements (D’Amico et al., 2017). With a daily intake of 250 g of bread/day/person, bread with amaranth would cover the requirement of adults (over eighteen, with a weight range of 55–60 kg) according to the adult protein requirements declared by FAO/WHO/UNU (2007). Sanz-Penella et al. (2013) reported that bread with A. cruentus (up to 40% substitution) had a protein content of 16.3 g/100 g, d.b.; in the current investigation the results were higher, even when the level of replacement was lower (25%), with both A. hypochondriacus and A. spinosus (Table 1.2). The same trend was observed by Iglesias-Puig et al. (2015) in bread with 50% replacement with quinoa, which had a protein content of 12.4 g/100 g. In general, the protein content in pseudocereals is usually higher in amaranth, followed by quinoa and buckwheat, and higher in pseudocereals than in common cereals such as wheat (D’Amico et al., 2017).

Effect of formulation on myo-inositol phosphate profile in bread

The amount of phytates in the *A. hypochondriacus* and *A. spinosus* whole flours was 18 and 21 mol/g dry matter, respectively. Similar values in *A. cruentus* were reported by Sanz-Penella et al. (2013), in contrast with other investigations, which reported values between 4.8 and 9.4 mol/g in *A. cruentus*, *A. hypochondriacus*, and *A. hybridus* (Lorenz & Wright, 1984). The inclusion of amaranth flour in the bread formulations increased the phytic acid content from negligible values in the control sample to 3.6/3.7 µmol/g in WAs/WAh-25 and 8.5/8.8 µmol/g in the WAh/WAs-50 formulations (Table 1.2). The reduction of chelating capacity (expressed as phytic acid content) in these breads was around 54–58% in bread with *A*. *spinosus* and *A. hypochondriacus* flour substituted at 50% and around 80–83% in bread at 25%. However, the reduction of InsP_6 is lower in the current study than in the formulations with *A. cruentus*, probably owing to the longer fermentation time used in that investigation (Sanz-Penella et al., 2013). The phytates could be hydrolyzed as a result of the action of endogenous phytase enzymes during the cereal/pseudocereal fermentation step, and as the fermentation time increases the phytic acid content decreases (Siwatche et al., 2019).

Table 1.1

Effect of the inclusion of whole amaranth flour on bread composition

Sample	Units	Formulations				
		Control	**WAs-25**	**WAs-50**	**WAh-25**	**WAh-50**
***Physicochemical parameters*[a]**						
Moisture	%	27.8±0.2 a	28.6±1.7 ab	28.9±0.2 ab	28.9±0.2 ab	29.3±0.3 b
Proteins	% d.m.	16.5±1.4 a	18.1±0.6 ab	18.4±0.3 c	17.6±0.1bc	19.0±0.8 bc
Lipids	% d.m.	0.07±0.0 a	0.70±0.1b	1.45±0.1c	0.78±0.1b	1.54±0.1c
Ash	% d.m.	1.7 ± 0.3 a	3.0±0.1 b	3.5±0.2 c	3.0±0.2 b	4.0±0.1 c
***Dietary Fiber*[a]**						
Total Dietary Fiber	g/100 g d.m.	6.96±1.3 a	9.50±0.04 b	11.7±0.8 c	9.9±0.9 bc	11.3±0.3 bc
Soluble fiber	g/100 g d.m.	3.1±0.7 a	3.2±0.2 a	2.9±0.1 a	3.4±0.9 a	3.4±0.7 a
Insoluble fiber	g/100 g d.m.	3.9±0.5 a	6.3±0.2 b	8.7±0.6 c	6.5±0.0 b	7.9±0.4 c
Soluble/Insoluble Fiber Ratio[b]	g/g	1:1.2	1:2	1:3	1:1.9	1:2.3
AI[c] contribution	%	33/50	45/68	54/83	46/70	53/80
***Myo-inositol phosphates*[a]**						
InsP_6	mmol/g	n.d.	3.6±0.3b	8.8±0.8c	3.7±0.6b	8.5±0.5c
InsP_5	mmol/g	n.d.	0.75±0.1b	1.59±0.4c	0.87±0.2b	2.0±0.5c
InsP_4	mmol/g	n.d.	0.40±0.1b	0.61±0.1c	0.34±0.1b	0.61±0.1c
InsP_3	mmol/g	n.d.	0.16±0.04a	n.d.	0.27±0.04b	n.d.
InsP_6 + InsP_5	mmol/g	n.d.	4.4±0.4a	10.40±0.5b	4.5±0.8a	10.1±0.5b

Tabla 1.1 (continuación)

Sample	**Units**	**Formulations**				
		Control	**WAs-25**	**WAs-50**	**WAh-25**	**WAh-50**
Minerals						
Ca[a]	mg 100 g dm^{-1}	37.7±0.0 a	88.2±0.8 b	139.7±0.0 d	92.7±0.5 c	152.6±0.7e
DRI contribution[d]	%**	7	16	21	16	27
InsP_6 /Ca >0.24[e]	mol mol^{-1}	n.d.	0.16	0.24	0.16	0.22
Fe[a]	mg 100 g dm^{-1}	1.5±0.0 a	3.2±0.1 b	4.9±0.3 d	3.3±0.1 c	4.9±0.3 e
DRI contribution[d]	% man/woman	35/15	72/32	108/48	74/33	108/48
InsP_6 /Fe >1[e]	mol mol^{-1}	n.d.	6.2	9.7	6.2	9.7
Zn[a]	mg 100 g dm^{-1}	1.2±0.0 a	2.1±0.2 b	2.6±0.2 c	1.9±0.0 b	2.3±0.2 bc
DRI contribution[d]	% man/woman	19/27	34/47	43/59	31/43	37/50
InsP_6 /Zn >5[e]	mol mol^{-1}	n.d.	11.2	20.9	12.4	24.3

Codes: C, Control Bread; WAs-25, 25% whole *A. spinosus* flour; WAs-50, 50% whole *A. spinosus* flour; WAh-25, 25% whole *A. hypochondriacus* flour; WAh-50, 50% whole *A. hypochondriacus* flour; d.m., dry matter; InsP_3 to InsP_6: *myo*-inositol containing 3–6 phosphates per inositol residue; n.d., not detected.

[a] Mean±SD. n=3. Values followed by the same letter in the same column are not statistically different at 95% confidence level, *n.d.* not detected

[b] Ratio of soluble/insoluble fiber, 1:2 (Jaime, Mollá, Fernández, Martín-Cabrejas, López-Andreu, & Esteban, 2001); 1:3 (Salas-Salvadó, Bulló, Pérez-Heras, & Ros, 2006).

[c] AI (adequate intake) contribution (%) for a daily average intake of 250 g of bread. AI in g per day for dietary fiber in adult man/woman is (38/25). The values in parentheses are recommended dietary allowances and adequate intakes for adults for each gender between 19 and 50 years; Food and Nutrition Board, Institute of Medicine (2004).

[d] DRI (dietary reference intakes) contribution (%) for a daily average intake of 250 g of bread if mineral absorption inhibitors are absent. The values in parentheses are recommended dietary allowances and adequate intakes for individuals between 19 and >70 years, except for: * (between 31 and >70 years), and ** (men between 19 and 70 years, women between 19 and 50 years) (Food and Nutrition Board, 2004).

[e] Threshold ratios (InsP_6/mineral) for mineral availability inhibition (Ma et al., 2005); InsP_6, *myo*-inositol hexakisphosphate; minerals Ca, Fe or Zn.

Effect of bread formulation on minerals and mineral dietary reference intake contribution

The mineral content increased significantly as a result of the replacement of wheat flour, as was expected, owing to the flour composition. The substitution of WAh/WAs-25 and WAh/WAs-50 contributed between two and four times more Ca, Fe, and Zn than the control bread. In addition, as a result of the incorporation of whole amaranth flour the contribution of the minerals intake increased the dietary reference intakes (DRIs) for a daily average intake of 250 g of bread if the mineral absorption inhibitors are absent, according to the Food and Nutrition Board of the Institute of Medicine, National Academy of Science (NAS, 2004). With regard to Zn, consumption of the control bread would provide only 27% or less of the daily requirement in adults, while the bread made with WAh/WAs could provide nearly 50% of the daily requirement for women. The same tendency was observed with Fe, where WAh-25/WAs-25 could supply males with more than 70% of the daily requirement of this mineral. With respect to macrominerals such as calcium, they could supply nearly four times as much as wheat bread. However, the Fe and Zn would not be bioavailable in their entirety because of the high presence of phytates, as shown by the InsP6/mineral molar ratio (Table 1.2).

Technological quality of fresh bread

Owing to the lack of gluten in the whole amaranth flour, the specific volume decreased from 4.05 to 2.38 mL/g as a result of the addition of amaranth flour at different levels (Figure 1.1 and Table 1.3). A similar trend was observed by Sanz-Penella et al. (2013) and by Almeida, Chang, & Steel (2013) in bread with wheat flour replaced with A. cruentus and A. caudatus, respectively. This behavior was observed in other studies as a result of the inclusion of ingredients such as fiber in bread formulations, owing to a gluten dilution effect (Iglesias-Puig et al., 2015). On the other hand, the slice shape did not show significant differences compared to the control sample (Figure 1.1). The inclusion of whole amaranth flour produced a significant change in crumb firmness, reaching 2.16/2.46 for 50% substitution (Table 1.3). The same effect was observed in bread supplemented with other pseudocereals (Sanz-Penella et al., 2013; Iglesias-Puig et al., 2015). Whole pseudocereal flours are rich in dietary fiber and do not provide gluten, but their proteins, such as albumin, have the capacity to interact with wheat glutenin protein through disulfide bonds, which does not weaken the gluten network overmuch (Oszvald et al., 2009). The high polar lipid content in amaranth may have functionality as a gas stabilizing agent during breadmaking, which probably improves bread elasticity (D’Amico et al., 2017). In fact, Meullenet et al. (1998) found a direct relation between dough elasticity/crumb chewiness and crumb firmness. In the current investigation, the same tendency was observed between the chewiness and firmness parameters (Table 1.3).

The mean cell area for the WAs-25 and WAh-25 formulations was approximately the 50-70% of the control and WAh-50/WAs-50 areas, but they had a higher amount of cells/m^2 than the others. Therefore, their specific volume was significantly higher than that of WAh-50/WAs-50, and lower, but not significantly lower, than that of the control sample (Table 1.3). Again, this effect could be due to the low amount of gluten and consequent decrease in dough elasticity in the formulations with 50% replacement (Table 1.3 and Figure 1.1). The color parameters of the crust and crumb showed a tendency to decrease in terms of lightness and hue, whereas the chroma tended to increase, especially in formulations substituted with 50% of whole amaranth flour (Table 1.3 and Figure 1.1). In general, the temperature at the center of the bread crumb does not reach 100 °C during baking, but this temperature is greatly exceeded in the crust, so the crust color is mainly due to the effect of the Maillard reaction and sugar caramelization (Martínez et al., 2013). In the current study, it is noteworthy that, as the level of substitution of wheat flour increased, the $L^*$and h_{ab} parameters in the crust and crumb decreased compared to the control sample. Therefore, in all the formulations there were significant differences in crust and crumb color (ΔE^*) compared to the control sample, with values greater than 5 and clearly perceptible to the consumer according to the criteria of the International Commission on Illumination (1978) (Figure 1.1).

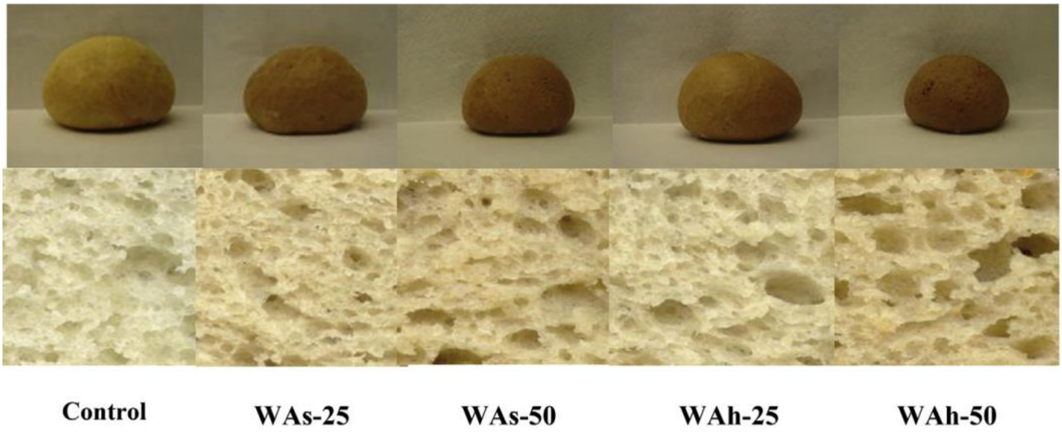

Fig. 1.1. Appearance of the loaf bread and crumb structure. Bread formulations: C, control bread; WAs-25, WAs-50, WAh-25, and WAh-50, whole A. spinosus and A. hypochondriacus replaced at 25% and 50%, respectively.

Table 1.2
Effect of inclusion of whole amaranth flour on bread quality

Sample	**Units**	**Formulations**				
Technological parameters[a]		**Control**	**WAs-25**	**WAs-50**	**WAh-25**	**WAh-50**
Loaf volume	mL	334 ±13 d	305 ± 36 c	195 ± 23 a	298 ± 16 c	223 ± 17 b
Specific volume	mL/g	4.05 ± 0.16 b	3.74 ± 0.52 b	2.38 ± 0.28 a	3.63 ± 0.07 b	2.71 ± 0.20 a
Shape ratio	cm/cm	1.70 ± 0.08 a	1.61 ± 0.04 a	1.65 ± 0.08 a	1.64 ± 0.04 a	1.64 ± 0.07 a
Crumb textural parameters[a]						
Firmness	N	0.77 ± 0.17 a	0.91 ± 0.12 a	2.16 ± 0.28 b	1.05 ± 0.19 a	2.46 ± 0.35 c
Springiness		0.95 ± 0.10 a	1.00 ± 0.01 b	0.97 ±0.02ab	1.00 ± 0.01 b	0.95 ± 0.02 a
Cohesiveness		0.89 ± 0.03 ab	0.83 ± 0.01 a	0.84 ± 0.01a	0.83 ± 0.01 a	0.84 ± 0.10 a
Chewiness		0.66 ± 0.17a	0.84 ± 0.13 ab	1.74 ± 0.12 c	0.87 ± 0.09 b	1.94 ± 0.13 c
Gumminess	N	0.68 ± 0.14 a	0.81 ± 0.12 a	1.80 ± 0.16 b	0.87 ± 0.15 a	2.06 ± 0.23 c
Crust color parameters[b]						
L^*		73 ± 2 d	61 ± 2 c	54 ± 2 b	58 ± 3 c	50 ± 2 a
C^*		31 ± 3 a	37 ± 1 c	34 ± 1 b	36 ± 1 c	33 ± 1 b
h_{ab}		86 ± 2 c	72 ± 1 b	66 ± 1 a	70 ± 1 b	65 ± 1 a
ΔE^*		-	16 ± 1 a	22 ± 1 c	18 ± 1 b	26 ± 1 d

Tabla 1.2 (Continuación)

Sample	Units	Formulations				
Crumb color parameters^b		**Control**	**WAs-25**	**WAs-50**	**WAh-25**	**WAh-50**
L^*		70 ± 3 d	68 ± 2 b	65 ± 1 b	67 ± 2 c	61 ± 1 a
C^*		16 ± 1.1 a	21.1 ± 1.0 cd	25.1 ± 0.5 c	21.9 ± 0.6 b	25.8 ± 0.8 c
h_{ab}		95.1 ± 0.5 e	90.1 ± 0.4 d	85.5 ± 0.7 b	88.8 ± 0.4 c	83.7 ± 0.8 a
ΔE^*		-	5.9 ± 0.6 a	11.1 ± 0.4 c	6.8 ± 0.2 b	14.2 ± 0.9 d
Crumb grain(digital image analysis)^b						
Cell Area/Total Area	cm^2/cm^2	0.53±0.04 c	0.37±0.06 a	0.46±0.05 b	0.34±0.04 a	0.45±0.06 b
Wall Area/Total Area	cm^2/cm^2	0.49±0.04 a	0.63±0.06 c	0.56±0.06 b	0.67±0.04 c	0.57±0.06 b
Cells/cm^2		110±17 a	160±46 b	129±42 ab	148±13 b	134±25 ab
Mean Cell Area	mm^2	5.13±0.01 c	2.63±0.01 a	4.00±0.02 bc	2.50±0.01 a	3.50±0.01 ab
Sensory evaluation (Hedonic Scale)^c						
Overall acceptability		7.5±1.4 e	5.8±0.6 b	5.6±0.7 a	6.9 ± 0.9 d	6.1 ± 0.9 c

Codes: C Bread control; WAs-25, 25% whole *Amaranth spinosus* flour, WAs-50, 50% whole *Amaranth spinosus* flour, WAh-25, 25% whole *Amaranth hypochondriacus* flour, WAh-50, 50% whole *Amaranth hypochondriacus* flour; d.m., dry matter.

Mean±SD, ^{a}n=3. ^{b}n=4. ^{c}n=25.Values followed by the same letter in the same column are not statistically different at 95% confidence level.

Sensory evaluation

In this study a preliminary sensory analysis was performed with a nine-point hedonic scale for the overall acceptability of the products after they had been tasted by consumers. In general, the products made with *A. hypochondriacus* flour had better acceptance than the *A. spinosus* breads. The WAh-25 sample showed 20% more acceptability than the WAh-50 sample (Table 1.3), and the WAs-25 and WAs-50 formulations showed 58% and 55% of acceptance, respectively. This result indicated that the consumers preferred the products made with *A. hypochondriacus* at a 25% level of substitution rather than the other amaranth formulations (Table 1.3). Some of the comments of the tasters who described the flavor of the bread with amaranth flour, a new flavor that they had not tasted before, were: "*moisture flavor*," "*mold flavor*," "*strange flavor*," "*sand flavor*," "*indifferent flavor*,", "*slightly acid*," "*not very pleasant*," "*new flavor/different*" (data not shown). The new flavor could be due to the presence of saponins, although amaranth grains have a lower amount of saponins than quinoa grains, so a product made with amaranth flour would be less bitter than one with quinoa (Reguera & Haros, 2017). Thus, the lower scores were due not only to the taste but also to the appearance and color of the slices, and their texture and palatability.

Effect of the inclusion of amaranth on starch thermal properties

The bread samples showed a peak in the thermogram, corresponding to the process of gelatinization of the amorphous phase of starch. It was observed between 62.7 and 75.9 °C, with an enthalpy of 1.6 J/g in the control sample and values significantly higher in the formulations with 25% replacement (Table 1.4). However, in the formulation with 50% replacement the gelatinization onset temperature did not have significant differences compared to the formulations with 25% replacement, but the enthalpy values were 1.7/1.9 J/g for WAs-25/WAh-25, significantly different from the values of 1.2/1.3 J/g for WAs-50/WAh-50, respectively (Table 1.4). This reduction could be due to the higher amount of water absorbed by the amaranth grain, and the greater swelling power and solubility of the amaranth starch granule compared with the wheat starch granule (Pérez-Rea & Antezana-Gómez, 2018).

In the second heating cycle, after storage at 20 °C, two peaks were observed. The first one was the amylopectin retrogradation peak, while the second one was the amylase–lipid complex melting transition. In the bread substituted with whole amaranth flour there was practically no alteration in the transition temperature range. Moreover, the retrogradation enthalpy after 4 days of storage tended to be significantly lower in the WAs-50/WAh-50 formulations compared to WAs-25/WAh-25 and the control sample. In fact, there are findings that show that amaranth starch has slower retrogradation enthalpy than other types of starches belonging to corn, wheat, and rice (Repo-Carrasco-Valencia et al., 2017). It was observed that the high enthalpy retrogradation value for the control bread was directly related with the aging of the bread during storage owing to the reorganization of the amylopectin crystals. In general, the retrogradation kinetics for the bread with whole amaranth flour showed a tendency to decrease, but there were no significant differences between formulations (Table 1.4).

However, after 10 days of storage the amylopectin retrogradation in samples with amaranth was slightly and significantly lower than in the control bread (Fig. 1.2).

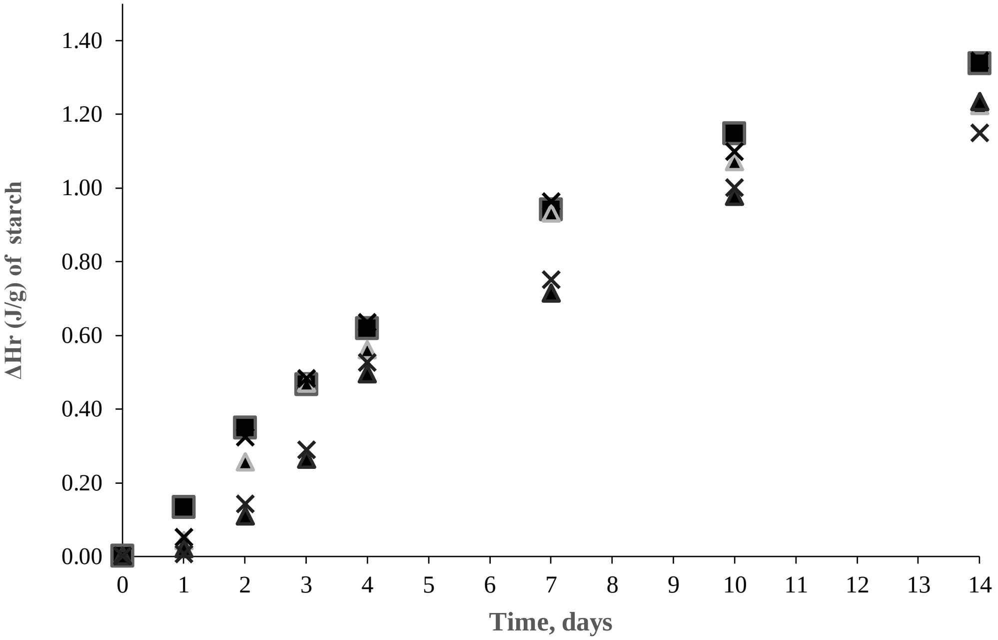

Fig. 1.2. Effect of the inclusion of Whole Amaranth flour on amylopectin retrogradation: □, C, Bread control wheat flour; △ ,WAs-25, 25% whole A. spinosus flour; ▲, WAs-50, 50% whole A. spinosus flour; **X,** WAh-25, 25% whole A. hypochondriacus flour; **X,** WAh-50, 50% whole A. hypochondriacus flour.

Table 1.3

Effect of amaranth flour on starch thermal properties of dough.

Sample	**Units**	**Control**	**Amaranth (%)**			
			WAs-25	**WAs-50**	**WAh-25**	**WAh-50**
Starch gelatinization[a]						
Onset temperatura	°C	62.7±0.6 c	64.2±0.7 b	65.1±1.0 ab	64.5±1.0 ab	65.4±0.8 a
Peak temperatura	°C	68.8±0.6 c	70.51±0.46 b	71.1±0.7 bc	71.04±0.9 bc	71.4±0.6 bc
Conclusion temperatura	°C	75.9±1.2 b	78.6±2.4ª	78.6±2.4 a	79.80±3.44a	79.1±1.5 a
Gelatinization enthalpy, ΔH_G	J/g	1.6±0.2 b	1.7±0.2 bc	1.2±0.1 a	1.9±0.2 c	1.3±0.1 a
PHI	J/g°C	0.26±0.04 a	0.27±0.05 b	0.20±0.03 c	0.29±0.04 b	0.21±0.02 c
Amylopectin retrogradation, 4 days[b]						
Onset temperatura	°C	55.2±0.4 a	55.45±0.1 a	55.4±0.4 a	55.3±0.4 a	55.2±0.7 a
Peak temperatura	°C	62.9±0.1 a	63.5±0.02 a	63.2±0.1 a	63.0±0.5 a	63.7±0.4 a
Conclusion temperatura	°C	76.4± 2.5 a	82.0±0.8 a	74.5±2.9 a	78.8±0.7 a	76.6±1.0 a
Retrogradation enthalpy, ΔH_R	J/g	0.62±0.02 bc	0.56±0.00 ab	0.50±0.00 a	0.63±0.06 c	0.53±0.00 a
RI		0.39±0.01 b	0.34±0.00 a	0.42±0.00 b	0.34±0.03 a	0.42±0.00 b

Codes: C, Control Bread; WAs-25, 25% whole *Amaranth spinosus* flour, WAs-50, 50% whole *Amaranth spinosus* flour, WAh-25, 25% whole *Amaranth hypochondriacus* flour, WAh-50, 50% whole *Amaranth hypochondriacus* flour; d.m., dry matter.

Mean±SD. [a]n=14, [b]n=3; values followed by the same letter in the same column are not statistically different at 95% confidence level; DSC differential scanning calorimeter, PHI, Peak Height Index $\Delta H_G/(T_p-T_o)$; RI, Retrogradation Index $\Delta H_R/\Delta H_G$(J/g).

Previous investigations concluded that the presence of the higher lipid and fiber content in bread doughs makes the recrystallization of amylopectin more difficult and reduces retrogradation enthalpy (Mondragón, et al., 2006). Therefore, in general, bread aging could be delayed in bread formulations with amaranth flour.

CONCLUSIONS

Bread formulated with *A. spinosus* and *A. hypochondriacus* flours had higher levels of protein and dietary fiber than wheat bread, and was close to the recommended intake for adults. Bread with amaranth flours exhibited soluble/insoluble fiber ratios close to 1:2, which presents the most effective physiological action. The levels of lipids, minerals, and *myo*-inositol phosphates were also increased significantly by the incorporation of this pseudocereal. The intake of products with high substitution with amaranth could supply a high percentage of the DRIs of Zn and Fe in adults, but the high amount of phytates in these formulations could inhibit mineral availability. Consumers preferred the bread with 25% replacement, with whole *A. hypochondriacus* rather than with *A. spinosus* at the same level of substitution. The inclusion of either amaranth species delayed aging of the bread. The level of inclusion of whole flours of the two species studied, *A. spinosus* and *A. hypochondriacus*, in bakery products could be limited to a maximum proportion of 25 g/100 g, providing acceptable technological characteristics and sensory quality with high nutritional value.

As a general conclusion, the use of *A. hypochonriacus* or *A. spinosus* as a bakery ingredient allowed to develop similar breads compared to the ones made with others Amaranth species, as *A. caudatus or A. cruentus,* in terms of technological and nutritional quality. However, *A. spinosus* presented a significant lower overall acceptability which is necessary to study the minimum substitution level in bread formulations with a high acceptability and a positive impact in the human health.

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CAPÍTULO 2

Effect of Chia as Breadmaking Ingredient on Nutritional Quality, Mineral Availability, and Glycemic Index of Bread

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ABSTRACT

Chia seeds and chia flour could be used as ingredients to enrich foods owing to their high amount of nutrients. The goal of this investigation was to provide further information about how replacing wheat flour with chia ingredients (seeds, whole flour, semi-defatted, and low-fat flours) affects the nutritional and functional value of bread. The higher levels of proteins, lipids and minerals determined in raw chia flours directly affected the increase of these nutrients. High levels of phytates were found in chia ingredients (5.1–6.6 µmol/g d.b.), which affect Zn and Fe bioavailability, as predicted by phytate/mineral molar ratios. Bread with chia had a high amount of linoleic acid, especially in bread with chia seeds, owing to protection of seed integrity during baking. Chia ingredients did not show limiting essential amino acids such as lysine, which is deficient in cereals. Glycemic index was lower in bread with chia ingredients compared to control. The beneficial effects on glucose metabolism together with the nutritional and functional characteristics could be clinically important for prevention of metabolic diseases.

INTRODUCTION

The increasing consumer demand for nutritious, healthy food has made the food industries examine their own resources to discover and take advantage of functional ingredients. Nowadays, with regard to bakery goods, wheat bread has been enriched with new food ingredients (Fernandes, 2017**;** Sayed-Ahmad et al., 2018). Numerous epidemiological and experimental studies suggest that changes in the diet are decisive in the prevention of various metabolic disorders included in the so-called metabolic syndrome, such as type 2 diabetes, insulin resistance, hypertension, obesity, and cardiovascular disease (Capitani et al., 2012). Furthermore, intake of prebiotic foods, wholegrain, high fiber, seed breads, or high amounts of omega-3 leads to lower blood cholesterol and consequently reduces the risk of cardiovascular disease (Tenenbaum and Fisman, 2018).

The seeds of *Salvia hispanica* (chia) have high nutritional and functional characteristics. Its oil has predominantly unsaturated fatty acids, such as α-linolenic acid (64.4%) and linoleic acid (21.5%), and less than 10% of saturated fatty acids (Timilsena et al. ,2017). Chia seed oil has a low n-6/n-3 ratio, therefore intake of it could help to get the ratio between 5:1 and 9:1, in accordance with WHO/FAO (World Health Organization/Food and Agriculture Organization) (FAO, 2008) and EFSA (European Food Safety Authority Food) Panel on Dietetic Products and Allergies (Agostoni et al., 2010) recommendations to prevent the development of metabolic disorders, tumor cells, and chronic diseases (Mansara et al., 2015). The seeds are also a more abundant source of proteins (19%–27%)

than conventional crops such as rice (5.95%), oat (13.15%), and wheat (9.61%) (US Department of Agriculture, 2016; US Department of Agriculture, 2018). Chia proteins contain high concentrations of essential amino acids such as lysine, leucine, isoleucine, and valine (Kulczynski, 2019). These proteins have a complete amino acid profile, unlike cereals, which are particularly deficient in lysine in comparison with the scoring pattern for children (1–2-years-old) which is taken as a reference (Agostoni et al., 2012; FAO, 2007]. In addition, the seeds have a high amount of fiber (18%–40%), more than other grains such as cereals and legumes, and it is mainly soluble [Capitani et al., 2012, Lazaro, 2018]. Soluble fiber (gums, pectins, and mucilages) has bioactive effects, such as enhancing the immune function, lowering cholesterol and delaying starch digestion and glucose release from foods, with a consequent decrease in post-prandial glycaemia (Schuchardt et al., 2016). Furthermore, the mucilage of chia seeds could be linked to starch in bread baking products, impeding starch gelatinization and thus enzymatic vulnerability, and lowering the glycemic index [Schuchardt et al., 2016; Iglesias-Puig, 2013]. Moreover, it has been found that chia seeds contain a high number of phenolic compounds and high concentrations of natural antioxidants, such as quercetin and kaempferol, while caffeic and chlorogenic acids are present in low concentrations (Reyes-Caudillo et al., 2008). Chia can be considered a seed with antioxidative potential and could be used as an antihypertensive substance (Orona-Tamayo et al., 2015). Chia has a high concentration of minerals, but the bioavailability of di- or trivalent cations, such as calcium, iron, or zinc, depends on the phytate concentration, which may decrease during food processing (Garcia-Mantrana et al., 2014).

Because of its high nutritional properties, consumption of chia has spread widely in the European Union (EU), and in the EU list of novel foods EFSA has authorized the use of chia seeds up to maximum inclusion levels (Turck, 2019). Whole chia seeds may be marketed in the European community as a food ingredient for use in baked products and breakfast cereals up to 10%; ground chia seeds up to 5% in bread; whole chia seeds up to 5% in sterilized ready-to-eat meals based on cereal/pseudo-cereal grains and/or pulses; pre-packaged chia seed as such, and fruit/nut/seed mixes; and chia in confectionery products and chocolates; edible ices; fruit and vegetable products; non-alcoholic beverages and puddings (<120 °C in their preparation) without limit, according to the European Commission (European Union, 2020). Recently, the use of two partially defatted powders of chia enriched with proteins or fibers was authorized as food supplements for the adult population (up to 7.5 and 12 g/day, respectively), or as nutritional ingredients in a variety of foods (yogurt, vegetable beverages, energy drinks, chocolate, fruit, and pasta) at a level of 0.7%–10% (Turck et al., 2019). The partial replacement of wheat by chia seeds, whole chia flour and defatted chia flour in bread (up to a level of 5%–6%) obtained high consumer acceptance in earlier studies, and it could extend the shelf life of bread, since it inhibits the kinetics of retrogradation of amylopectin during storage (Sayed-Ahmad et al., 2018; Iglesias-Puig and Haros, 2013). However, the breadmaking process may affect the stability and/or bioavailability of nutrients/bioactive compounds owing to various chemical and enzymatic reactions during kneading, fermentation, and baking, and accordingly this enriched bread would provide a greater or lesser health benefit (Benitez et al., 2018).

The nutritional value of food depends on many factors, but mainly on the amounts of macronutrients (proteins, fats, carbohydrates) and micronutrients (minerals and vitamins). The lack or excess of some of these substances can have detrimental effects on health, and therefore the EFSA (EFSA (European Food Safety Authority), 2017) and FAO/WHO/UNU (United Nations University) (FAO, 2007) have developed and applied dietary reference intakes (DRIs), which are the minimum amount of a particular nutrient that can be consumed daily without health risks in order to maintain the health and well-being of the body.

The objective of this study was to characterize and analyze the potential of chia seeds, whole chia flour, and the defatted chia flour obtained after extraction of chia oil (currently considered as waste in the EU) as nutritional and functional bakery ingredients. Further aims were to study the effect of baking on the amino acid and fatty acid profiles, mineral availability and the contribution to nutrient DRIs, and to estimate the glycemic index of the bakery products developed.

MATERIALS AND METHODS

Materials

Commercial Spanish wheat flour (W) was purchased from La Meta (Lleida, Spain). Chia seeds (CWS), chia whole flour (CWF), semi-defatted chia flour (CSDF), and low-fat chia flour (CLFF) were donated by Chia S.A. Company (Valencia, Spain). CSDF and CLFF were obtained by supercritical CO2 extraction. The characteristics of wheat flour (W) and chia ingredients were described in an earlier study by Iglesias-Puig and Haros. Compressed yeast (Saccharomyces cerevisiae; Levamax, Valencia, Spain) was used as a starter for the breadmaking process.

Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), compressed yeast (2.5% flour basis), sodium salt (1.6% flour basis) and distilled water (up to optimum absorption, 500 Brabender Units). The ingredients were mixed for 4 min, rested for 10 min, divided (100 g), kneaded and then rested (15 min). The breadmaking process was carried out according to the method previously described by Iglesias-Puig and Haros. The various bread products studied were control bread (WB); whole seed bread (CWSB5 and CWSB10); whole flour bread (CWFB5 and CWFB10); semi-defatted flour bread (CSDFB5 and CSDFB10) and low-fat chia flour bread (CLFFB5 and CLFFB10), where 5 and 10 mean with 5% and 10% of chia ingredient on flour basis.

Composition of Raw Materials and Breads

Moisture was determined by a method of the AACC (American Association of Cereal Chemists) (AACC, 1995), ash content was determined in a muffle by incineration at 910 °C (ICC, 1990), and protein was quantified by the Kjeldahl method of the AACC (AACC, 1983). Lipid content was extracted with hexane or petroleum ether reflux conditions by the Soxhlet technique, and dietary fiber content was measured by the soluble, insoluble, and total dietary fiber assay procedure (AOAC, 2003; Lee et al., 1992). Minerals were measured with a flame atomic absorption spectrometer at the Analysis of Soils, Plants and Water

Service in the Institute of Agricultural Sciences, Madrid (Spain). The caloric value of the loaves was calculated using the Atwater coefficient based on the caloric coefficient corresponding to the protein, carbohydrate and lipid contents.

Determination of Myo-Inositol Phosphates

InsP6 (phytic acid or phytates) present in the raw materials and the remaining InsP6 and lower myo-inositol phosphates generated during the breadmaking process (InsP5, InsP4, and InsP3) were purified by ion-exchange chromatography and measured by the HPLC (High Performance Liquid Chromatography) method in reverse phase described by Türk et al. (Türk et al., 1996), as modified by Sanz-Penella et al. (Sanz-Penella et al., 2008). The myo-inositol phosphates were identified by comparison with standards of phytic acid di-potassium salt (Sigma-Aldrich, St. Louis, MO, USA). Samples were analyzed in triplicate.

Amino Acid Composition

The amino acid composition of the samples tested was analyzed by reverse phase liquid chromatography after acid hydrolysis according to AOAC (Association of Official Analytical Chemists) 994.12 and derivatized with diethyl ethoxymethylenemalonate to obtain the amino acids compound N-(2,2-bis(ethoxycarbonyl) vinyl) (Alaiz et al., 1992). Tryptophan was determined by basic hydrolysis and neutralization and analysis by reverse-phase HLPC with spectrophotometric determination, using an isocratic elution system consisting of sodium acetate and sodium azide/acetonitrile, according to Yust et al. (Yust et al., 2004).

Amino Acid Score

The amino acid score (AAS) was obtained by dividing the amino acid content of the raw materials or bread (mg/g protein) by the scoring pattern for children (1–2-years-old) given by FAO/WHO/UNU (FAO) and EFSA (Agostoni et al., 2012), according to Equation (1):

$$\text{AAS}=\frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}} \tag{1}$$

Fatty Acid Profile

The samples were transesterified to convert triglycerides into fatty acid methyl esters (FAMEs), following the methodology previously described by Garces and Mancha (Garces and Mancha, 1993). The fatty acid composition and quantification were analyzed by GC (Gas chromatography) (7890A; Agilent, Santa Clara, CA USA) fitted with a capillary column (30 m length; 0.32 mm internal diameter; 0.20 µm film thickness) of fused silica (Supelco, Bellafonte, PA, USA) and a flame ionization detector according to International Union of Pure and Applied Chemistry (IUPAC) method 2.302 (IUPAC, 1992). Measurements were carried out in triplicate.

Estimation of In Vitro Glycemic Index

To evaluate the in vitro rate of starch hydrolysis, the method described by Goni et al. (Goni et al.,1997) was employed, with slight modifications according to Sanz-Penella et al. (Sanz-Penella et al., 2014). The hydrolysis index (HI) of the samples was calculated from the area under the curve (AUC) from 0 to 120 min as a percentage of the corresponding reference area (wheat bread; $HI = AUCsample/AUCwheat\ bread \times 100$). The glycemic index (GI) was calculated using the equation $GI = 0.549 \times HI + 39.71$. The measurements were carried out in triplicate. The predicted glycemic load (pGL) for a 100 g bread portion was calculated from the glucose-related GI, with $pGL = glycemic\ index \times total\ carbohydrates/100$, taking into account the total carbohydrates of each sample (Wolter et al., 2014).

Statistical Analysis

Multiple sample comparison of the means (ANOVA) and Fisher's least significant differences (LSD) were applied to establish significant statistical differences between treatments. All statistical analyses were carried out with the Statgraphics Centurion XV.II software (Virginia, VA, USA), and the significance level was established at $p < 0.05$.

RESULTS AND DISCUSSION

The protein and ash contents in CSDF and CLFF were higher than in chia seeds or whole chia flour, while the lipid contents were lower, as was expected after the defatting process. A similar trend was found by Capitani et al., where the nutrients were concentrated after the extraction of chia oil. The bread with chia seeds and chia-by products showed a significant ($p < 0.05$) increase in the levels of ash, total dietary fiber (TDF), lipids, and proteins, and a decrease ($p < 0.05$) in the starch content in comparison with the control bread as shown in Tables 2.1–2.5.

Evaluation of Quality Proteins

The levels of proteins in the raw materials were in the following descending order, CLFF > CSDF > CWF > CWS > W, with the chia flours after oil extraction (CLFF and CSDF) showing significantly higher protein contents than the wheat flour and chia, as was expected (Table 2.1). On the other hand, the protein content in CWS (20.2 ± 0.2 g/100 g d.m.) was higher than in other oilseeds, such as sunflower (19.33 g/100 g) and sesame seed (17.73 g/100 g), and even than chia seed in other studies (16.54 g/100 g) (De Lamo and Gómez, 2018). This variation can be attributed to various factors, such as growing region, stage of plant development, genotype, temperature, light, and soil (Porras-Loaiza et al., 2014). As mentioned above, the protein contents in the defatted chia flours CSDF (22.5 ± 0.5g/100 g) and CLFF (23.5 ± 0.1 g/100 g d.m.) were significantly higher. These values could vary, depending on the defatting process or type of oilseed, for example, but they were higher than in sesame by-products after seed defatting and dehulling (10.23 ± 0.32 g/100 g d.m.), and lower than in defatted flax by-product (which ranged from 35% to 40%) (Elleuch et al., 2007).

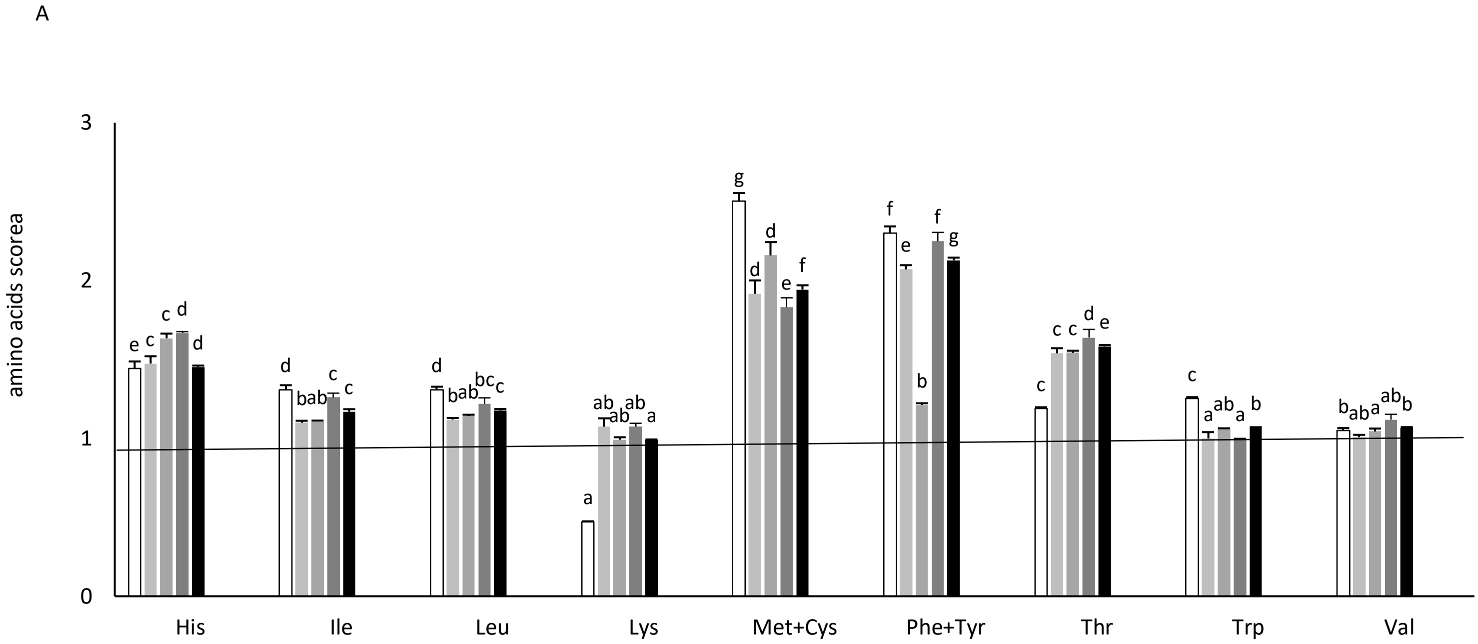
The nutritional contribution of food proteins to the maintenance of consumer health depends on their biological quality, given by the presence of all the essential amino acids (EAAs) (FAO, 2007; Engelking, 2015]. The chia seed and chia flours had up to two times more EAAs than wheat flour, with the highest values corresponding to defatted and semi-defatted chia flour (Table 2.1). In the current investigation, the total amount of essential amino acids in chia seeds was 38%, similar to the results found in the literature (Kulczynski, 2019; Coelho and Salas-Mellado, 2015; Coelho and Salas-Mellado, 2018). There were notable increases in the amounts of tyrosine (Tyr), histidine (His), methionine (Met), tryptophan (Trp), lysine (Lys), and cysteine (Cys) after the defatting process, as can be seen in the CSDF and CLFF samples in comparison with the chia seeds and whole chia flour (Table 2.1). Furthermore, with regard to the stability of certain amino acids, the defatting process could be advantageous because of the high susceptibility of lipids to oxidation. The generation of lipid-free radicals can induce the release of protein-free radicals, which form protein–protein or lipid–protein complexes. Moreover, lipid oxidation products, such as peroxides and hydroperoxides, could damage amino acid residues (Wąsowicz et al., 2004).

Given that protein quality is to directly associated with the essential amino acid profile, it is important to note that the scores for histidine, isoleucine, leucine, lysine, methionine+cysteine, phenylalanine+tyrosine, threonine, tryptophan, and valine in the chia protein ingredients were higher than in the protein reference pattern for children (1–2-years-old) and adults (Agostoni et al., 2012; FAO, 2007). In the case of lysine, which is the limiting amino acid in cereals, the score in the chia ingredients was around 1 (Figure 1.1A). On the other hand, the non-essential amino acids in the chia ingredients had abundant amounts of glutamic acid+glutamine (37.8–46.7 mg/g), arginine (19.6–23.4 mg/g), and aspartic acid+asparagines (17.9–21.8 mg/g), corresponding to 60% of the amount of non-essential amino acids, which was similar to the percentage observed in defatted chia flour (Coelho et al., 2018) and chia seed (Ding et al., 2018).

In other studies, chia seeds and defatted flour contained limiting amino acids such as threonine, lysine, and leucine (Olivos-Lugo et al., 2010; Ziemichod et al., 2019), but in the current study limiting amino acids did not appear. This discrepancy could be due to the different varieties, soils, and climatic conditions of the crop, as was reported by Ayerza (2013). However, the chia proteins in this study contained all the essential amino acids in quantities corresponding to human requirements according to the scoring patterns for the 1–2-year-old, 11–14-year-old, and adult age groups (Agostoni et al.,2012; FAO, 2007). The wheat protein, as is also the case with whole wheat flour (US Department of Agriculture, 2019), showed a deficient protein quality for all age groups in comparison with the chia proteins (Figure 2.1B), mainly because of the low lysine content. Besides, the purpose of food made with nutritional ingredients is to contribute to the recommended dietary allowance (RDA) of each nutrient, taking into account the age group and body weight when setting the daily consumption (Agostoni et al.,2012; FAO, 2007). In the case of proteins/amino acids, intake of 15 g of chia seeds or chia whole flour (3 g of protein) for an adult weighing 70 kg would provide 7% of the adult RDA of Ile, Try, Val, and Lys, 17% of the adult RDA of Met+Cys, and 21% of the adult RDA of Phe+Tyr. Taking into account the recommendation of EFSA (see Introduction), intake of 12 g of the defatted chia flours (CSDF/CLFF; 2.6 g of protein) would provide 6% of the adult RDA of Lys and Val, 13% of the adult RDA of Met+Cys, and 19% of the adult RDA of Phe+Tyr in the same individual. Consequently, chia ingredient intake could provide a high percentage of the adult RDA of sulfur and aromatic amino acids in the diet.

The bread formulations with chia ingredients showed significantly higher protein contents than the wheat bread, particularly in formulations with 10% substitution (Table 2.1).

Intake of 100 g of bread with 5% of chia by a 70 kg adult who performs normal activity could cover 19% of the PRI (popular reference intake; EFSA, 2017) or RDA (recommended dietary allowance; FAO/WHO/UNU, 2007) of protein, similar to the contribution of the control bread (18%). The contribution of protein to the PRI would depend on the ingredient and level in the formula, all of which provided a better contribution than the control sample (Table 2.1).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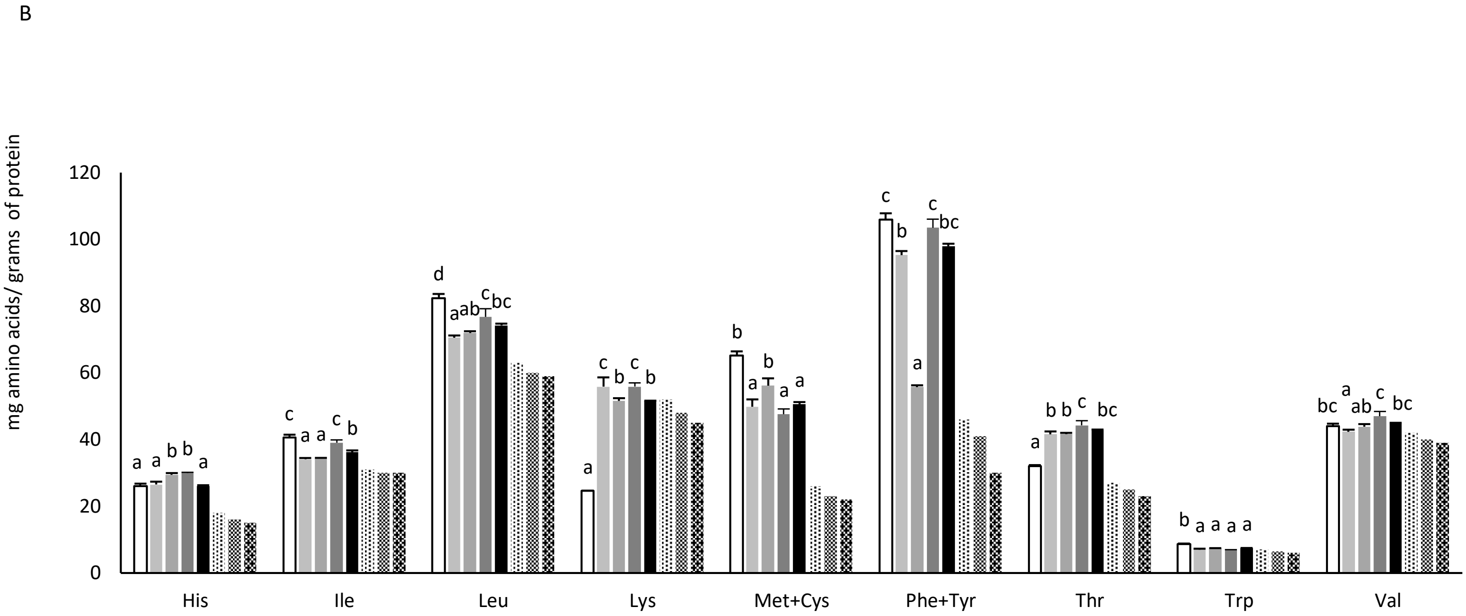

Fig. 2.1. Evaluation of essential amino acids in chia ingredients. **A. Amino acids score (mg/g protein**): essential amino acid pattern requirement for children as high-quality protein. **B. Composition of amino acids (mg/g protein d.m.**): based on FAO/WHO/UNU (Food and Agriculture Organization/World Health Organization/United Nations University) standard (2007), 1–2-year-old reference pattern (mg/g protein): His-18, histidine; Lys-52, lysine; Ile-31, isoleucine; Leu-63, leucine; Met+Cys-26, methionine + cysteine; Phe+Tyr-46, phenylalanine + tyrosine; Thr-27, threonine; Trp-7, Tryptophan; Val-42, Valine. Wheat flour (W); CWS, whole chia seed; CWF, whole chia flour; CSDF, semi-defatted chia flour; and CLFF, low-fat chia flour. Values are expressed as mean ± standard deviation (n = 3). Bars followed by the same letter are not significantly different at 95% confidence level.

Taking in account the amino acid composition, although the chia seed and flours had a higher amount of essential amino acids than the wheat flour, the bread formulations with 5% and 10% replacement showed only a slight increase compared to the control bread (Table 2.1).

The presence of abundant hydrophobic interactions of chia proteins, which could delay their denaturalization in a thermal process (Olivos-Lugo et al., 2010), could explain why no lysine losses were observed in the bread formulations (Table 2.1). However, in bread enriched with legumes there was a reduction of nutritional quality in terms of essential amino acids after the breadmaking process, especially with regard to lysine, which reacts with reducing carbohydrates to form amino acid–sugar compounds, which could not make up for the deficiency of lysine in the control bread (Turfani et al., 2017). There were slight increases in the amounts of methionine and histidine in comparison with the control bread, although they decreased or remained constant when the proportion of chia increased (Table 2.1). This behaviour was also observed by Oğur (Oğur, 2014) when she evaluated the changes in the amounts of amino acids in bread with partial replacement of flour by washed fish mince. The reduction of some amino acids may have been due to reactions with other bread components, as in Maillard reactions. Amino acids are consumed during fermentation, and then their concentration increases at the end of this stage as a result of yeast activity, which adapts to the nutritional conditions of the medium. Moreover, there are factors, such as fermentation time and dough pH, that can affect the amounts of amino acids during fermentation (Oğur, 2014; Arendt, 2007). With regard to the amounts of non-essential amino acids, there were significant increases in the amounts of glutamic acid + glutamine, glycine, arginine, and alanine in the breads with 5% and 10% replacement, particularly in the formulations with chia flour from which oil had been extracted (CSDF and CLFF). There was a decrease in proline in all the formulations with chia ingredients, as was expected because of the lower amount of this amino acid in the raw materials in comparison with wheat flour, in agreement with results reported by Diana et al. (Diana et al., 2014) and Turfani et al. (Turfani et al., 2017).

Evaluation of Fatty Acid Profile

The chia seeds and flour had higher lipid concentrations than the wheat flour, as was expected (Table 2.2). In addition, chia oil had a higher ($p < 0.05$) amount of saturated fatty acids (SFAs) than wheat oil, in which they were mainly palmitic and stearic acids (Table 2.2). Of course, the defatting process affected the amount of fatty acids and may have affected the fatty acid profile of the CSDF and CLFF chia ingredients (Table 2.2). In the semi-defatted chia flour the amount of PUFAs was still higher than the amount of SFAs, consisting mainly of linoleic and α-linolenic acids, just as in the chia seeds and whole flour. Moreover, the defatting process increased the PUFA:SFA ratio from 3.1:1/3.6:1 (chia flour/chia seeds) to 4.3:1 (CSDF) and 5.4:1 (CSFF). The efficiency of chia oil extraction and its fatty acid profile depend on the supercritical carbon dioxide extraction conditions such as pressure, temperature, and extraction time (Ixtaina et al, 2010). It was observed that extraction time had a significant effect on the percentages of linoleic acid (12.7 and 14.3 g/100 g of chia oil) and linolenic acid (32.8 and 34.7 g/100 g of chia oil) and on the PUFA:SFA ratios (4.3 and 5.4) and the ω-3/ω-6 ratios (2.6 and 2.4) in the CSDF and CLFF samples. As the unsaturated fatty acids (PUFAs) were much more concentrated in the chia seeds and flours than in the wheat flour, better ω-3/ω-6 ratios were observed in the chia seeds and chia flours (between 3.0 and 2.4) than those recommended by WHO/FAO (FAO, 2008) or EFSA (Agostoni et al., 2010) (1:5 and 1:8, respectively). A prolonged diet with a low ω-3/ω-6 ratio could lead to the development of chronic diseases such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2008). Accordingly, the inclusion of chia ingredients in food formulations could be a good strategy to promote the intake of healthy lipids, which could help to reduce the risk of developing diseases, have a beneficial effect on brain function, and help to avoid cardiovascular disease, arthritis and some types of cancer (Pizarro et al., 2015). In addition, there are studies on experimental animals and humans in which intake of chia seeds reduced the plasma triglycerides level, owing to their high α-linolenic content (Ayerza and Coates, 2005; Tenore et al., 2018). However, the stability of these unsaturated fatty acids is sometimes compromised, depending on the process used, as explained below.

Table 2.1

Amino acid composition of raw materials used in this study, mg/g of bread in dry matter [a.]

Amino Acid	Raw Materials					Bread Formula								
	Wheat (W)	Chia Whole Seeds (CWS)	Chia Whole Flour (CWF)	Chia Semi-Defatted Flour (CSDF)	Chia Low-Fat Flour (CLFF)	Control (WB)	Chia Seeds (CWSB)		Chia Whole Flour (CWFB)		Chia Semi-Defatted Flour (CSDFB)		Chia Low-Fat Flour (CLFFB)	
							5%	10%	5%	10%	5%	10%	5%	10%
Proteins, % d.m.	10.1 ± 0.1 a	20.2 ± 0.2 b	20.0 ± 0.1 b	22.5 ± 0.5 c	23.5 ± 0.1 d	16.1 ± 0.1 a	16.87 ± 0.5 b	22.4 ± 0.1 c	17.09 ± 0.4b	24.0 ± 0.1 d	16.93 ± 0.1 b	24.3 ± 0.2 e	17.24 ± 0.5 b	25.2 ± 0.1 f
	Essential Amino Acids (EAA)													
Histidine	2.6 ± 0.1 a	5.2 ± 0.2 b	5.8 ± 0.2 c	6.7 ± 0.1 d	6.1 ± 0.1 c	2.4 ± 0.0 a	2.9 ± 0.2 bc	2.9 ± 0.0 bc	2.9 ± 0.2 bc	2.7 ± 0.0b	3.1 ± 0.0 c	3.0 ± 0.1 bc	3.1 ± 0.1 c	2.9 ± 0.1 bc
Threonine	3.2 ± 0.0 a	8.3 ± 0.3 b	8.3 ± 0.1 b	9.7 ± 0.4 c	10.0 ± 0.1 c	3.6 ± 0.0 a	3.9 ± 0.1 b	4.1 ± 0.0 de	3.8 ± 0.1 b	4.2 ± 0.0ef	4.0 ± 0.1 c	4.3 ± 0.0 f	4.1 ± 0.0 cd	4.2 ± 0.0 ed
Tyrosine	4.9 ± 0.1 a	8.2 ± 0.3 b	8.3 ± 0.2 b	9.5 ± 0.5 c	9.8 ± 0.1 c	4.3 ± 0.0 a	4.9 ± 0.1 b	5.0 ± 0.0 b	5.0 ± 0.4 b	4.9 ± 0.1b	5.1 ± 0.1 b	5.7 ± 0.1 c	5.1 ± 0.2 b	5.2 ± 0.3 b
Valine	4.4 ± 0.1 a	8.5 ± 0.2 b	8.7 ± 0.2 b	10.4 ± 0.5 c	10.5 ± 0.1 c	4.7 ± 0.1a	5.2 ± 0.1 bcd	4.9 ± 0.1 ab	5.2 ± 0.0 bcd	4.9 ± 0.3ab	5.5 ± 0.0 cde	5.6 ± 0.1 de	5.9 ± 0.0 e	5.1 ± 0.5 abc
Methionine	1.9 ± 0.1 a	3.2 ± 0.5 b	4.6 ± 0.3 c	3.4 ± 0.2 b	4.1 ± 0.2 bc	2.1 ± 0.1 ab	2.5 ± 0.1 abc	2.6 ± 0.4 abc	2.9 ± 0.1 c	2.0 ± 0.4a	2.8 ± 0.2 bc	2.8 ± 0.1 c	3.1 ± 0.4 c	2.1 ± 0.3 b
Phenylalanine	5.7 ± 0.1 a	10.9 ± 0.0 b	11.1 ± 0.2 b	13.4 ± 0.3 c	13.1 ± 0.1 c	5.8 ± 0.0 a	6.2 ± 0.1 bc	6.4 ± 0.1 cd	6.2 ± 0.1 b	6.7 ± 0.2ef	6.5 ± 0.0 de	6.7 ± 0.1 f	6.6 ± 0.0 def	6.6 ± 0.1 def
Tryptophan	0.9 ± 0.0 a	1.3 ± 0.1 b	1.5 ± 0.0 c	1.6 ± 0.0 d	1.8 ± 0.0 d	0.7 ± 0.0 b	0.4 ± 0.0 a	1.0 ± 0.0 c	0.4 ± 0.1a	1.0 ± 0.0c	0.4 ± 0.0 a	1.0 ± 0.1 c	0.4 ± 0.0 a	1.0 ± 0.1 c
Isoleucine	4.0 ± 0.1 a	6.8 ± 0.1 b	6.8 ± 0.1 b	8.7 ± 0.3 c	8.4 ± 0.2 c	4.1 ± 0.1 a	4.4 ± 0.1 abc	4.1 ± 0.1 a	4.4 ± 0.1 abc	4.0 ± 0.3a	4.7 ± 0.0 cd	4.6 ± 0.0 bc	5.1 ± 0.0 d	4.3 ± 0.5 ab
Leucine	8.2 ± 0.2 a	14.2 ± 0.2 b	14.3 ± 0.2 b	16.9 ± 0.8 c	17.4 ± 0.2 c	8.4 ± 0.0 a	8.9 ± 0.2 bc	8.9 ± 0.0 bc	8.7 ± 0.1 b	9.1 ± 0.1cd	9.2 ± 0.0 cd	9.6 ± 0.2 e	9.5 ± 0.0 e	9.3 ± 0.2 de
Lysine	2.5 ± 0.0 a	10.9 ± 0.8 bc	10.2 ± 0.2 b	12.4 ± 0.4 d	12.1 ± 0.1 cd	2.0 ± 0.1 a	3.4 ± 0.0 b	3.6 ± 0.0 c	3.4 ± 0.1 b	3.8 ± 0.1cd	3.6 ± 0.0 c	3.9 ± 0.1 d	3.8 ± 0.1 cd	3.9 ± 0.1 d
Total EAAs	38	78	80	93	93	39	43	44	43	43	45	47	47	45

Table 2.1 *(Continuación)*

Amino Acid	Raw Materials					Bread Formula								
	Wheat (W)	Chia Whole Seeds (CWS)	Chia Whole Flour (CWF)	Chia Semi-Defatted Flour (CSDF)	Chia Low-Fat Flour (CLFF)	Control (WB)	Chia Seeds (CWSB)		Chia Whole Flour (CWFB)		Chia Semi-Defatted Flour (CSDFB)		Chia Low-Fat Flour (CLFFB)	
							5%	10%	5%	10%	5%	10%	5%	10%
	Non-Essential Amino Acid (NEAA)													
Aspartic acid + asparagine	4.6 ± 0.0 a	17.9 ± 0.6 b	17.7 ± 0.1 b	20.0 ± 0.7 c	21.8 ± 0.3 d	5.2 ± 0.1 a	6.1 ± 0.2 bc	6.9 ± 0.1 ef	5.8 ± 0.1 b	7.1 ± 0.1fg	6.3 ± 0.2 cd	7.5 ± 0.2 h	6.7 ± 0.3 de	7.3 ± 0.1 gh
Glutamic acid + glutamine	38.8 ± 0.5 a	37.8 ± 0.6 a	37.4 ± 0.3 a	43.1 ± 1.0 b	46.7 ± 0.5 c	38.2 ± 0.3 ab	38.9 ± 0.8 bc	38.9 ± 0.5 bc	38.0 ± 0.2 a	39.0 ± 0.2bcd	39.8 ± 0.2 de	40.1 ± 0.2 e	39.2 ± 0.1 cd	40.2 ± 0.3 e
Serine	6.7 ± 0.1 a	14.5 ± 0.5 b	14.2 ± 0.1 b	16.0 ± 0.6 c	17.6 ± 0.1 d	6.8 ± 0.1 ab	7.1 ± 0.0 ab	7.8 ± 0.1 c	6.8 ± 0.2 a	7.9 ± 0.1c	7.2 ± 0.1 b	8.3 ± 0.4 d	7.2 ± 0.1 ab	7.9 ± 0.1 c
Glycine	4.4 ± 0.0 a	11.6 ± 0.4 c	10.7 ± 0.2 b	12.1 ± 0.2 c	13.6 ± 0.1 d	4.6 ± 0.0 a	4.9 ± 0.2 bc	5.3 ± 0.1 e	4.7 ± 0.0 ab	5.3 ± 0.0e	5.0 ± 0.1 cd	5.4 ± 0.0 e	5.1 ± 0.1 d	5.5 ± 0.1 e
Arginine	4.2 ± 0.2 a	19.6 ± 0.7 b	22.0 ± 0.4 c	26.3 ± 0.5 d	23.4 ± 1.0 c	4.7 ± 0.0 a	5.8 ± 0.2 c	6.5 ± 0.0 d	5.5 ± 0.2 bc	6.3 ± 0.2d	5.8 ± 0.2 c	6.4 ± 0.0 d	5.4 ± 0.1 b	6.4 ± 0.2 d
Alanine	3.9 ± 0.1 a	11.8 ± 0.2 bc	11.1 ± 0.3 b	12.5 ± 0.4 c	14.7 ± 0.4 d	4.0 ± 0.0 a	4.4 ± 0.1 a	5.0 ± 0.1 bc	4.4 ± 0.0 a	5.2 ± 0.1bc	4.8 ± 0.2 b	5.7 ± 0.4 bc	5.2 ± 0.1 c	5.3 ± 0.2 c
Proline	10.3 ± 0.9 c	5.9 ± 0.4 ab	5.2 ± 0.1 a	7.0 ± 0.1 b	7.7 ± 0.5 b	11.0 ± 0.6 d	6.7 ± 0.4 a	6.3 ± 0.2 a	8.3 ± 0.4 b	7.9 ± 0.4b	9.9 ± 0.6 c	8.3 ± 0.1 b	8.4 ± 0.0 b	7.7 ± 0.0 b
Cysteine	4.6 ± 0.1 a	6.5 ± 0.2 b	6.3 ± 0.3 b	7.1 ± 0.3 bc	7.7 ± 0.1 c	3.9 ± 0.0 a	4.1 ± 0.1 ab	4.4 ± 0.1 abc	4.3 ± 0.5 abc	4.4 ± 0.2bc	4.1 ± 0.2 ab	4.7 ± 0.2 c	4.0 ± 0.0 ab	4.7 ± 0.1 c
Total NEAAs	77	126	125	144	153	78	78	82	78	83	83	86	81	85

[a] Values are expressed as mean ± standard deviation (*N* = 3), values followed by the same letter in the same row are not significantly different at 95% confidence level. The statistical analysis of the raw materials was carried out separately from the statistical analysis of the bread samples, d.m. dry matter.

One of the highest values of monounsaturated acid (MUFA) was found in WB, consisting mainly of oleic and elaidic acids. In general, the MUFA content in the bread with wheat flour partially replaced by chia was less than in the control sample, with the exception of the sample CWSB10 (Table 2.2). It is well known that unsaturated fatty acids are affected by high temperature and the presence of oxygen as a result of the breadmaking process, because the double bonds in their chemical structure are sensitive to oxidation (Wąsowicz et al., 2004). Consequently, the level of PUFAs in the bread with wheat flour partially replaced by CWF, CSDF, or CLFF was much less than in the bread with wheat flour partially replaced by chia seeds (Table 2.2). This could be due to protection of lipids by the seed outer cover structure, which remained intact throughout the whole process. However, all the formulations with chia had a significantly higher amount of PUFAs than in the control bread, mainly due to the α-linolenic acid (ALA) concentration ($p < 0.05$). Coelho and Salas-Mellado found that bread made with partial replacement of wheat flour by chia flour had lower SFA amounts and higher PUFA amounts than control bread in more drastic baking conditions, 20 min at 220 °C, than those used in the current investigation (Coelho and Salas-Mellado, 2015). In general, the substitution of wheat flour by chia flour produced a worse ω-3/ω-6 ratio than in the chia seed bread, regardless of the proportion of substitution, and even more so in the case of the defatted chia flours, in which the ratio decreased from 1:1 to 1:8 (Table 2.2). However, they were better than in the control bread and are near the recommended values (FAO, 2008; Agostoni et al., 2010]. Dietary recommendations (adequate intake (AI)) exist for polyunsaturated fatty acids, which are expressed as a percentage of total dietary energy (E%) (EFSA, 2017). The AI for linoleic acid (LA) is 4 E% and for α-linolenic acid (ALA) it is 0.5 E% (EFSA, 2017). The amounts of LA and ALA in the breads with chia made a greater contribution to dietary AI E% than the control bread, and CWSB10 made a higher contribution to the AI E% of LA (~6%) and ALA (~46%) than the other bread formulations, assuming an intake of 100 g of bread.

On the other hand, the caloric values of the breads with 5% replacement of wheat flour by chia ingredients presented a range between 256 and 270 kcal/100 g (CLFFB5 and CSDFB5, respectively), whereas the values of the breads with 10% replacement were slightly lower (232–268 kcal/100 g). CSDFB10 (232 kcal/100 g) and CLFFB10 (237 kcal/100 g) had lower values than the control bread (254 kcal/100 g), and also than the bread with chia, flax and sesame seeds (250 kcal/100 g) reported by USDA (United States Department of Agriculture) (US Department of Agriculture,2018). The main component in semi-defatted or low-fat chia flour is mucilage, so the breads made with those flours had the lowest values, as was expected. A similar trend was found by Fernandes and Salas-Mellado (Fernandes and Salas-Mellado, 2017), who made breads and cakes with vegetable fat replaced by chia mucilage, which decreased the caloric value.

Mineral Contribution to Popular Reference Intake/Recommended Dietary Allowances (PRI/RDAs) and Prediction of Mineral Bioavailability

The ash content in the chia ingredients was higher than in the wheat flour, and the ash contents of the semi-defatted and low-fat chia flours were approximately two times greater than the ash content of the chia flour. Furthermore, the higher levels of minerals found in the raw chia flours in comparison with wheat flour led to higher levels of these nutrients in the bread samples, as was expected (Table 2.3). The substitution of wheat flour by chia ingredients contributed between two and four times more Ca, Fe, and Zn than in the control bread (data not shown). Accordingly, if mineral absorption inhibitors are absent, the incorporation of chia in bread could increase the percentage contribution of important minerals, assuming an average intake of 100 g of bread per day, according to the PRI (popular reference intake (EFSA, 2017)) or the recommended dietary allowances (RDAs)). Ca was the only case in which the PRI contribution of the CLFFB formulation was higher (12%–14%) than the contribution of the control bread (9%). The same tendency was observed in the case of Fe, for which the contribution to PRI of bread with chia was higher than that of the control bread, and the formulation with CLFF was the best (Table 2.3). However, Ca, Fe, and Zn would not be bioavailable in their entirety because of the high presence of phytates, as shown by the InsP6/mineral molar ratio, which can predict the effect of phytate on the absorption of minerals (Ma et al., 2005). High levels of phytates were found in the chia ingredients (5.4–6.6 μmol/g on dry basis; Table 2.3). It is widely known that phytates have adverse effects on the bioavailability of di- and trivalent cations because of the formation of insoluble complexes in the intestinal tract of human and monogastric animals. However, there is a decrease in phytate content during the breadmaking process, mainly caused by the activity of phytase, which depends on many factors, such as temperature and pH, among others. Despite this reduction, it is generally not enough to make much improvement in the bioavailability of some minerals (Sanz-Penella et al., 2008). Consequently, the high phytate levels in the bread with 5% or 10% of chia could affect the mineral bioavailability of Zn, Fe, and Ca, as predicted by the phytate/mineral molar ratios (Sanz-Penella et al., 2008).

All the calcium present in the chia breads would be bioavailable, because the InsP6:Ca molar ratio was lower than 0.24, which means that its bioavailability would not be compromised. However, for the molar ratio found for InsP6, Fe was higher than 1.0 (1.8–10), which indicates that these breads would not be good sources of iron, because it would not be bioavailable. With regard to the bioavailability of Zn, FAO reported it in terms of three categories (high, moderate, and low) (FAO, 2001), whereas EFSA classified it according to the amount of phytate present in the diet (300, 600, 900, or 1200 mg/day) (FAO, 2001). For the breads with 5% of chia had $InsP_6$, Zn molar ratios that were <5, and they could provide at least 50% of the RDAs (FAO high bioavailability). With respect to the chia bread with 10% replacement had InsP6, Zn molar ratios between 5 and 15, which corresponds to FAO moderate bioavailability (FAO, 2001). It is important to note that the predicted bioavailability of zinc in all the breads formulated with chia ingredients was high or moderate because the $InsP_6$:Zn molar ratio was less than 15 (FAO, 2001) (Table 2.3).

Table 2.2

Fatty acid composition of raw material and bread with 5% and 10% replacement used in this study, g/100 g d.m. [a]

Fatty Acid	**Raw Materials**					**Bread Formula**								
	Wheat (W)	**Seeds (CWS)**	**Whole Flour (CWF)**	**Semi-Defatted Flour (CSDF)**	**Chia Low-Fat Flour (CLFF)**	**Control (WB)**	**Chia Seeds (CWSB)**		**Chia Whole Flour (CWFB)**		**Chia Semi-Defatted Flour (CSDFB)**		**Chia Low-Fat Flour (CLFFB)**	
							5%	**10%**	**5%**	**10%**	**5%**	**10%**	**5%**	**10%**
Lipids, % d.m.	2.09 ± 0.3 a	39.3 ± 1.3 d	41.5 ± 2.3 d	20.6 ± 0.7 c	13.5 ± 0.2 b	1.78 ± 0.1 bc	2.88 ± 0.1 de	3.5 ± 0.0 e	1.44 ± 0.2 abc	1.88 ± 0.01 c	1.39 ± 0.15 ab	1.44 ± 0.3 abc	1.14 ± 0.02 a	1.68 ± 0.1 bc
∑ SFA	0.53	3.36	4.33	2.16	1.23	0.54	0.58	0.61	0.49	0.60	0.54	0.52	0.44	0.50
Palmitic acid (C16:0)	0.48 ± 0.00 a	2.11 ± 0.04 d	2.65 ± 0.12 e	1.34 ± 0.05 c	0.83 ± 0.05 b	0.38 ± 0.00 abc	0.43 ± 0.01 c	0.43 ± 0.02 bc	).39 ± 0.02 ab	0.43 ± 0.03 bc	0.40 ± 0.02 bc	).39 ± 0.02 ab	0.35 ± 0.02 a	).38 ± 0.04 ab
Stearic acid (C18:0)	0.02 ± 0.02 a	1.08 ± 0.00 d	1.46 ± 0.10 e	0.72 ± 0.00 c	0.40 ± 0.02 b	0.14 ± 0.01 efg	0.13 ± 0.00 de	0.15 ± 0.02 g	0.09 ± 0.00 ab	0.15 ± 0.01 fg	0.12 ± 0.01 cde	0.11 ± 0.01 cd	0.07 ± 0.00 a	).10 ± 0.00 b
Arachidic acid (C20:0)	0.02 ± 0.00 a	0.12 ± 0.00 c	0.15 ± 0.01 d	0.07 ± 0.00 b	n.d.	0.02 ± 0.00 c	0.01 ± 0.00 bc	0.02 ± 0.00 c	).01 ± 0.00 ab	0.01 ± 0.00 bc	0.01 ± 0.00 abc	).01 ± 0.00 ab	0.01 ± 0.00 a	).01 ± 0.00 ab
Behenic acid (C22:0)	0.01 ± 0.00 a	0.05 ± 0.01 c	0.07 ± 0.07 c	0.03 ± 0.00 b	n.d.	n.d.	0.01 ± 0.00 a	0.01 ± 0.00 a	n.d.	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a
∑ MUFA	0.77	4.63	5.93	2.12	0.72	0.78	0.47	0.80	0.35	0.54	0.43	0.44	0.25	0.51
Elaidic acid (C18:1n9t)	0.15 ± 0.06 b	0.81 ± 0.02 c	0.97 ± 0.09 d	0.27 ± 0.02 b	n.d.	0.10 ± 0.01 cd	0.10 ± 0.01 cd	0.17 ± 0.00 f	0.06 ± 0.00 b	0.12 ± 0.01 e	0.09 ± 0.01 c	0.11 ± 0.01 d	0.03 ± 0.00 a	).10 ± 0.01 cd
dOleic acid (C18:1n9c)	0.62 ± 0.13 a	3.82 ± 0.08 d	4.96 ± 0.43 e	1.85 ± 0.13 c	0.72 ± 0.05 b	0.68 ± 0.03 e	0.37 ± 0.03 cd	0.63 ± 0.02 e	0.29 ± 0.01 b	0.42 ± 0.04 d	0.34 ± 0.03 bc	0.33 ± 0.00 bc	0.22 ± 0.00 a	0.41 ± 0.03 d
∑ PUFA	0.79	12.1	13.3	9.37	6.61	0.36	0.7	1.27	0.6	0.44	0.39	0.48	0.45	0.61
Linoleic acid (C18:2n6c)	0.75 ± 0.01 a	3.02 ± 0.07 c	3.81 ± 0.33 d	2.61 ± 0.18 c	1.93 ± 0.12 b	0.34 ± 0.02 a	0.36 ± 0.01 ab	0.65 ± 0.03 c	0.43 ± 0.12 ab	0.31 ± 0.09 a	0.32 ± 0.00 a	0.37 ± 0.01 ab	0.40 ± 0.08 ab	).54 ± 0.06 b
α-Linolenic acid (C18:3n3)	0.04 ± 0.00 a	9.09 ± 0.20 d	9.52 ± 0.83 d	6.76 ± 0.46 c	4.68 ± 0.29 b	0.02 ± 0.00 a	0.34 ± 0.00 f	0.62 ± 0.02 g	0.17 ± 0.00 e	0.13 ± 0.03 de	0.07 ± 0.01 bc	0.11 ± 0.01 cd	0.05 ± 0.00 ab	).07 ± 0.01 b
PUFA:SFA Ratio	1.3:1	3.6:1	3.1:1	4.3:1	5.4:1	0.66	1.21	2.1	1.22	0.7	0.7	0.92	1.0	1.34
ω-3/ω-6 Ratio Recommended Ratio 1:5 [1]; 1:8 [2]	1:19	3.0:1	2.5:1	2.6:1	2.4:1	1:17	1:1	1:1	1:3	1:2	1:5	1:3	1:8	1:8

[a] Values are expressed as mean ± standard deviation (*N* = 3), values followed by the same letter in the same row are not significantly different at 95% confidence level. The statistical analysis of the raw materials was carried out separately from the statistical analysis of the bread samples, d.m.: dry matter, n.d.: not detected; codes: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [1] WHO/FAO (World Health Organization/Food and Agriculture Organization), 2010; [2] EFSA (European Food Safety Authority), 2010.

Table 2.3

Effect of bread formulation on mineral dietary reference intake contribution and mineral availability prediction.

Parameter *	Units			Wheat Flour (W)	Chia Ingredients							
					Seeds (CWS)		Whole Flour (CWF)		Semi-Defatted (CSDF)		Low-Fat (CLFF)	
Ash ^{a}	g 100 g^{-1}			0.6 ± 0.0 a	2.0 ± 0.4 b		1.9 ± 0.5 b		3.5 ± 0.0 c		4.9 ± 0.1 d	
Ca ^{a}	mg 100 g dm^{-1}			106 ± 4 a	524 ± 4 b		659 ± 3 c		860 ± 10 e		805 ± 14 d	
Fe ^{a}	mg 100 g dm^{-1}			1.5 ± 0.1 a	7.3 ± 0.6 b		10.3 ± 0.2 d		8.0 ± 0.2 bc		8.3 ± 0.0 c	
Zn ^{a}	mg 100 g dm^{-1}			1.8 ± 0.0 a	8.7 ± 1.1 c		7.0 ± 0.1 b		7.7 ± 0.2 bc		8.1 ± 0.1 bc	
InsP_6	µmol g dm^{-1}			n.d.	5.4 ± 1.2 b		6.6 ± 1.3 c		5.1 ± 1.0 a		6.6 ± 0.7 c	
					Bread with Chia Ingredients							
		PRI/RDA, mg day^{-1} or InsP_6/Mineral, mol mol^{-1}		Control Bread (WB)	Seeds (CWSB)		Whole Flour (CWFB)		Semi-Defatted (CSDFB)		Low-Fat (CLFFB)	
					5%	10%	5%	10%	5%	10%	5%	10%
Ash^a	g 100g^{-1}			2.0 ± 0.1 a	2.5 ± 0.1 b	2.7 ± 0.2 b	2.4 ± 0.1 b	2.7 ± 0.2 b	2.5 ± 0.1 b	3.2 ± 0.0 c	2.4 ± 0.2 b	3.7 ± 0.0 d
Ca Contribution	%	FAO ^{b}	1,000	9	12	12	7	12	9	11	12	14
		EFSA ^{b}	950	9	12	13	7	13	10	11	13	15
Fe Contribution	%	FAO ^{b}	14/29	10/5	11/5	13/6	12/6	14/7	13/6	14/7	14/7	15/7
		EFSA ^{b}	11/16	12/9	14/10	17/11	16/11	18/12	17/11	18/12	18/12	19/13
Zn Contribution	%	FAO ^{b} High										
		bioavailability FAO ^{b}	4.2/3	40/56	43/61	53/75	49/69	50/70	48/67	50/70	50/69	54/75
		Moderate bioavailability	7/4.9	23/19	25/20	30/25	28/23	28/23	27/22	28/23	28/23	30/25
		FAO ^{b} Low	14/9.8	12/17	13/19	16/23	15/21	15/21	14/21	15/21	15/21	16/23
		bioavailability	9.4/7.5	18/22	19/24	24/30	22/27	22/28	21/27	22/28	22/28	24/30
		EFSA ^{b} $_{300}$	11.7/9.3	14/18	16/20	19/24	18/22	18/22	17/22	18/22	18/22	19/24
		EFSA ^{b} $_{600}$	14/11	12/15	13/17	16/20	15/19	15/19	14/18	15/19	15/19	16/21
		EFSA ^{b} $_{900}$	16.3/12.7	10/13	11/14	14/18	13/16	13/16	12/16	13/16	13/16	14/18
		EFSA ^{b} $_{1200}$										
InsP_6	µmol g dm^{-1}			n.d	1.2 ± 0.3 a	4.1 ± 0.8 bc	0.8 ± 0.3 a	3.6 ± 0.3 b	0.9 ± 0.2 a	3.6 ± 0.4 b	0.9 ± 0.1 a	4.8 ± 0.6 c

InsP_5	µmol g dm^{-1}		n.d	0.18 ± 0.02 a	1.1 ± 0.1 c	0.10 ± 0.05 a	0.76 ± 0.06 b	0.12 ± 0.03 a	0.76 ± 0.13 b	0.1 ± 0.00 a	0.83 ± 0.08 b
InsP_4	µmol g dm^{-1}		n.d	0.04 ± 0.01 a	0.38 ± 0.04 e	0.06 ± 0.09 a	0.37 ± 0.02 cd	0.04 ± 0.04a	0.33 ± 0.04 be	0.05 ± 0.05a	0.30 ± 0.03 b
InsP_3	µmol g dm^{-1}		n.d	0.05 ± 0.01 a	0.20 ± 0.03 cd	0.27 ± 0.09 cd	0.53 ± 0.02 e	0.16 ± 0.04 b	0.30 ± 0.03 d	0.22 ± 0.05 bc	0.31 ± 0.04 d
InsP_6/Ca [c]	mol mol^{-1}	<0.24	0	0.03	0.11	0.03	0.09	0.03	0.10	0.02	0.09
InsP_6/Fe [c]	mol mol^{-1}	<1	0	3.4	10.0	1.8	7.4	2.0	7.1	2.0	9.1
InsP_6/Zn [c]	mol mol^{-1}	<5	0	3.4	9.54	1.8	8.13	2.0	7.89	2.0	9.87

[*] Values are expressed as mean ± standard deviation (N = 3). [a] Values followed by the same letter in the same row are not significantly different at 95% confidence level; d.m.: dry matter; n.d. not detected; [b] FAO (Food and Agriculture Organization)/RDAs (recommended dietary allowances); EFSA (European Food Safety Authority)/PRIs (popular reference intakes) contribution (%) for a daily average intake of 100 g of bread if mineral absorption inhibitors are absent. PRIs/RDAs in mg per day for males (M)/females (F) ≥18. The FAO considers three levels of bioavailability of zinc, depending on the phytate (InsP_6) content in the diet: high, FAO$_{\text{high}}$ (InsP_6/mineral < 5); moderate, FAO$_{\text{moderate}}$ (InsP_6/mineral 5–15); and low bioavailability, FAO$_{\text{low}}$ (InsP_6/mineral > 15) [61]. EFSA contemplates four levels of phytate intake per day (300, EFSA$_{300}$; 600, EFSA$_{600}$; 900, EFSA$_{900}$; and 1200 mg per day, EFSA$_{1200}$) [24]; [c] Threshold ratios (InsP_6/mineral) for mineral availability inhibition [60]; InsP_6, *myo*-inositol hexakisphosphate; minerals Ca, Fe, or Zn.

Adequate Intake of Total Dietary Fiber in Breads

The amount of total dietary fiber in the breads with 5% or 10% of chia seed or chia flour varied between 6.1% and 8.7%, which was higher than in the control bread (4.1%; Table 2.4). These results were even higher than those of other formulations of bread with chia (up to 11% of substitution), which had 5.7% of total dietary fiber (Coelho and Salas-Mellado, 2015). The differences could be due to the great differences in the composition of chia seeds, depending on their origin. In the European Union, the current regulations concerning the composition of chia seeds marketed in Europe state that they should have no less than 18% of crude fiber, defined as the part of fiber made mainly of indigestible cellulose, pentosans, and lignin (European Union, 2013). The amount of dietary fiber in the chia seeds used in the current investigation varied between 30.9% and 36.2%, and was even higher after lipid extraction (Iglesias-Puig and Haros, 2013).

Intake of dietary fiber produces physiological activity that is most effective when the soluble/insoluble ratio is 1:2 (Jaime et al., 2002). The formulations with 10% of chia had ratios close to this value (Table 2.4) . These bakery products could be included in the diet, especially in the diet of people who do not achieve adequate intake of total dietary fiber, and they could have healthy effects such as reducing cholesterol, preventing constipation, and lowering the risk of developing diabetes or cardiovascular disease (Nohra and Bochicchio, 2015; Slavin, 2013]. Most of the fiber in chia seeds is soluble fiber, owing to the high proportion of mucilage, which can absorb up to 35.2 times its weight in water. This water holding capacity increases the viscosity of foods and also of the alimentary bolus, which could delay gastric emptying and thus reduce the accessibility of nutrients such as glucose (Lazaro et al., 2018) (Table 2.4).

From a nutritional point of view, assuming an intake of 100 g of bread per day, the breads with 10% of chia would provide between 33% and 34% of the AI of total dietary fiber for adults, which is 25 g/day (EFSA, 2017; Nishida et al., 2004).

Evaluation of Glycemic Index of Bread

The glycemic index could be affected by different factors such as food texture, source of starch, degree of starch gelatinization, and food processing, and by interaction with other ingredients. The control bread showed a high perentage of starch hydrolysis, ~56.9% (at 90 min in the in vitro test), in comparison with the 5% and 10% chia breads (between 5 and 9 units and between 9 and 15 units, respectively). The glycemic index and the glycemic load were lower in the loaves with 10% replacement than in those with 5%, and the latter had lower values than that of the control bread at 90 min in the in vitro test (Table 2.5). This behaviour corresponds to the lower amount of starch and higher amount of fiber in the bread with 10%

Table 2.4

Dietary fiber content and contribution to adequate intake in bread formulated with chia ingredients.

Parameter [a]	Units	Control (WB)	Bread with Chia Ingredients							
			Seeds (CWSB)		Whole Flour (CWFB)		Semi-Defatted (CSDFB)		Low-Fat (CLFFB)	
			5%	10%	5%	10%	5%	10%	5%	10%
Total Dietary Fiber [a]	g/100g d.m.	4.1 ± 0.1 a	6.1 ± 0.6 b	8.7 ± 0.2 c	6.3 ± 0.2 b	8.5 ± 0.5 c	7.0 ± 0.9 b	8.2 ± 0.1 c	7.1 ± 0.6 b	8.5 ± 0.1 c
Soluble Fiber [a]	g/100g d.m.	1.0 ± 0.0 a	1.7 ± 0.4 ab	2.4 ± 0.4 bc	1.5 ± 0.2 ab	2.8 ± 0.8 c	1.8 ± 0.3 ab	3.2 ± 0.3 c	1.8 ± 0.0 ab	2.9 ± 0.7 c
Insoluble Fiber [a]	g/100g d.m.	3.1 ± 0.1 a	4.4 ± 0.5 ab	5.0 ± 0.2 c	4.8 ± 0.0 bc	4.7 ± 0.7 bc	5.1 ± 0.6 bc	5.0 ± 0.4 bc	5.4 ± 0.6 bc	5.6 ± 0.8 bc
Soluble/Insoluble Fiber ratio [b]	g/g	1:3	1:3	1:2	1:3	1:2	1:3	1:2	1:3	1:2
AI[c] contribution	%	16	24	35	25	34	28	33	28	34

[a] Values are expressed as mean ± standard deviation (*N* = 3). Values followed by the same letter in the same row are not significantly different at 95% confidence level. d.m., dry matter; [b] Ratio of soluble/insoluble fiber, 1:2 [64]. [c] AI (Adequate Intake) contribution (%) for a daily average intake of 100 g of bread. AI in g per day for dietary fiber in adult ≥18 is 25 [24].

substitution than in the bread with 5% substitution or in the control formula.

Increasing the amount of chia in the bread formulation produced a decrease in the total amount of starch, from 79.1% (control bread) to 76.4%–74.2% (5% replacement) and 70.7%–72.9% (10% replacement), owing to the dilution effect, since chia ingredients are practically devoid of starch. All the formulations with chia ingredients had a lower GI than that of the control sample. This demonstrates the influence of the chia ingredients on glycemic response. This behaviour could be due to the contribution of the amount of chia mucilage in the bread. A similar trend was found in studies on biscuits fortified with soluble fiber, which had a lower glycemic index than the counterpart without soluble fiber. On the other hand, a significant decrease in the GL (Glycemic Load) value was observed when the proportion of chia seeds in the bread increased from 5% to 10%.

There was a significant decrease in the GI of all the bread formulations when the proportion of chia increased from 5% to 10%. Although the bread formulations with chia ingredients had an in vitro GI greater than 70 (high-glycemic food), further studies are needed to elucidate the real mechanism of action of chia ingredients in the lowering of GI. Laparra and Haros (Laparra and Haros, 2018) observed that when female rats were fed with a bread formulation containing 5% of chia seed the release of glucose in the blood was slow. Consumption of this type of bread may be beneficial in controlling the weight of obese people and it could help to prevent dysfunction in glucose metabolism.

Table 2.*5*

Effect of chia by-products in bread formulation on in vitro glycemic index estimation [a.]

Formulation		Total starch (%)	TSH_{90} (%)	AUC	GI	GL
Chia Ingredient	**Level (%)**					
Control (W)	0	79.1 ± 1.1 g	56.9 ± 5.5 c	5013	100 ± 2 d	57.0 ± 7.1 e
Chia Seeds (CWS)	5	74.5 ± 0.8 de	57.2 ± 0.0 c	4720	91.6 ± 2.0 c	52.4 ± 1.6 de
	10	71.7 ± 0.6 ab	48.1 ± 0.4 ab	3712	80.5 ± 3.1 ab	38.7 ± 1.7 abc
Whole Chia Flour (CWF)	5	75.6 ± 0.5 ef	48.4 ± 0.2 a	4228	86.1 ± 1.8 bc	41.4 ± 1.5 cd
	10	72.6 ± 0.0 b	42.1 ± 2.8 a	3215	75.0 ± 1.8 a	31.5 ± 1.0 ab
Semi-Defatted Chia Flour (CSDF)	5	74.2 ± 0.4 cd	51.5 ± 0.8 bc	4408	88.4 ± 2.5 c	45.5 ± 2.8 de
	10	70.7 ± 0.3 a	45.0 ± 3.0 ab	3293	77.0 ± 1.5 a	34.7 ± 4.2 abc
Low-Fat Chia Flour (CLFF)	5	76.4 ± 0.2 f	49.2 ± 0.9 abc	4308	87.3 ± 3.0 bc	42.9 ± 1.3 bcd
	10	72.9 ± 0.7 bc	43.8 ± 3.0 ab	3213	76.3 ± 3.0 a	29.9 ± 5.5 a

[a] Mean ± standard deviation, *N* = 3. Values followed by the same letter in the same column are not significantly different at 95% confidence level. TSH_{90}, Total starch hydrolyzed at 90 min; AUC, area under the curve of starch digestion; GI, glycemic index; GL glycemic load

CONCLUSIONS

The incorporation of chia seeds or chia flour increased the nutritional value of bread products with regard to the concentrations of proteins with higher biological value, lipids with a higher proportion of omega fatty acids, and minerals compared to the control sample. It is important to emphasize that chia seeds and chia flour contain a high concentration of the basic amino acid lysine, which is an essential amino acid from a nutritional standpoint and deficient in cereals. Consequently, chia ingredients are beneficial in cereal products. The higher linolenic acid content of the samples containing chia seeds, due to the protection that the integrity of the seed cover provides against oxidation during baking, should be taken into account when formulating baked foods enriched with omega-3; encapsulation of the oil is imperative if fortification is required. However, the chia seeds and chia flour provided a better ω-3/ω-6 ratio according to the recommendations of WHO/FAO and EFSA, despite the loss of unsaturated acids in the oven. The contribution of iron was deficient, whereas the contributions of calcium and zinc were higher than in wheat bread, taking into account the fact that their bioavailability was conditioned by the molar ratio (InsP_6/mineral). The CSDFB10 and CLFFB10 breads could be included in the daily diet to control obesity and to prevent constipation owing to their low fat content and high amount of dietary fiber.

The breads with chia provide higher contributions to intake of calcium, iron, and zinc than wheat bread. Accordingly, bread with chia seed or chia flour meets almost all the daily requirement of these minerals in women and men, which is not the case with wheat bread. The bread with 10% chia could be used in a weight control diet because of its low glycemic load.

In the light of the present data, chia seed and chia flour could be used as a partial replacement of wheat flour in bread formulations, increasing the nutritional and functional value of the products, with important implications that could help to enhance their role in the prevention of metabolic diseases.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations:

American Association of Cereal Chemists	AACC
Adequate intake	AI
Amino acid score	AAS

Alpha-linolenic acid	ALA
Association of Official Analytical Chemists	AOAC
Chia seeds	CWS
Chia whole flour	CWF
Control bread	WB
Cysteine	Cys
Dietary reference intakes	DRIs
Dry matter	d.m.
Essential amino acids	EAAs
European Food Safety Authority	EFSA
European Union	EU
Females	F
Food and Agriculture Organization	FAO
Gas chromatography	GC
Glycemic index	GI
Glycemic load	GL
High Performance Liquid Chromatography	HPLC
Histidine	His
Hydrolysis index	HI
Inositol tetrakisphosphate	InsP_4
Inositol trisphosphate	InsP_3
Inositol pentakisphosphate	InsP_5
Isoleucine	Ile

Linoleic acid	LA
Low-fat chia flour	CLFF
Low-Fat chia flour bread, replaced at 5%	CLFFB5
Low-fat chia flour bread, replaced at 10%	CLFFB10
Lysine	Lys
Males	M
Methionine	Met
Metionine+Cysteine	Met+Cys
Monounsaturated acid	MUFA
Non-essential amino acid	NEAA
Phenylalanine+Tyrosine	Phe+Tyr
Phytic acid	InsP_6
Polyunsaturated fatty acids	PUFAs
Popular reference intake	PRI
Recommended dietary allowance	RDA
Saturated fatty acids	SFA
Semi-defatted chia flour	CSDF
Semi-defatted flour bread replaced at 5%	CSDFB5
Semi-defatted flour bread replaced at 10%	CSDFB10
Total dietary fiber	TDF
Tryptophan	Trp
Tyrosine	Tyr
United Nations University	UNU

United States Department of Agriculture	USDA
Valine	Val
Wheat flour	W
Whole seed bread, replaced at 5%	CWSB5
Whole Seed Bread, replaced at 10%	CWSB10
Whole flour bread, replaced at 5%	CWFB5
Whole flour bread, replaced at 10%	CWFB10
World Health Organization	WHO

Chemical compounds studied in this article:

L-histidine	PubChem CID: 6274
L-threonine	PubChem CID: 6288
L-tyrosine	PubChem CID: 6057
L-valine	PubChem CID: 6287
L-methionine	PubChem CID: 6137
L- phenylalanine	PubChem CID: 6140
L-tryptophan	PubChem CID: 6305
l-isoleucine	PubChem CID: 6306
L-leucine	PubChem CID: 6106
Lysine	PubChem CID: 5962
L-aspartic acid	PubChem CID: 5960
L-asparagine	PubChem CID: 6267
Glutamic acid	PubChem CID: 33032
L-glutamine	PubChem CID: 5961

Serine	PubChem CID: 5951
Glycine	PubChem CID: 750
L-arginine	PubChem CID: 6322
L-alanine	PubChem CID: 5950
L-proline	PubChem CID: 145742
L-Cysteine	PubChem CID: 5862
Palmitic acid	PubChem CID: 985
Stearic acid	PubChem CID: 5281
Arachidic acid	PubChem CID: 10467
Behenic acid	PubChem CID: 8215
Elaidic acid	PubChem CID: 637517
Oleic acid	PubChem CID: 445639
Linoleic acid	PubChem CID: 5280450
alpha-Linolenic acid	PubChem CID: 5280934
Phytic acid	PubChem CID: 890
Myo-inositol	PubChem CID: 892
D-Glucose	PubChem CID: 5793

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CAPÍTULO 3

Combined effect of chia, quinoa and amaranth incorporation on physico-chemical, nutritional and functional quality of fresh bread

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ABSTRACT

With regard to constant technological innovations in the bakery sector in order to increase bread nutritional value without affecting its technological and sensory characteristics, we applied pseudocereals/oilseeds to obtain an optimal formulation. A factorial design 3^3 was used and the independent factors were chia flour (levels: 0, 10, 20% flour basis), quinoa flour (levels: 0, 20, 40% flour basis), and amaranth flour (levels: 0, 20, 40% flour basis). Their effects and interactions were studied through the response surface methodology to optimise the bread formulation from a holistic viewpoint, which included the nutritional, technological and sensory characteristics. The optimum formulation with the highest quality was the blend made with 10, 4, and 20% of chia, quinoa, and amaranth, respectively. The results showed a significant increase in protein amount, ash, lipids, and crumb firmness compared to wheat bread. The calorie value of the control sample and the optimised formula were significantly similar, bearing in mind the high lipid amounts present in raw materials. Loaf-specific volume slightly decreased in comparison to control bread, as expected in formulations with gluten-free raw materials and a large amount of fibre. The optimised formula presented nutritionally/functionally higher indexes and similar overall acceptability to the control bread ($p < 0.05$).

INTRODUCTION

In the last few years, scientific studies have demonstrated that the regular intake of wholemeal or whole grain products prevents certain chronic diseases from developing, such as cardiovascular diseases, type 2 diabetes, and certain cancer types. Hence, consumer interest in such products has grown, although consumer acceptability is conditioned by their sensorial aspects despite being nutritional food with biological functionality in our organism (Parenti et al., 2020; Ye et al., 2012). Thanks to efforts to develop healthy and appealing bread products to supply nutritional, technological, and sensorial quality requirements, researchers have studied different strategies in order to develop products that use wholemeal flours with coadjuvants/additives that cover these requirements. These include adding baking enhancers, such as enzymes and/or chemical compounds (Parenti et al., 2020; Rebellato et al., 2017; Sanz-Penella et al., 2014), using wholemeal flours with different granulometries to increase sensorial quality (Bin and Peterson, 2016; Protonotariou et al., 2020), and/or partially replacing flour with more nutritional and healthy ingredients such as legumes (Bedrníček et al.,2020; Guardado-Félix et al., 2020) oilseeds (Iglesias-Puig et al., 2013; Ahmad et al., 2018), and pseudocereals (Ballester-Sánchez et al., 2019; Miranda et al., 2019). The use of wholemeal flours of legumes, oilseeds, cereals, and pseudocereals increases their mineral content, but this increase comes with higher levels of phytic acid (InsP6), forming insoluble compounds that inhibit their bioavailability. Some strategies can increase the bioavailability of minerals when using sourdough (Karaman et al., 2018; Sanz-Penella et al., 2012), or exogenous phytases, which are bread fermentation starters that produce phytases (Sanz-Penella et al., 2014; Iglesias-Puig et al., 2015), or chemical agents such as ferric sodium ethylene diamine tetra acetic acid (Rebellato et al., 2017), among other strategies.

Consequently, the industry in this sector has closely examined the strategy of substituting refined wheat flour for wholemeal ingredients with high added value, such as pseudocereals, legumes, and/or oilseed so that more wholemeal foods offering better technological properties are eaten (loaf-specific volume, and crumb and crust colour and texture) with better nutritional properties (better amino acid and lipid profile, higher mineral content, better protein digestibility, and less starch digestibility) (Liu et al., 2017). According to a considerable number of studies, baking products supplemented with wholemeal quinoa, amaranth, or chia flours have a higher nutritional value, but the end product's technological and sensorial quality is lost (Miranda-Ramos et al., 2020; Iglesias-Puig et al., 2015; Haros and Schoenlechner, 2017; Penella et al., 2013; Zettel and Hitzmann, 2018). Generally speaking, loss of quality with formulations enriched with different ingredients to wheat is due to gluten dilution, which affects all the bread-making process steps in accordance with the substitution level and the ingredient in question (De Lamo and Gómez, 2018). Although quality is compromised with such products, their nutritional value increases. This occurs in the bread formulations replaced with quinoa wholemeal flour, which not only contributes to daily diet fibre intake and daily Fe and Zn requirements, but also improves the ω-6/ω-3 ratio and protein quality (Ballester-Sánchez et al., 2019; Iglesias-Puig et al., 2015). Nevertheless, loaf-specific volume is reduced with textural changes in crumbs such as crumb firmness; crumb grain with bigger pores and thin walls; low resilience, cohesion, and elasticity; and a bitter taste (Gostin, 2019; Xu et al., 2019). The behaviour of the baking products replaced partially with different amaranth species was similar (Miranda et al., 2019).

Replacing wheat flour with chia did not lead to loss of end product quality, and consumer acceptability was higher (Iglesias-Puig et al., 2015). However, this tendency did no remain when the level of this oilseed was raised, taking in account the current EU regulations of a maximum level of 10% in bakery products (Miranda-Ramos et al., 2020), European Food Safety Authority (EFSA, 2020). Regarding the nutritional profile, as with bread products made with pseudocereals, bread made with chia contained more minerals, lipids, dietary fibre, and proteins, all with a higher biological value. Including chia in products results in lower glycaemic index (GI) and better saturated fatty acid (SFA)/polyunsaturated fatty acid (PUFA) ratios (Miranda-Ramos et al., 2020).

Hence, the main objective of this study was to develop top quality bread by substituting wheat flour for an optimum mixture of wholemeal quinoa, amaranth, and chia flours to maximise its physico-chemical, technological, nutritional, and sensorial properties by a factorial design 33 and by following the response surface methodology (RSM). Another aim was to evaluate the nutritional value of the optimised formulation by considering its contribution to the daily recommended intake of fatty acids (omega), minerals (Ca, Fe, Zn), and dietary fibre (soluble and insoluble) and its protein quality, and to estimate its glycaemic index (GI) values in vitro.

MATERIALS AND METHODS

Materials

Quinoa (*Chenopodium quinoa* Willd), black chia (*Salvia hispanica* L.), and amaranth (*Amaranth caudatus*) flour (Inca´s treasure, Quito, Ecuador) were milled in a hammer type cyclone mill and at standard sieve (0.8 mm) (Lab Mill 3100, Perten Instruments, Huddinge, Sweden) and stored at 14 °C. Dehydrated yeast (*Saccharomyces cerevisiae*, Maizena, Spain) was used as a starter. Commercial wheat flour and whole wheat flour obtained from HARINERA LA META S.A. (part of La Meta Group, the Vall Companys Group's flour division, Barcelona, Spain) was employed for the bread-making process.

Bread-making procedure

The control bread dough formula consisted of wheat flour (300 g), compressed yeast (3% flour basis), sodium salt (1.6% flour basis), and distilled water (up to optimum absorption, 500 Brabender Units). The 27 bread formulations with amaranth, quinoa, and/or chia, obtained by factorial design 3^3, were mixed for 7 min, left for 10 min, divided (100 g), kneaded, and then left again (15 min). Dough was manually rolled, proven (up to optimum volume increase at 28 °C, 85% relative humidity), and baked at 180 °C/29 min. Temperature and volume increase of dough was monitored at regular intervals during fermentation. After fermentation, dough was baked in an electric oven and cooled at room temperature for 60 min for subsequent analyses.

Composition of flours and bread

Proximate analyses of raw materials and breads were performed in terms of moisture, total dietary fibre (TDF), and starch according to the approved Association of Official Agricultural Chemistry 925.09, 991.43, and 996.11, respectively (AOAC, 1996). Protein determination was carried out by the Dumas combustion method and a nitrogen conversion factor: 5.7/wheat flour; 5.53/quinoa, amaranth, chia whole flours; 5.83/wheat wholemeal; and 6.25/breads according to ISO (International Organization for Standardization)/TS(Technical Specification)16634-1 and ISO/TS 16634-2 (ISO/TS, 2016). Lipid and ash contents were established according to Official Methods 30-10 and 08-03, respectively, from the American Association of Cereal Chemists (AACC, 2000). Measurements were taken in triplicate.

Technological parameters

The analysed technological parameters were as follows: loaf-specific volume (cm^3/g) by measuring volume (cm^3) by seed displacement (volume-meter, Chopin, France) and weight (g), the width/height ratio of the central slice (cm/cm), and colour tristimulus parameters (Chromameter CR-400, Konika Minolta Sensing, Japan). From the colour parameters, we calculated the total colour difference (ΔE^*) by the Equation (1): Samples were analysed at least in triplicate (Iglesias-Puig et al., 2013).

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

Crumb texture was determined by the texture profile analysis using a TA-XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom). A 2 cm-thick slice of bread was compressed twice by a stainless steel 0.5 cm diameter plunger, moving 1.0 min/s to a penetration distance of 50%, with an interval of 50 s between compressions. The following parameters were evaluated: firmness, springiness, cohesiveness, and chewiness.

The digital image analysis was used to measure bread crumb structure. Digital images were taken by an EVOCAM-II Macroscope (Vision engineering, Woking, United Kingdom). Images were processed and analysed by the Nis Elements BR 3.2 software (Nikon Corporation, Japan) and also Fiji (ImageJ 1.49q Software, National Institutes of Health, Bethesda, MD, USA). A single 10 × 10 mm square field of view of two central slices (10 mm thick) of both loaves was used to yield three digital images per treatment. Data were processed using the Statgraphics Plus 16.1.03 software (Bitstream, Cambridge, MN, USA). The chosen crumb grain features were cell area/total area, cm^2/cm^2; wall area/total area, cm^2/cm^2; number of cells per cm^2; and mean cell area, mm^2.

Fatty acid profile

Samples were transesterified to convert triglycerides into fatty acid methyl esters (FAMEs), following the methodology previously described by the American Oil Chemists' Society (AOCS, 1992). The fatty acid composition and quantification were determined by gas chromatography with a capillary column (SP 2330 on 100/120 WAW-60 m × 0.25 mm × 0.5 mm) and a flame ionisation detector according to the International Union of Pure and Applied Chemistry Method 2.302 (IUPAC. Fats and Derivatives, 1992). Measurements were taken in triplicate.

Mineral Composition

The total Ca, Fe, and Zn concentrations were determined in a flame absorption spectrometer at the Analysis of Soils, Plants and Water Service of the Institute of Agricultural Sciences, Madrid (Spain). Each sample (0.5 g) was placed in a Teflon perfluoroalkoxy vessel and digested with HNO_3 (4 mL, 14 M) and H_2O_2 (1 mL, 30% *v/v*) attack. Samples were irradiated at 800 W (15 min at 180 °C) by a Microwave Accelerated Reaction System (MARS, Charlotte, NC, USA). At the end of the digestion programme, the digest was placed in a polypropylene tube and made up to final volume with distilled water. Measurements were taken in triplicate (Miranda-Ramos et al., 2020).

Determination of myo-inositol hexakisphosphate

The *myo*-inositol hexakisphosphate or phytic acid (InsP_6) present in raw materials and the residual in the bread formulations after the bread-making process was measured as phosphorus released by phytase and alkaline phosphatase by a simple quantity K-PHYT method (McKie and McCleary, 2019). This method consists of acid extraction of phytates, followed by treatment with phytase and alkaline phosphatase enzymes to release phosphates from the myo-inositol ring. The total released phosphate was measured by a colorimetric technique according to the AOAC method 986.11 (AOAC, 1996). Samples were analysed in triplicate.

Amino Acids Profile

For the amino acid analysis, 10 mL of hydrolysed sample was prepared with 4 mL of 6 N HCl. Solutions were capped in a nitrogen atmosphere for 24 h. Amino acids were determined by acid hydrolysis after derivatisation with diethyl ethoxymethylenemalonate in a high-performance liquid chromatography (HPLC) Model 600E multisystem with a 484 UV–VIS detector (300 mm × 3.9 mm) and a reversed-phase column (Novapack C18, 4 m; Waters), acetonitrile in the binary gradient, detection at 280 nm, with D,L-α-aminobutyric acid as the internal standard. Solvents were injected into the column at a flow rate of 0.9 mL/min. Temperature remained at 18 °C (Alaiz et al., 1992).

In Vitro Protein Digestibility, Essential Aminoacids and Nutritional Index

The in vitro gastric digestion of bread samples was carried out according to the methodology described by Sanz-Penella et al. (Sanz-Penella et al., 2012). The dry and ground ~~power~~ of bread was subjected to a simulated gastrointestinal digestion, beginning by a simple digestion with the addition of pepsin (800–2500 Units/mg protein), pancreatin (activity, 4×; United States Pharmacopeia (USP)/reference standard specifications), and bile extract, which were demineralised with Chelex-100 before use. Briefly, 6 mL of an isotonic saline solution (140 mM NaCl, 5 mM KCl) was added to the sample breads (1.000 ± 0.001 g), and mixtures were acidified to pH 3.0 with 0.1 mol/L HCl. Then, 0.96 mL of a pepsin solution (0.01 g/mL) was added, and the mixture was incubated for 1 h at 37 °C (gastric digestion). Later, the protein contained in the gastric digestion solution was measured by the Bradford method with bovine serum albumin as the standard.

The essential amino acid index (EAAI) was calculated according to Motta et al. (Motta, et al., 2019) by applying the following Equation (2):

$$EAA = 0.1\left[log\left(\frac{a_1}{a_{1s}} \times 100\right) + log\left(\frac{a_2}{a_{2s}} \times\ 100\right) + \cdots log\left(\frac{a_n}{a_{ns}}\right) \times 100\right] \tag{2}$$

where $a_1, a_2, \ldots, a_n$ are the amino acid contents in the sample, and $a_{1s}, a_{2s}, \ldots, a_{ns}$ are the essential amino acid requirements in the protein standard (FAO/WHO/UNU, 2007).

The nutritional index (NI, Equation (3)) normalises the qualitative and quantitative variations of the test protein compared to its nutritional status. The NI was calculated by the equation of Crisan and Sands (Crisan and Sands, 1978), which considers all the factors to be of equal importance:

$$NI = \frac{EAAI\ Protein\ (\%)}{100} \tag{3}$$

In vitro Glycaemic Index estimation

To evaluate the in vitro rate of starch hydrolysis, we followed the method described by Goni et al. (Goni, et al., 1997) with slight modifications according to Sanz-Penella et al. (Sanz-Penella et al., 2014). The hydrolysis index (HI) was calculated from the area under the curve (AUC) from 0 to 120 min for samples as a percentage of the corresponding area of reference (wheat bread) (HI = AUC sample/AUC wheat bread × 100). The glycaemic index (GI) was calculated by the equation GI = 0.549 × HI + 39.71. Measurements were taken in triplicate. The predicted glycaemic load (pGL) was calculated for a 100 g bread portion from the glucose-related GI according to pGL = glycaemic index × total carbohydrates/100, and by taking into account the total carbohydrates of each sample (Wolter et al., 2014).

Preliminary sensory evaluation

The parameters measured in the control and optimised bread formulae were appearance, texture, taste, and overall acceptability, evaluated by a panel of 50 untrained tasters who usually purchase wheat bread using a 9-point hedonic scale of global acceptance: (9) “Especially like”; (8) “Very much like”; (7) “Moderately like”; (6) “Somewhat like”; (5) “Neither like nor dislike”; (4) “Slightly dislike”; (3) “Moderately dislike”; (2) “Very much dislike”; (1) “Especially dislike” (Iglesias-Puig et al., 2015).

Factorial design

In order to study the effect of replacing wheat flour with nutritious ingredients on the physico-chemical, nutritional, technological, and sensory properties, we used a factorial design 3^3. The 3 studied factors were the percentage of wheat flour replacement with whole chia flour at 3 levels (0, 10, and 20%), whole quinoa flour at 3 levels (0, 20, and 40%), and amaranth flour at 3 levels (0, 20, and 40%). The run conditions of the factorial design in terms of the experimental conditions and coded values are shown in Table 3.1.

Table 3.1
Factorial design.

Trial	Name	% of Substitution in Flour Basis			Variables Codes		
		Chia Flour	**Quinoa Flour**	**Amaranth Flour**	**Chia *x1***	**Quinoa *x2***	**Amaranth *x3***
1	CB	0	0	0	−1	−1	−1
2	Ch$_{10}$	10	0	0	0	−1	−1
3	Ch$_{20}$	20	0	0	1	−1	−1
4	Q$_{20}$	0	20	0	−1	0	−1
5	Ch$_{10}$Q$_{20}$	10	20	0	0	0	−1
6	Ch$_{20}$Q$_{20}$	20	20	0	1	0	−1
7	Q$_{40}$	0	40	0	−1	1	−1
8	Ch$_{10}$Q$_{40}$	10	40	0	0	1	−1
9	Ch$_{20}$Q$_{40}$	20	40	0	1	1	−1
10	A$_{20}$	0	0	20	−1	−1	0
11	Ch$_{10}$A$_{20}$	10	0	20	0	−1	0
12	Ch$_{20}$A$_{20}$	20	0	20	1	−1	0
13	Q$_{20}$A$_{20}$	0	20	20	−1	0	0
14	Q$_{20}$A$_{20}$Ch$_{10}$	10	20	20	0	0	0
15	Q$_{20}$A$_{20}$Ch$_{20}$	20	20	20	1	0	0
16	Q$_{40}$A$_{20}$	0	40	20	−1	1	0
17	Q$_{40}$A$_{20}$Ch$_{10}$	10	40	20	0	1	0
18	Q$_{40}$A$_{20}$Ch$_{20}$	20	40	20	1	1	0
19	A$_{40}$	0	0	40	−1	−1	1
20	A$_{40}$Ch$_{10}$	10	0	40	0	−1	1
21	A$_{40}$Ch$_{20}$	20	0	40	1	−1	1
22	Q$_{20}$A$_{40}$	0	20	40	−1	0	1
23	Q$_{20}$A$_{40}$Ch$_{10}$	10	20	40	0	0	1
24	Q$_{20}$A$_{40}$Ch$_{20}$	20	20	40	1	0	1
25	Q$_{40}$A$_{40}$	0	40	40	−1	1	1
26	Q$_{40}$A$_{40}$Ch$_{10}$	10	40	40	0	1	1
27	Q$_{40}$A$_{40}$Ch$_{20}$	20	40	40	1	1	1

CB: control bread; Ch: whole chia flour; Q: whole quinoa flour; A: whole amaranth flour.

The design enabled us to approximate the experimental data (Y_{obs}) with a response surface model expressed as coded values according the Equation (4):

$$Y_{obs} = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{22}x_2^2 + a_{23}x_2x_3 + a_{33}x_3^2 + \varepsilon \quad (4)$$

where x_1 is the design factor whole chia flour; x_2 is the whole quinoa flour; x_3 is whole amaranth flour; and coefficients a_1, a_2, and a_3 are the main effects of x_1, x_2, and x_3, respectively. The square coefficients (a_{ii}) indicate whether any of the variables have a maximum or minimum in the experimental domain, whereas the mixed coefficients (a_{12}), (a_{13}), and (a_{23}) represent interactions between factors. The difference between the experimental data (Y_{obs}) and model Y_{calc} gives the residual (ε). For each response, the RS-Q (squared correlation coefficient) was calculated, which is the fraction of variation of the response explained by the model.

The response variables were content of lipids, ash, proteins, and calorie value of bread (nutritional characteristics); piece-specific volume, crumb and crust colour, crumb firmness, and shape ratio (technological qualities); and sensory evaluation, such as the product's appearance, texture, taste, and overall consumer acceptability. Twenty-seven formulations were studied with different proportions of amaranth, quinoa, and/or chia, as shown in Table 3.1.

Statistical analysis

One-way ANOVA and Fisher's least significant differences (LSD) were applied to establish significant differences between samples (*p* < 0.05). Homogeneity of variances was tested using Levene's tests and normally distributed on the basis of the Shapiro–Wilk test. All statistical analyses and optimisation of multiple responses were carried out with the Statgraphics Plus 16.1.03 software (Bitstream, Cambridge, MN, USA).

The desired objective was selected for each dependent variable on the basis of the values obtained by the control condition (wheat bread). For the numerical optimisation, all the independent variables were left within their predetermined range, while the dependent variables were optimised by taking into account the nutritional, technological, and sensory aspects.

RESULTS AND DISCUSSION

Flour composition

The chemical composition of the flours herein employed is found in Table 3.2. The protein content of the quinoa, amaranth, and chia flours was significantly higher than the protein content of both the refined wheat and commercial wholemeal wheat flours. The protein contents of amaranth, quinoa, and chia in this study fell in line with those previously reported by other researchers: 13.1–21.5%, 8.0–22.0%, and 18.2–25.3%, respectively (Marineli et al., 2014; Martínez-Villaluenga et al., 2020; Montemurro et al., 2019). Variations in protein content and the amino acid profile depend on growth conditions and genotype (Reguera and Haros, 2017). This means that wholemeal flours from the crops of Andean origin used in the present study could cover the protein requirements recommended by both the Food and Agriculture Organisation (FAO)/World Health Organisation (WHO), which stress chia protein (Miranda-Ramos et al., 2020; Grancieri et al., 2019). The high lysine content in the amino acid profile is present in quinoa, amaranth, and chia grains, whereas is deficient in cereals (Montemurro et al., 2019; Grancieri et al., 2019; Singh et al., 2019). In grains, proteins are distributed heterogeneously, and have a different biological quality depending on grain parts. For instance, in amaranth, 65% of lysine-rich proteins are found in the germ, but only 35% of lysine-poor protein lie in the endosperm. The exact opposite occurs in cereals where 85% of lysine-poor protein appears in the endosperm (Martinez-Lopez et al., 2020). This is why it is so important to eat food made with wholemeal flours because they supply much nutrition, including protein quality and quantity.

Lipid content in the quinoa, amaranth, and chia flours was significantly higher than the lipid content in refined wheat flours because the germ is removed during the refining process, as are outer bran layers, which is where the biggest quantity of fat and fibre is found (Bressiani et al., 2017). Nonetheless, the lipid content of chia seeds of Andean origin can vary according to agronomic conditions (30.7–41.5%) (Miranda-Ramos et al., 2020; Valdivia-López and Tecante, 2015). The high percentage of lipids in wholemeal quinoa and amaranth flours is because the germ remains after milling whole grains. These results fall in line with those found in the literature, lying between 2% and 11% in quinoa, and between 5.6% and 19.3% in amaranth [40], and are similar to the results obtained for wholemeal wheat flour (1–2.5%) (Solah et al., 2016).

Table 3.2
Chemical composition of raw materials.

Parameters [a]	Units	Whole Wheat Flour	Wheat Flour	Whole Amaranth Flour	Whole Quinoa Flour	Whole Chia Flour
Moisture	%	13.25 ± 0.011 e	11.38 ± 0.09 b	12.41 ± 0.04 c	12.62 ± 0.04 d	7.95 ± 0.01 a
Protein	% d.m.	11.76 ± 0.07 a	12.54 ± 0.1 b	17.02 ± 0.10 c	17.51 ± 0.50 c	19.63 ± 0.3 d
Lipids	% d.m.	1.45 ± 0.03 b	1.00 ± 0.02 a	6.60 ± 0.20 d	6.45 ± 0.02 c	34.2 ± 0.4 e
Ash	% d.m.	1.43 ± 0.02 b	0.58 ± 0.01 a	2.65 ± 0.04 d	2.82 ± 0.05 c	4.69 ± 0.05 e
Starch	% d.m.	73.3 ± 3.00 b	68.9 ± 2.90 b	55.0 ± 0.30 a	54.30 ± 1.70 a	ND
Total fibre	% d.m.	6.58 ± 0.06 b	3.90 ± 0.10 a	15.6 ± 2.90 c	14.20 ± 0.60 c	39.0 ± 0.1 d
Soluble dietary fibre	% d.m.	0.88 ± 0.07 a	1.06 ± 0.46 b	3.08 ± 1.50 b	4.10 ± 1.20 b	6.60 ± 1.30 c
Insoluble dietary fibre	% d.m.	5.70 ± 0.05 b	2.81 ± 0.35 a	12.6 ± 1.4 d	10.2 ± 0.5 c	32.4 ± 1.3 e

[a] Mean ± SD *n* = 3. Values followed by the same letter in the same column are not statistically different at 95% confidence level; d.m., dry matter; ND, not detected.

Variation in lipid content is generally due to inter-species differences, environmental factors, and crop-growing practices (Miranda-Ramos et al., 2020; Grancieri et al., 2019; Martínez et al., 2018). The quality of the lipids presents in the herein used flours is characterised by their high content of PUFA and their suitable linoleic acid (LA)/alpha-linolenic acid (ALA) ratios, whose low values positively impact health. The lowest ratio of the employed ingredients was found for chia for its high alpha-linolenic acid content (50–57%), followed by quinoa, then amaranth, and finally by wheat (Martínez-Villaluenga et al., 2020). Thus, employing these crops could help to promote healthy eating and prevent cardiovascular diseases (Valdivia-López and Tecante, 2015).

Total fibre content was significantly higher in the chia flour compared to the wholemeal quinoa, amaranth, and wheat flours (Table 3.2). Of the total chia fibre, 17% corresponded to soluble dietary fibre (SDF) and the rest to insoluble dietary fibre (IDF), which agrees with values reported in the literature (7–15%/SDF; 85–93%/IDF) (Grancieri et al., 2019; Kulczyński et al., 2019). No significant differences were found between total fibre content for quinoa and amaranth, which was higher than in wheat. The same trend was observed with both soluble and insoluble fibre, which also agrees with the values reported in the literature for soluble fibre content in quinoa and amaranth versus wheat. Moreover, the percentage ratio between soluble and insoluble fibre, compared to total fibre, in the wholemeal quinoa (29%/SDF; 71%/IDF) and amaranth (20%/SDF; 80%/IDF) flours were similar to those shown in the literature (Haros and Schoenlechner, 2017; Montemurro et al., 2019).

Generally speaking, the wholemeal flours made with ancient crops presented a significantly higher ash concentration, which was directly related to a higher mineral content, where chia stood out, followed by quinoa and amaranth, and finally by wheat (Table 3.2). Regarding starch content, this main carbohydrate found in cereals/pseudocereals was not detected in chia and was significantly lower in wholemeal flours of pseudocereals than in wheat flours (Table 3.2). This may be particularly relevant in cereal food formulations to lower their GI values (Miranda-Ramos et al., 2020).

Effect of the Independent Variables on Bread Nutritional Properties

When optimising a bread product formulation, the intention is to maximise lipid content because it is known that amaranth, quinoa, and chia flours have high polyunsaturated fatty acid (PUFA) contents with beneficial health effects, as previously reported (Haros and Schoenlechner, 2017; De Lamo and Gómez, 2018). The lipid content of all the studied formulations varied from 1% to 12% d.m. (dry matter) (Figure S1). This means that the isolated inclusion of chia flour in the formulation increased this parameter, just as its linear coefficient indicated (a_1: 0.214, $p < 0.01$), followed by the quinoa (a_2: 0.061, $p < 0.01$) and amaranth (a_3: 0.002, $p < 0.01$) flours (Table 3.3). A significant interaction was observed between the amaranth and chia flours (a_{13}: 0.002, $p < 0.05$), which was not detected with quinoa (Table 3.3). This effect could be shown in the baked products requiring high temperatures when baked, such as bread products containing amaranth and chia, as they present better lipid stability because both matrices contain high concentrations of antioxidants, unlike quinoa (Haros and Schoenlechner, 2017;

Marineli et al., 2014; Martínez-Villaluenga et al., 2020; Grancieri et al., 2019). Natural antioxidants are present, such as tocopherol and squalene, although the latter is only present in amaranth (D'Amico et al., 2017) and can help to reduce lipid oxidation in chia (Bodoira et al., 2017). Therefore, it would be worthwhile to mix chia and amaranth flours to protect the lipid fraction in formulations. Moreover, this interaction effect that took place in the chia–amaranth mixture, but not in the quinoa flour, could also be affected by quantities of polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFAs), and ratios. As the PUFA/SFA ratio increases, lipid stability diminishes in oxidative rancidity terms (Jiménez, et al., 2020). The quinoa PUFA/SFA ratio was 4, while that of amaranth was 3 (Martínez-Villaluenga et al., 2020).

The calorie value is related mainly to lipid content. It rose as chia flour quantity increased, and, as expected, the linear coefficient was positive and significant (a_1: 0.906; $p < 0.01$), followed by amaranth flour (a_3: 0.307; $p < 0.0$), and finally by quinoa flour (a_3: 0.129; $p < 0.01$). Protein and ash contents varied between 14.25–20.26% d.m. and 0.91–3.27% d.m., respectively. The nutritional value of formulations rose as the amount of chia, quinoa, and/or amaranth wholemeal flour did in formulations. This increase mostly responded to the effects of the linear coefficients in each studied ingredient $a_1 > a_2 > a_3$, respectively (Table 3). However, the nutritional criterion was not the only one used to optimise bread product formulations because a direct relation appeared between the amount of each flour and the nutritional value. Moreover, the healthy fat content, proteins with higher biological values, and minerals were maximised, but the calorie value was minimised (Figure S2). Other quality criteria were also considered, such as the product's technological and sensorial characteristics, in order to seek a compromise formulation that lived up to all expectations from a holistic viewpoint, as shown below.

Effect of the Independent Variables on Bread Technological Characteristics

The specific volume of all the studied formulations varied from 0.90 ± 0.06 mL/g, which corresponded to the formulation with a higher substitution percentage ($Ch_{20}Q_{40}A_{40}$), to 5.5 ± 0.1 mL/g (CB). This parameter was negatively affected when gluten-free ingredients were included and contained a higher proportion of fibre, which led to a poor retention of the carbon dioxide produced during fermentation (Peressini and Sensidoni, 2009; Quiles et al., 2018). As expected, the effect of linear coefficients indicated a smaller specific volume when the replacement rate rose for the amaranth (−0.087; $p < 0.01$), quinoa (−0.059; $p < 0.01$), and chia (−0.039; $p < 0.01$) flours (Figure S3). The highest specific volume value among formulations, after the control sample, was for the formulations with chia (Ch_{10}: 5.0 ± 0.1 mL/g and Ch_{20}: 4.9 ± 0.1 mL/g). Increasing chia substitution in wheat flour lowered gluten content, and the specific volume was also reduced, but not as significantly as in the formulations with amaranth or quinoa at the same substitution level (Am20%: 4.3 ± 0.1 and Q20%: 4.6 ± 0.1 mL/g, respectively). Moreover, the effect of gluten dilution and the difference in the specific volume among the formulations with chia, amaranth, and/or quinoa flours could have been due to the high proportion of mucilage that chia seeds possess, forming hydrophilic complexes among ion groups and gluten proteins (Iglesias-Puig and Haros, 2013). This tendency has been observed in wheat bread with a 5% chia flour substitution whose specific volume was higher than for the chia-free control sample (Iglesias-Puig and Haros, 2013).

The crumb firmness parameter varied from 0.96 ± 0.03N (CB) to 29.59 ± 0.71 N (formulation with a higher degree of substitution, Ch20Q40A40). This parameter positively correlated with chia flour (0.381; $p < 0.01$), but with no interactions; that is, the more this ingredient was added, the higher crumb firmness values became (Ch10: 1.12 ± 0.06 N and Ch20: 9.0 ± 0.3 N).

Table 3.3

Factorial design coefficients of nutritional, technological, and sensory properties of bread.

	Physicochemical Properties				Technological Properties					Sensory Evaluation			
Source	**Lipids**	**Ash**	**Protein**	**Caloric Value**	**Specific Volume**	**Crumb Colour, *ΔE ****	**Crust Colour, *ΔE ****	**Crumb Firmness**	**Shape Ratio**	**Appearance**	**Texture**	**Taste**	**Overall Acceptability**
Units	**% d.m.**	**% d.m.**	**% d.m.**	**kcal/100 g**	**mL/g**	**—**	**—**	**N**	**cm/cm**	**—**	**—**	**—**	**—**
a_o	−0.227	0.900	14.877	250.345	5.691	2.100	0,345	1.287	2.144	7.711	7.423	8.090	8.008
a_1	0.214 **	0.041 **	0.070 **	0.906 **	−0.039 **	0.721 **	0.815 **	0.381 **	−0.037 **	0.005 **			
a_2	0.061 **	0.035 **	0.047 **	0.129 **	−0.059 **	0.349 **	0.656 **	−0.129 **	−0.014 **	−0.051 **	−0.0001 **	−0.053 **	−0.055 **
a_3	0.059 **	0.027 **	0.048 **	0.307 **	−0.087 **	0.144 **	0.165 *	−0.254 **			0.037 **		0.042 **
a_{11}	0.005 *			0.033 **					0.001 *	−0.009 *			
a_{12}						−0.007 **	−0.006 *						
a_{13}	0.002 *	0.001 *							−0.001 **	0.003 *	0.003 **	0.003 *	0.004 **
a_{22}							−0.007 **		0.001 **				
a_{23}					0.001 *		−0.006 **	0.011 **	−0.0003 **				
a_{33}		−0.0004 **						0.008 **			−0.002 **	−0.003 **	−0.001 **
R-SQ	0.985	0.967	0.979	0.982	0.931	0.933	0.925	0.931	0.936	0.789	0.789	0.653	0.703

Codes: 0.05 (*) and 0.01 (**) indicate statistical significance at the 95 and 99% confidence levels, respectively. d.m., dry matter, "—" without units. *ΔE*:* total colour difference, a_1, a_2, and a_3 are the coefficients of the main single effects of x_1, x_2, and x_3, respectively (x_1 is whole chia flour, x_2 is whole quinoa flour, and x_3 is whole amaranth flour). The square coefficients (a_{ii}) indicate if any of the variables has a maximum or minimum in the experimental domain, whereas the mixed coefficients (a_{ij}) represent the interactions between factors. R-SQ: adjusted square coefficient of the fitting model.

This result indicates that the inclusion of chia/mucilage does not always reduce/maintain crumb firmness, and it depends on the substitution level in formulations (Iglesias-Puig and Haros, 2013). Firmness also increased with a bigger quantity of the quinoa (Q_{40}: 2.29 ± 0.32 N) and amaranth (A_{40}: 1.22 ± 0.41 N) wheatmeal flours, but to a lesser extent in the latter. Given the interaction between the inclusion of quinoa and amaranth flours, a synergy was generated with increased crumb firmness ($Q_{20}A_{20}$: 3.28 ± 0.13 N; $Q_{40}A_{20}$: 7.67 ± 0.43 N; $Q_{20}A_{40}$: 15.70 ± 0.08 N; $Q_{40}A_{40}$: 23.24 ± 0.42 N), as shown in Figure S4. A minimum value was obtained in this context according to the quadratic coefficient of the amaranth flour factor (a_{33}: 0.008; $p < 0.01$), which would imply that the formulations substituted only for amaranth flour would have closer values to the control bread's firmness values (0.96 ± 0.03 N).

The shape ratio parameter (the width/height ratio of the central slice of a loaf) showed an interaction coefficient between the amaranth and chia flours (a_{13}) with a reduction from 2.13 ± 0.02 cm/cm (CB) to 1.66 ± 0.03 cm/cm ($Am_{20}Ch_{20}$). Conversely, the interaction coefficient between the amaranth and quinoa flours (a_{23}) left this parameter at 2.14 ± 0.28 cm/cm ($Q_{20}Am_{40}$); that is, loaves with the same shape, but a smaller volume. This would mean that formulations with amaranth and chia flours could be used to obtain more circular-shaped loaves. A similar tendency has been previously reported when substituting wheat for wholemeal amaranth flour (Miranda et al., 2019). An increase in the proportion of chia also affected the shape of the central slice, and a minimum was obtained according to the quadratic coefficients (0.001; $p < 0.01$) [11] (Figure S5).

The difference in crust colour and crumb colour (ΔE^*) varied within the 3.4 ± 0.8 to 25.0 ± 0.3 and 5.6 ± 0.3 to 20.2 ± 0.3 ranges, respectively, compared to the control sample. A loaf's crust colour is given mostly by non-enzymatic browning, by the Maillard reaction, and by caramelisation to a lesser extent. Differences in crust colour among formulations were due mainly to raw materials' colour, followed by different chemical reactions while baking that depend on the employed flour to a greater or lesser extent (Figure 3.1). Crumb showed fewer colour differences versus the control than crust because the speed of browning reactions slowed down due to higher humidity (Martínez et al., 2013; Mohammadi et al., 2014). The linear coefficients of each DE* factor for crust and crumb were significantly relevant (Table 3.3). The most significant ingredient ($p > 0.05$) was chia flour, which can be easily explained by the raw materials' colour parameter values (Figure 3.1). Chia flour displayed a more reddish (a^*) and darker (lower L^*) colouring than the other flours (chia: $L^* = 3.0 \pm 1.3$, $a^* = 2.2 \pm 0.1$, $b^* = 6.6 \pm 0.1$; quinoa: $L^* = 71.98 \pm 0.07$, $a^* = 1.42 \pm 0.06$, $b^* = 15.25 \pm 0.01$; amaranth: $L^* = 54.4 \pm 2.1$, $a^* = 2.13 \pm 0.03$, $b^* = 13.7 \pm 0.4$; wheat: $L^* = 65.8 \pm 1.0$, $a^* = -0.64 \pm 0.04$, $b^* = 7.5 \pm 0.2$) (Figure 3.1). The effect of the quinoa/amaranth interaction, which led to less marked crust colour differences, was lost when chia flour was added. Those formulations containing only amaranth and quinoa obtained crust ΔE^* values of 11.5 ± 0.5, 12.1 ± 0.6, and 13.0 ± 1.0 ($Q_{20}A_{40}$, $Q_{40}A_{20}$, and $Q_{40}A_{40}$, respectively). However, this tendency was the opposite to that observed in flours, and the highest colour change value was found for amaranth flour ($\Delta E^* = 14.7 \pm 0.3$) instead of for quinoa flour ($\Delta E^* = 9.66 \pm 0.06$) when both were compared to wheat flour. The biggest colour difference for amaranth flour can be explained by the red-violet pigment of the betacyanins present in *Amarantus caudatus*, which confers its grain a slightly redder colour, unlike white quinoa in which this pigment was not detected (Martínez-Villaluenga et al., 2020; Cai et al., 2005; Escribano et al., 2017). Therefore, the less marked colour change in the formulations with amaranth flour could be because this pigment inhibits the Maillard reaction and avoids final glycosylation products from being produced (Coy-Barrera, 2020). Finally, the formulations substituted for up to 40% quinoa flour reached a maximum with chia in the $Q_{40}Ch_{20}$ formulation ($\Delta E^* = 22.4 \pm 0.4$), as the quadratic coefficient indicated.

Preliminary Sensorial Evaluation

The scores in all the studied formulations for all the investigated sensorial attributes were similar to or lower than those obtained by the control bread, but were never superior. The linear coefficients of the sensorial attributes studied for the quinoa factor indicated that the more this ingredient was added, the worse the loaf, texture, flavour, and the product’s overall acceptability became (Table 3.3). However, when amaranth was included, consumers gave crumb texture and the product’s overall acceptability acceptable values. According to the 9-point hedonic scale, the texture attribute scores varied from 3.40 ± 0.05 to 8.5 ± 0.7, and the product’s overall acceptability score went from 2.7 ± 0.5 to 8.4 ± 0.2. The higher scores of these two attributes were for the control bread, followed by formulation A_{20} (texture: 6.3 ± 0.2 and overall acceptance: 7.3 ± 0.3 between “Moderately like” and “Very much like”), which remained some way behind those obtained in control bred (CB) (texture: 8.5 ± 0.7 and overall acceptance: 8.4 ± 0.2, between “Very much like” and “Especially like”) (Table 3.3). No significant interaction was detected between either quinoa and chia (a_{12}) or quinoa and amaranth (a_{23}).

Fig. 3.1. Raw materials: wheat grains (**a**); chia seeds (**b**); amaranth grains (**c**); quinoa grains (**d**); wheat flour (**e**); whole chia flour (**f**); whole amaranth flour (**g**); whole quinoa flour (**h**).

According to the linear coefficient (a_1), as the proportion of chia flour increased in the formulation, bread appearance slightly improved, with a maximum in the experimental domain ($Ch_{10}A_{20}$: 7.67 ± 0.08) in accordance with the quadratic coefficient (a_{13}), but it had no effect on texture, flavour, and acceptability. Conversely, for higher quinoa substitutions, sensorial parameters were negatively influenced (Figure S6).

Optimising Bread Formulation

To obtain the optimum formulation, we assigned conditions per response: (1) for the nutritional value, the lipid, protein, and ash contents were maximised, and the product's calorie value was minimised; (2) for technological quality, the loaf-specific volume was maximised, while the central slice aspect/crumb firmness ratio, as well as differences in the crust colour and crumb colour parameters compared to the control sample, were minimised; (3) consumer scores given to the sensorial attributes appearance, texture, flavour, and the baked product's overall acceptability were maximised. The formulation with all these attributes was formulation $Ch_{10}Q_4A_{19}$ (chia 10%, quinoa 4%, amaranth 19%), that is, a loaf made with wheat flour substituted for 33% wholemeal chia, quinoa, and amaranth flours at the 10%, 4%, and 19% flour basis proportions, respectively.

To validate the model, the optimum formulation was experimentally obtained and indicated $Ch_{10}Q_4A_{19}$. Its nutritional, technological, and sensorial characteristics were compared to the values that the model predicted. Hence, the obtained experimental results differed from those predicted by a percentage error between 0.52% and 6.7%, except for the two sensorial attributes (Table 3.4). A comparative study was also conducted between the characteristics of the optimised formulation and the bread made with the refined wheat and wholemeal wheat flours (Table 3.4).

Evaluating the Quality Parameters of the Optimised Bread

A comparison of the physico-chemical and sensorial characteristics of the wholegrain wheat bread, refined wheat (the control sample), and optimised bread can be found in Table 3.4. The addition of quinoa, chia, and/or amaranth flours significantly increased soluble, insoluble, and total fibre compared to those found in the wholemeal and control breads. In the latter, the difference was obvious because refined flours lack both the germ fraction and outer cereal layers. Unrefined flours are rich in dietary fibres, lipids, and minerals, and are complete in nutrients and beneficial bioactive compounds that benefit our health (Zhou et al., 2014). As expected, the optimised formulation indicated similar protein and lipid contents to the wholemeal bread, but higher ones than in the control bread. The optimised bread's calorie value (259 ± 4 Kcal/100 g) was lower than that for the wholemeal bread (279 ± 1.2 Kcal/100 g) despite similar lipid contents. This was due to the high fibre content in the optimised formulation (15.0 ± 1.3% d.m) compared to the wholemeal wheat bread (7.0 ± 0.5% d.m), and also due to its lower carbohydrate content, which would imply a considerable reduction in its calorie value. This means that the optimised bread could be considered fibre-rich food with a similar common bread value despite the high degree of substitution for flours with high lipid proportions.

Table 3.4 *Physico-chemical and sensory characteristics of bread formulations*

Parameters	**Units**	**Bread Formulation**				
		Whole Wheat	**Control**	**Optimal Formulation**	**Predicted Value**	**D%**
Physico-chemical parameters [a]						
Lipids	% d.m.	3.6 ± 0.6 b	0.23 ± 0.03 a	3.4 ± 0.1 b	3.500	−2.941
Ash	% d.m.	2.3 ± 0.5 b	0.91 ± 0.10 a	2.6 ± 0.1 b	2.500	3.846
Protein	% d.m.	12.3 ± 1.6 a	14.3 ± 0.04 ab	15.8 ± 0.1 b	16.400	−3.145
Soluble dietary fibre	% d.m.	1.6 ± 0.1 a	1.07 ± 0.04 a	4.1 ± 0.7 b	n.i.	--
Insoluble dietary fibre	% d.m.	5.4 ± 0.4 a	4.4 ± 0.3 a	10.9 ± 0.6 b	n.i.	--
Total dietary fibre	% d.m.	7.0 ± 0.5 a	5.4 ± 0.4 a	15.0 ± 1.3 b	n.i.	--
Caloric values	Kcal/100g	279 ± 1.2 b	250 ± 4 a	259 ± 4 a	268	-3.520
InsP_6	mg/100g	10.7 ± 1.8 b	1.5 ± 0.6 a	3.4 ± 0.4 a	n.i.	--
Technological parameters [a]						
Specific volume	mL/g	3.03 ± 0.03 a	4.5 ± 0.3 b	3.6 ± 0.4 ab	3.690	−2500
Shape ratio	cm/cm	1.6 ± 0.1 a	2.13 ± 0.02 a	1.7 ± 0.2 a	1.840	−8.235
Crumb textural parameters (TPA) [a]						
Firmness	N	2.26 ± 0.02 c	0.96 ± 0.03 a	1.95 ± 0.03 b	1.991	2.513
Springiness	mm	1.00 ± 0.00 a	1.70 ± 0.11 a	1.01 ± 0.26 a	1.000	1.478
Cohesiveness	m/m	0.79 ± 0.00 ab	0.84 ± 0.00 b	0.73 ± 0.03 a	0.750	−2.740
Chewiness	N	1.90 ± 0.01 a	1.46 ± 0.61 a	2.06 ± 0.96 a	2.020	1942
Crust colour parameters [b]						
L^*	—	49.7 ± 1.0 a	61.2 ± 2.0 b	51.7 ± 1.9 a	51.390	0.523
C^*	—	31.99 ± 0.02 b	34.4 ± 1.08 c	27.4 ± 0.4 a	28.860	−5.175
h_{ab}	—	60.7 ± 0.5 a	74.0 ± 2.0 b	71.8± 0.4 a	68.870	4.081
ΔE^*	—	17.9 ± 0.2 b	—	10.5 ± 0.5 a	10.030	4.111

Table 3.4 (Continuación)

Parameters	Units	Bread Formulation				
		Whole Wheat	**Control**	**Optimal Formulation**	**Predicted Value**	**D%**
Crumb colour parameters [b]						
L^*	—	57.6 ± 1.3 b	61.5 ± 1.2 b	52.6 ± 0.1 a	52.140	0.875
C^*	—	20.7 ± 0.9 b	13.3 ± 0.8 a	15.0 ± 0.2 a	15.280	−1.867
h_{ab}	—	78.5 ± 1.2 a	95.1 ± 0.8 c	85.9 ± 0.1 b	86.670	−0.896
ΔE^*	—	18.5 ± 2.1 b	—	9.35 ± 0.08 a	9.980	−6.738
Crumb structure [a]						
Cell area/total area	cm^2/cm^2	0.40 ± 0.10 a	0.24 ± 0.00 a	0.21 ± 0.00 a	n.i.	--
Wall area/total area	cm^2/cm^2	0.60 ± 0.00 a	0.76 ± 0.00 b	0.79 ± 0.00 c	n.i.	--
Cells/cm^2	—	402 ± 6 b	85 ± 7 a	109 ± 6 a	n.i.	--
Mean cell area	mm^2	0.41 ± 0.02 c	0.28 ± 0.02 b	0.19 ± 0.01 a	n.i.	--
Sensory analysis (hedonic scale) [c]						
Aspect	—	n.d.	8.5 ± 0.7 a	8.8 ± 0.8 a	8.540	2.955
Texture	—	n.d.	8.5 ± 0.7 a	8.5 ± 0.8 a	7.100	16.471
Taste	—	n.d.	8.7 ± 0.5 a	7.9 ± 0.7 a	7.460	5.570
Overall acceptability	—	8.1 ± 0.9 a	8.4 ± 0.2 a	8.7 ± 0.8 a	7.170	17.586

d.m.: dry matter; n.d.: not determined; n.i.: not included in the optimisation; InsP_6: *myo*-inositol hexakisphosphate or phytic acid; N: newton; L^*: lightness; C^*: chroma; h_{ab}: hue angle; ΔE^*: total colour difference, $\Delta E^* = [(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$; a^*: redness to greenness; b^*: yellowness to blueness; "—" without units. Mean ± SD, [a] n = 3, [b] n = 4, [c] n = 50. Values followed by the same letter in the same column are not statistically different at 95% confidence level.

Regarding crumb texture, the optimised formula generally had a similar profile to that of the wholemeal wheat bread. Although elasticity and chewiness presented no significant differences among formulations, a lowering trend was observed in both parameters compared to the control bread. The crumb firmness parameter differed significantly among formulations, where the optimised bread formulation obtained an intermediate value between the control and wholemeal wheat breads (Table 3.4).

For crumb structure, the mean crumb cell area in the wholemeal wheat bread was significantly higher than the values obtained for the control and optimised breads (Table 3.4). This tendency was also found by Angioloni and Collar (Angioloni and Collar, 2009) when they compared crumb cell distributions in the bread made with wholemeal and refined wheat flours. No significant differences appeared among the formulations for the cell area/total area parameter (Table 3.4). Nonetheless, in the optimised bread, this parameter was slightly lower with the subsequent significant increase in the wall area/total area compared to the control. This result might be related to the 33% gluten dilution in the optimised formulation, which negatively interfered with CO_2 retention capacity during bread-making fermentation and oven impulse, which leads to more compact crumbs and a smaller specific volume in the optimised product versus the control (Figure 3.2).

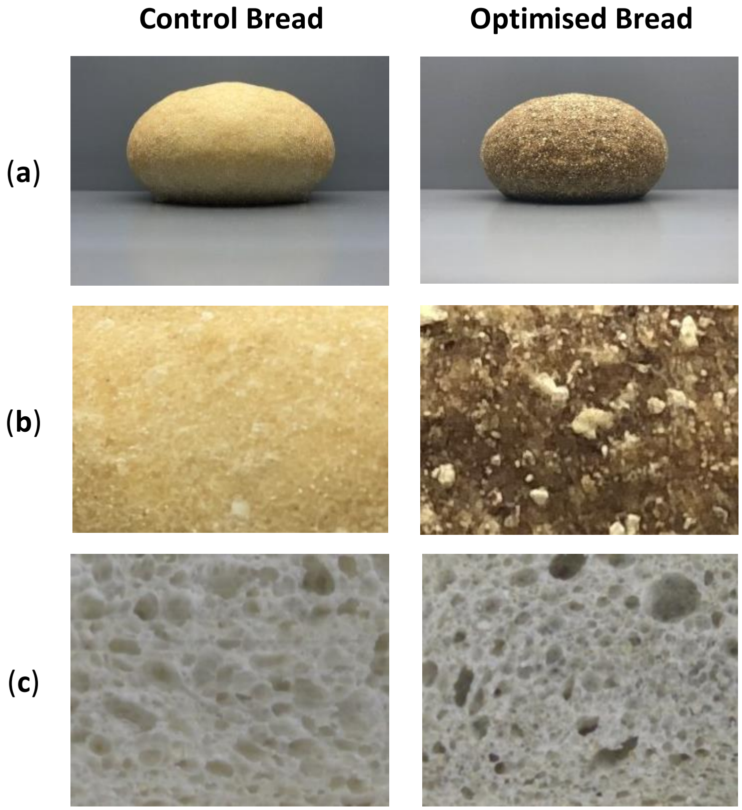

Fig. 3.2. Photographic images of control bread and optimised bread: (**a**) bread roll, (**b**) crust, (**c**) crumb structure.

The difference in the crumb/crust colour (DE*) of the optimised and wholemeal wheat breads, in comparison with the control bread, exceeded a value of 5. This indicates that consumers would notice colour differences at a glance (Table 3.4). The results of colour parameters L^*, C^*, and h_{ab} for the wholemeal bread crust were similar to those for the optimised bread (L^* and h_{ab}), but significantly differed from those of the control bread. The optimised bread crust was a darker colour, conferred mostly by the chia flour included in the formulation (Figure 3.2). A similar tendency was found when replacing 5% wholemeal flour with wholemeal chia flour (Iglesias-Puig and Haros, 2013). Nevertheless, the crumb luminosity in the optimised formulation was lower than for the wheat bread, with similar saturation to the control bread, along with an intermediate tone somewhere between the control formulation and wholemeal wheat.

Overall consumer acceptability showed no significant differences among formulations, with scores ranging from 8.1 ± 0.9 (wholemeal bread) to 8.7 ± 0.8 (optimised formulation) between "Very much like" and "Especially like".

Nutritional Properties of the Optimised Bread

Contribution of Bread Minerals to Diet

The nutritional quality of wholemeal flours with these ancient seeds is generally higher than those of cereals, not only for their high mineral content but also for their content of dietary fibre, unsaturated fatty acids, and proteins of high biological value (Miranda-Ramos et al., 2020). Nonetheless, the high concentration of some antinutrients, including phytic acid or phytates ($\mathrm{Ins}P_6$), affects the bioavailability of the minerals in the human intestine, which is indicated by the inhibition threshold values given for the $\mathrm{Ins}P_6$/mineral molar ratio (Ma et al., 2005). Phytate inhibition is basically due to insoluble complexes forming in the digestive tract, and solubility is a major requirement when these complexes are absorbed by enterocytes in the intestine (Laparra et al., 2008). Nonetheless, phytate content gradually decreases as the bread-making process advances owing to endogenous phytase activity. This activity depends on a number of factors, including fermentation time and temperature, dough pH, and baking time. Yet, the reduction of phytates via endogenous phytases during the bread-making process is not generally enough to extensively improve the bioavailability of minerals (Penella et al., 2008). Thus, in order to estimate the contribution that each mineral makes to adequate intake (AI) in our daily diet when we eat bread products it is necessary to quantify this antinutritional compound, as well as its possible inhibition in the bioavailability of minerals by focusing on Zn.

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Eating 100 g of bread made with the optimised formula on a daily basis can contribute to suitable Ca intake, which was threefold higher than in the control bread, by being completely bioavailable and having an InsP_6/Ca ratio below 0.24 (Table 3.5). With regards to Zn content, according to the FAO/WHO [66], its contribution to diet is contemplated in accordance with a high, moderate, or low degree of bioavailability, which depends on the InsP_6/Zn < 5, 5 < InsP_6/Zn < 15, or InsP_6/Zn > 15 molar ratio, respectively. The Zn content of the optimised formulation and the control bread displayed high bioavailability, and the former stood out for contributions of 29% in men and 41% in women (Table 3.5). Other bread formulations with 10% chia wholemeal flour or 25% wheatmeal quinoa flour substitutions have presented a moderate/high bioavailability with high contributions, but their contribution was not as high as that reported herein (Miranda-Ramos et al., 2020; Ballester-Sánchez et al., 2019).

With regards to Fe contribution, it can be stated that Fe bioavailability in both formulations is compromised. Studies about Fe bioavailability in vitro using the Caco-2 cell line model were studied. They revealed Fe bioavailability inhibition in bread to which wholemeal amaranth flour had been added up to the 40%

substitution level (Sanz-Penella, 2012). Nonetheless, 20% substitution with wholemeal amaranth flour led to better Fe absorption compared to the control bread; that is, although mineral absorption was inhibited, the higher Fe content favoured its higher bioavailability in the study model (Sanz-Penella, 2012). Thus, a higher proportion of Fe in the optimised formulation could contribute to increasing Fe bioavailability owing to other involved mechanisms apart from the inhibition caused by phytates. Low Fe bioavailability has been previously observed in the formulations of bread with InsP_6/Fe ratios above 1 and the following replacements: 25% wholemeal quinoa flour, 10% chia flour, 30% amaranth (Miranda-Ramos et al., 2020; Ballester-Sánchez et al., 2019; Penella et al., 2013).

Fatty Acid Quality of Bread

The optimised bread with a higher lipid proportion stood out for its high PUFA content compared to the control bread. The PUFA/SFA ratio went from 0.66 in the control bread to 5.8 in the optimised formulation. Therefore, given its bigger PUFA supply, it can be considered a healthy product (WHO/FAO, 2010). The majority PUFA content in the optimised bread corresponded to the alpha-linolenic acid content (C18:3n3; 1.5%), which was higher in the wheat bread with the 10% chia flour substitution (C18:3n3; 0.13%) (Miranda-Ramos et al., 2020). What this shows is that the chia, amaranth, and quinoa flours mixture enhances the PUFA/SFA ratio, even after the changes that the bread-making process entails. This means that such functional products could positively impact health after eating these flours because of the lower low-density lipoprotein (LDL) cholesterol concentration and due to the total cholesterol/high-density lipoprotein (HDL) cholesterol ratio, which reduce the risk of developing cardiovascular diseases (WHO/FAO, 2010; Coelho and Salas-Mellado, 2015).

Fatty acids omega-6 and omega-3 can be synthesised by means of their precursors alpha-linolenic acid (ALA) and linoleic acid (LA), respectively (Haros and Schoenlechner, 2017). For adults, the daily intakes of 10 g of LA/day and 2 g of ALA/day have been established (Bresson et al., 2009). As far as nutrition and preventing cardiovascular diseases are concerned, food agencies recommend maintaining the LA/ALA ratio in diet between 1:1 and 5:1 (FAO, 2008). The LA/ALA ratio of 0.6:1 obtained with the optimised formula was even better than these recommendations and the control bread ratio (LA/ALA, 17:1). Other formulations obtained higher ratios than the recommended ratio, e.g., those with 25% wholemeal quinoa flour of different varieties (LA/ALA, 9.2:1-9.6:1), and were better than in the wheat formulations (Ballester-Sánchez et al., 2019).

The formulations with 10% wholemeal chia flour obtained ratios that agree with recommendations (LA/ALA; 2.4:1), and a similar tendency has been reported for bread made with 15% salmon powder (LA/ALA, 3.4:1) (Miranda-Ramos et al., 2020; Desai et al., 2018).

Table 3.5

Nutritional composition of control and optimised breads.

Parameters		Reference Values (Male/Female)	Units	Control Bread	Optimised Bread
Average requirement (AR) contribution of minerals [a]					
Ca		1000 mg/d	%	3	9
Fe		14/29 mg/d	%	5/2.4	12/6
Zn	High bioavailability	4.2/3 mg/d	%	14/20	29/41
	Moderate bioavailability	7/4.9 mg/d	%	9/12	18/25
	Low bioavailability	14/9.8 mg/d	%	4/6	9/13
	InsP_6/Ca	<0.24	mol/mol	0.061	0.041
	InsP_6/Fe	<1.0	mol/mol	2.123	2.014
	InsP_6/Zn	<15.0	mol/mol	2.453	2.742
Fatty acids quality [b]					
	PUFA/SFA ratio			0.661	5.802
	% of contribution of AI_{LA} E% for linoleic acid	2.5–9%E	%	5.998	9.210
	% of contribution of AI_{ALA} E% for α-linolenic acid	>0.5%E	%	7.009	116,000
	Ratio ω-6/ω-3	5:1		17:1	0.6:1
Protein quality [c]					
	In vitro protein digestibility (IVPD)		%	77.1 ± 0.3 b	72.8 ± 0.7 a
	Protein digestibility corrected amino acid score (PDCAAS)			0.15 ± 0.01 a	0.44 ± 0.01 b
	Essential amino acid index (EAAI)			2.4 ± 0.14 a	2.73 ± 0.09 b
	Nutritional index (NI)			0.34 ± 0.02 a	0.43 ± 0.02 b
Adequate intake of dietary fibre [d]					
	Soluble/insoluble dietary fibre ratio	1:2	g/g	1:4.1	1:2.7
	Adequate intake (AI_F) contribution	25 g/d	%	21.673	60.026
In vitro starch digestibility [e]					
	Starch		% d.m.	66.5 ± 0.7 b	63.3 ± 0.1 a
	TSH_{90}: total starch hydrolysed at 90 min		%	84.6 ± 0.1 b	68.1 ± 2.0 a
	AUC: area under the curve of starch digestion			5934 ± 83 b	4903 ± 81 a
	GI: glycaemic index			95.0 ± 0.8 b	85.0 ± 0.8 a
	pGL: predicted glycaemic load		%	28.3 ± 1.2 b	21.9 ± 0.6 a

[a–e] Values are expressed as mean ± standard deviation (*n* = 3). Values followed by the same letter in the same line are not significantly different at 95% confidence level. [a] AR (average requirement) contribution (%) for a daily average intake of 100 g of bread. AR in milligrams per day for males/females ≥18. [b] PUFA: total polyunsaturated fatty acids, SFA: total saturated fatty acids AI (adequate intake) contribution (%) for a daily average intake of 100 g of bread. $AI_{LA\ or\ ALA}$ E% (percentage of energy intake) for LA (linoleic acid) or ALA (α-linolenic acid) for adult ≥ 18, respectively, E = (Kcal proteins + Kcal lipids + Kcal carbohydrates) in 100 g of bread (EFSA, 2017). c PDCAAS: ASS (lowest score of an individual amino acid) x in vitro protein digestibility of bread sample; amino acid pattern suggested by Food and Agriculture Organisation (FAO) for adults (g/100 g protein). [d] Soluble/insoluble dietary fibre ratio, 1:2 g/g (Jaime, 2002), AI_F (adequate intake) contribution (%) for a daily average intake of 100 g of bread. AI_F in adult ≥18 is 25 g/d (EFSA, 2017). [e] GI high > 70, medium 55–70, low < 55.

This indicates that a mixture with suitable levels of wholemeal flours from pseudocereals and oilseeds in a bread product formulation would improve this ratio, as we observed with our optimised bread. Moreover, a daily intake of 100 g of optimised bread would cover the recommended alpha-linolenic acid intake (ALA, >0.5%E) and supply 9% of the recommended linoleic acid intake (LA, 2.5%E) (Table 3.5). The daily intake of the optimised bread product could prevent coronary heart diseases and metabolic disorders (WHO/FAO., 2010).

In Vitro Protein Digestibility (IVPD), Protein Digestibility Corrected Amino Acid Score (PDCAAS), and Nutritional Index of Bread

The IVPD of the optimised bread was lower than in the control bread (Table 3.5), which could be explained by the presence of antinutrients such as phytic acid that form complexes with digestive proteins by lowering their digestibility and bioavailability for the metabolism, despite the content of this compound being lowered during the bread-making process. It is worth pointing out that the protein digestibility of the optimum formulation (IVPD, 73%) was similar to the true digestibility of ready-to-eat cereals, based on corn, wheat, rice, or oat (IVPD, 70–77%), as reported by Gilani et al. (Gilani et al., 200). The protein quality of the optimised bread was better than that of wheat bread, as indicated by the protein digestibility-corrected amino acid score (PDCAAS) used by FAO/WHO/United Nations University (FAO/WHO/UNU, 2007). Although the essential amino acids index (EAAI) of the optimised bread showed a significant difference with respect to the control bread, it tended to rise, indicating a protein of greater nutritional quality. Of the indices used to evaluate the nutritional value of foods, we found the nutritional index (NI), which combines qualitative and quantitative factors. It is considered an overall predictor of protein quality, and it was significantly ($p <$ 0.05) higher for the optimised bread than the control bread. This same tendency has been previously reported when adding quinoa flour to wheat bread (Lorusso et al., 2017).

Dietary Fibre Content in Bread

Generally, pseudocereals such as quinoa and amaranth, and oilseeds such as chia, are an excellent source of dietary fibre (Iglesias-Puig et al., 2015; Reguera and Haros, 2017) Alvarez-Jubete et al., 2009; Lazaro et al., 2018). The optimised formula obtained 54% and 64% more total fibre compared to the wholemeal and the control wheat bread, respectively (Table 3.4). The total dietary fibre of our optimised formulation (15.0 ± 1.3% d.m.) was higher than that of the wholemeal flour formulations, to which sugar beet pulp and apple marc were added to increase the fibre content in the end product without making product acceptability worse (Torbica et al., 2019). The higher proportion of soluble fibre in the optimised formulation compared to the wholemeal wheat bread could be due to the addition of amaranth, quinoa, and chia with a higher proportion of soluble fibre, as described in the literature and as obtained in the present study (Lazaro et al., 2018; Repo-Carrasco-Valencia and Valdez, 2017) [77,79]. Chia in particular contains a higher proportion of soluble fibre in the form of mucilage, which can absorb up to 35.2-fold its weight in water (Lazaro et al., 2018).

The physiological action that occurs after eating food containing dietary fibre is more effective when the soluble/insoluble fibre ratio remains at 1:2 (Jaime et al., 2002). This ratio of the optimised formulation came close to the recommended one, unlike the control bread (Table 3.5). Thus, eating 100 g/day of optimised bread would contribute up to 60% of the total suitable dietary fibre intake for adults (25 g/day) (EFSA, 2017). The combined use of wholemeal quinoa/amaranth/chia flours as ingredients to prepare bread products is an important source of balanced dietary fibre to cover, to a great extent, the daily recommended fibre intake, given its beneficial effects for the organism, such as lowering cholesterol, reducing the risk of developing diabetes, and regulating intestinal passage (Jaime et al., 2002).

In Vitro Starch Digestion Analysis

The optimised bread's starch content was significantly lower than in the control bread (Table 3.5). This could partly explain why after 90 min after eating starch in wheat bread, 84.6% of hydrolysed starch was recorded, but only 68.1% of hydrolysed starch was found in the formulation with the chia/quinoa/amaranth flours (optimised bread) (Table 3.5). These differences in starch hydrolysis speed did not only respond to the initial starch content, but also to granule type and size, the degree of gelatinisation, and other components present in the food matrix, such as fibre, proteins, and lipids, which wholemeal flours normally contain (Laparra-Llopis and Haros, 2018). All these factors influence the GI in foods to a greater or lesser extent. The control bread had a 95% GI, which was 85% for the optimised formulation (Table 3.5). These values are similar to the GI values of the buckwheat (GI = 80) or quinoa (GI = 95) formulations (Wolter et al., 2014). A lower GI can be explained by the higher soluble fibre content in the optimised bread compared to the control bread (Table 3.4), and a similar tendency has been observed in biscuits enriched with soluble fibre, which obtained a lower GI than their non-soluble fibre counterpart (Schuchardt at al., 2016). The authors suggested that the effectiveness of soluble fibre in regulating the postprandial glycaemia response was attributed to its capacity to delay gastric emptying. This tendency has also been observed in gluten-free bread products where, apart from replacing refined flour and starch, viscous diet fibres reduced the glycaemic response thanks to the capacity to form hydrogen links with water (Capriles and Arêas, 2016). Laparra and Haros (2018) confirmed that the inclusion of amaranth, quinoa, and chia flours in bread can significantly lower GI values. The reason for this could be the presence of antioxidants in amaranth and quinoa flours, activating the metabolism of carbohydrates and preventing diseases such as type 2 diabetes, obesity, and hypertension (Martinez-Lopez et al., 2020; Li and Zhu, 2017).

CONCLUSIONS

The response surface model herein employed was suitable for optimising bread formulations from the nutritional, technological, and sensorial points of view. Combining healthy ingredients such as chia, quinoa, and amaranth helped to develop a product with optimum characteristics ($Ch_{10}Q_4A_{19}$: chia 10%, quinoa 4%, amaranth 19%) as far as its nutritional characteristics were concerned (higher PUFA/SFA ratio, higher dietary fibre content by supplying soluble fibre, larger mineral supply, lower GI and better NI). It is a product with acceptable and better quality parameters than wholemeal wheat products (in terms of crumb firmness, loaf-specific volume, and the shape ratio of the central slice), and with excellent sensorial characteristics that are similar to products made with refined wheat flour. This optimised product can contribute to providing products with a beneficial impact on consumer health with high acceptability.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1: Figure S1. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on bread lipid yield. Figure S2. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on bread calorie value. Figure S3. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on bread specific volume. Figure S4. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on crumb firmness. Figure S5. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on shape ratio. Figure S6. Influence of interaction between the factors chia flour (X_1), quinoa flour (X_2), and amaranth flour (X_3) on sensorial evaluation aspect.

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CAPÍTULO 4

Influencia del prehorneado y almacenamiento congelado sobre la calidad tecnológica y nutricional de un pan multisemillas

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RESUMEN

La tenencia actual es elaborar productos panarios sustituyendo la harina de trigo por harinas integrales, sometiéndolos a procesos de elaboración que permitan su consumo en cualquier momento, garantizando las propiedades nutricionales y organolépticas, presentándose como un alimento de alto valor nutricional para el consumidor, quien cada vez es más exigente. En esta investigación se estudia el comportamiento de un producto panario multisemillas elaborado a partir de harinas de quínoa, chía y amaranto, aplicando un proceso de prehorneado, congelado y almacenado, fácil de preparar y consumir. Se investigaron las propiedades tecnofuncionales y nutricionales (biodisponibilidad mineral, contenido de aminoácidos y perfil de ácidos grasos) y el efecto del precocido y congelado en ellas. Se observó que los aminoácidos esenciales, tales como la lisina, valina y leucina, no se perdieron en el proceso de almacenamiento congelado, superando en 50%, 10%, y 5% respectivamente al producto control. En cuanto a la diferencia de color (ΔE) entre el producto control y el multisemillas. tras el hornedado/almacenamiento. fue inferior a 5, lo que indica que el consumidor podría no percibir la diferencia de color entre los dos productos. Por otro lado, la entalpía de retrogradación de la amilopectina tras el almacenamiento en congelación fue de hasta 33% inferior que en la muestra control, posiblemente debido a la acción de los ácidos grasos poliinsaturados y el contenido de fibra soluble presentes en el producto multisemillas. Esta formulación de pan podría satisfacer las necesidades nutricionales para adultos en cuanto a calcio y fibra dietética, con una ingesta diaria de 100 g.

INTRODUCCIÓN

En la actualidad, los consumidores buscan alimentos con mayor valor nutricional, y de fácil preparación, especialmente aquellos que son considerados básicos. Las expectativas se fundamentan en que los alimentos tras ser sometidos a diferentes procesos, conserven las características nutricionales y organolépticas durante su almacenamiento y posterior consumo. El procesamiento de panes prehorneados, congelados y su posterior almacenamiento, han ofrecido una alternativa para extender el tiempo de vida útil y asegurar la satisfacción del consumidor final (Bárcenas, et al., 2004; Cauvain & Young, 2010; Le-Bail & Gabric, 2012; Le-Bail, Leray et al., 2011). Sin embargo, existen diferentes factores, como las bajas temperaturas y tiempos de almacenamiento prolongado, así como los ciclos de congelación y descongelación, que afectan la calidad final del producto. Esto se debe a que la disminución del contenido de humedad favorece la formación de enlaces de hidrógeno entre los polímeros del almidón y las proteínas aumentando la dureza de la miga; la formación de cristales de hielo producen cambios en el gluten mermando la fuerza de la masa y su capacidad de retención de gas, provocando la reducción del volumen (Arthey, 2001). En consecuencia, durante el proceso de prehorneado y almacenamiento congelado, se afectan las propiedades tecnológicas, presentando una disminución en el volumen de la pieza panaria, y un incremento de la firmeza, cohesión, elasticidad y adhesividad de la miga. Estos cambios afectan las propiedades sensoriales y son percibidos por los consumidores (Bárcenas et al.,2004; Bosmans et al., 2013; Carr et al., 2006; Curti et al., 2014; Chen et al., 2013; Ronda et al., 2011).

Por otro lado, los productos panarios sin aditivos ni conservantes poseen una vida útil muy corta, contrario a la tendencia actual de los consumidores que es obtener un pan fresco en cualquier momento y que conserve su calidad tecnológica y nutricional al momento de consumir. Por consiguiente, existen diferentes investigaciones, realizadas con la inclusión de fibra y antioxidantes en panes de harina de trigo integral y con almacenamiento congelado; observando una notable mejora en las propiedades nutricionales y tecnológicas comparado con el pan de trigo (Bae et al., 2014; Jiang et al. 2019). Una tendencia similar fue encontrada en panes enriquecidos con fibra de harina de avena (Mandala et al., 2009). Por lo tanto, reemplazar la harina de trigo con otros ingredientes como harinas integrales de granos o semillas podrían contribuir a la mejora de la calidad de un producto panario prehorneado-congelado, debido a su alto contenido de fibra y otorgarle un alto valor nutricional. De la misma forma, el uso de ingredientes como la semilla de chía, que no posee almidón, y la harina de amaranto y quínoa, que poseen almidón resistente, retardan el envejecimiento del pan, con un bajo porcentaje de sinéresis, lo cual beneficia el procesamiento del producto durante los ciclos de congelado y descongelado, potenciando su uso en los productos de panadería (Grancieri, Martino, & de Mejia, 2019; Li & Zhu, 2018).

Debido a que existe escasa información sobre la influencia del proceso de prehorneado-congelado y conservación en la calidad nutricional del producto, el objetivo del presente trabajo fue analizar el efecto del proceso de prehorneado, congelado y el almacenamiento en congelación sobre el valor nutricional, las características tecnológicas y organolépticas de un pan de harinas integrales (quínoa, amaranto y chía). Los resultados se compararon con un producto control tratado en las mismas condiciones.

MATERIALES Y MÉTODOS

Materiales

Granos de quínoa (*Chenopodium quinoa Willd*), semillas de chía negra (*Salvia hispanica L.*) y granos de maranto (*Amaranth caudatus*) (Inca´s treasure, Quito, Ecuador) fueron molidos y almacenados a 14ºC en bolsas de polietileno con cierre hermético hasta su utilización. Se utilizó levadura deshidratada (*Saccharomyces cerevisiae*, Levital, España) como iniciador panario y harina de trigo comercial (La Meta, Barcelona, España) para el proceso de panificación.

Composición proximal

Todos los análisis se realizaron en las materias primas, pan fresco y panes prehorneados y congelados. El contenido de humedad, fibra dietética total y almidón se realizaron según los métodos 925.09, 991.43 y 996.11, respectivamente (AOAC, 1996). La determinación de proteínas se realizó mediante el método de combustión de Dumas, con el factor de conversión de nitrógeno: 5.7 harina de trigo; 5.53 quínoa, amaranto, chía; 6.25 para panes acorde a la ISO/TS (International Organization for Standardization/Technical Specification) 16634-1 and ISO/TS 16634-2 (Czech Republic & testing, 2016; Turkey, 2015). Los contenidos de lípidos y cenizas se determinaron de acuerdo con los métodos oficiales 30-10 y 08-03, respectivamente de la American Association of Cereal Chemists (AACC, 2000). Los ensayos se realizaron por triplicado.

Procedimiento de panificación

Las masas para el pan de trigo control, prehorneado y congelado (CWB), y pan de trigo prehorneado, congelado y almacenado (WB) fueron de 300 g de harina de trigo. Para el pan control multisemillas, prehorneado y congelado (CMB) y el pan multisemillas prehorneado, congelado y almacenado (MB), se reemplazó la harina de trigo en 19 % por harina integral de amaranto, 10% por harina integral de chía y 4% por harina integral de quínoa. A cada formulación de pan se le agregó 3% de levadura prensada, 1.6% de sal y agua hasta lograr una consistencia óptima de 500 Unidades Brabender. Los ingredientes se mezclaron y se amasaron, se dejaron reposar durante 10 min, y se dividieron en piezas de 70 g, se bolearon y formaron manualmente; la fermentación se llevó a cabo durante 45 min a 30±1 °C y 80% de humedad relativa, hasta alcanzar el volumen óptimo. El prehorneado se llevó a cabo a 170 °C durante 10 min, los panes se enfriaron hasta alcanzar los 40 °C en el centro de la miga, y posteriormente fueron congelados a -80 °C durante 1 hora. Los panes congelados se empacaron en fundas con cierre hermético de polietileno de baja densidad y se almacenaron a -20 °C durante 90 días. Para realizar los análisis los productos fueron descongelados hasta llegar a temperatura ambiente, posteriormente se completó su horneado a 185 °C durante 18 min y enfriados a temperatura ambiente durante 1h para su posterior análisis.

Calorimetría diferencial de barrido (DSC)

Las propiedades térmicas del almidón durante el horneado de la masa fermentada (gelatinización) y los cambios inducidos durante el almacenamiento del pan (retrogradación de la amilopectina) se midieron en un calorímetro diferencial de barrido (DSC-7, PerkinElmer). La calibración del DSC se llevó a cabo con indio, entalpía de fusión 28,41 J/g y punto de fusión 156,4 °C. Las muestras de masa fermentada fueron elaboradas como se indicó en el apartado anterior. Estas se pesaron directamente en cápsulas de acero inoxidable de manera precisa (±0,00002), entre 30 y 40 mg (DSC LVC 0319-0218, PerkinElmer) y se sellaron herméticamente (Quick-Press, 0990-8467, PerkinElmer). Las condiciones de barrido utilizadas fueron las de la metodología descrita por León et al. (1997) con ligeras modificaciones. En este estudio se siguió un procedimiento para imitar el proceso de panificación interrumpido. Las propiedades térmicas del almidón se analizaron mediante el proceso de prehorneado, almacenamiento en congelación y horneado final.

Etapa de prehorneado y almacenamiento en congelación. Para simular el prehorneado, se pesaron muestras de masa de pan (30-40 mg) en cápsulas de acero inoxidable como se indicó anteriormente. Se utilizó una cápsula vacía como referencia. Después de sellar las cápsulas se sometieron a un barrido de temperatura desde 30 a 90 °C a una velocidad de 11,7 °C/min. Al cabo de ese tiempo, las cápsulas se enfriaron a 30 °C a la velocidad de 50 °C/min. Las transiciones térmicas del pico de gelatinización del almidón de las muestras se definieron como temperatura de inicio (To), de pico (Tp), y de conclusión (Tc). La entalpía asociada a la gelatinización del almidón (ΔH_G), se calculó por integración del área bajo la curva del pico endotérmico, y se expresó en J/g de muestra seca. Posteriormente, las cápsulas se colocaron inmediatamente en un congelador a -80 °C durante 60 min, y se almacenaron a -20 °C durante 90 días, para su posterior análisis. Se realizaron doce réplicas por cada muestra.

Etapa de horneado final. Siguiendo con la simulación del proceso, las cápsulas fueron descongeladas hasta llegar a los 25 °C, posteriormente se sometieron a un segundo barrido de temperatura desde 30 a 105 °C a una velocidad de 11 °C/min, para completar el proceso de horneado. Se realizaron doce réplicas para cada muestra.

Etapa de envejecimiento y/o retrogradación de la amilopectina. Posteriormente, las cápsulas fueron almacenadas durante 0, 2, 4 y 7 días a 25ºC con el fin de estudiar la cinética de retrogradación de la amilopectina. Para ello, al cabo del tiempo de almacenamiento, se sometieron a un nuevo ciclo de calentamiento en el DSC desde 25 a 130 °C a una velocidad de 10 °C/min (Bárcenas et al., 2003; León et al., 1997). La entalpía de retrogradación de la amilopectina ($\Delta H_{R)}$ se calculó por integración del área bajo la curva del pico endotérmico, y se expresó en J/g de muestra seca (Durán, León, Barber & Benedito de Barber, 2001). Se realizaron tres réplicas para cada muestra y por cada tratamiento.

Análisis de los parámetros tecnológicos de las piezas panarias

Los parámetros tecnológicos analizados fueron: volumen específico de la pieza panaria (cm^3/g) por desplazamiento de semillas de nabo (medidor de volumen en cm^3, Chopin, Francia), peso de la pieza (g), y la relación de aspecto (ancho/altura) de la rebanada central (cm/cm)

Se midieron los parámetros triestímulos del color $L*$ (luminosidad), $a*$ (rojo a verde), $b*$ (amarillo a azul), ángulo de tono ($h*$), croma ($C*$) y la diferencia de color total ($\Delta E*$, Ecuación 1), de la corteza y miga de las piezas panarias horneadas (colorímetro digital Chroma Meter CR-400, Konika Minolta Sensing, Japón). Los ajustes del instrumento fueron los siguientes: iluminante C y ángulo de observación de 10°. Las muestras se analizaron por triplicado (Iglesias-Puig y Haros, 2013).

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½} \qquad \text{Ec. (1)}$$

El análisis de la textura se realizó en la miga de la debanada central de la pieza panaria en un texturómetro (TA-XT Plus Stable Micro Systems, Godalming, Reino Unido). Se tomó una muestra de rebanada de pan de 2 cm de espesor se comprimió dos veces utilizando un émbolo de acero inoxidable de 0,5 cm de diámetro, a la velocidad de 1,0 mm/s hasta una distancia de penetración del 50% de la miga, con un intervalo de 50s entre cada compresión (Haros, Rosell y Benedito, 2002). Las muestras se analizaron por triplicado.

En cuanto al análisis digital de imágenes, para medir la estructura de la miga se utilizó un microscopio EVOCAM-II (Vision engineering, Woking, Reino Unido). Las imágenes se procesaron y analizaron con los softwares NIS-Elements BR 3.2 (Nikon Corporation, Japón) y Fiji (Software ImageJ 1.49q, National Institutes of Health, EE. UU.), utilizándose un campo de visión de 10 mm x 10 mm x 10 mm. Los análisis se hicieron por duplicado. Los datos fueron procesados utilizando el software Statgraphics Plus 16.1.03 (Bitstream, Cambridge, MN, EE. UU.). Los parámetros elegidos para el análisis de la estructura de la miga fueron: área alveolo/área total, cm^2/cm^2; área de la pared/área total, cm^2/cm^2; número de alveolos/cm^2; y área media del alveolo, mm^2

Perfil de ácidos grasos

El perfil de ácidos grasos se analizó en las materias primas y en los productos terinados tras la precocción y el almacenamiento en congelación. Los triglicéridos de la fracción de lípidos de cada muestra fueron transesterificados para ser convertidos en ésteres metílicos de ácidos grasos (FAMEs), siguiendo la metodología descrita por Garcés & Mancha (1993). Para la composición y cuantificación de los ácidos grasos se utilizó el GC (cromatografía de gases) (7890A; Agilent, Santa Clara, CA, EE. UU.) equipado con una columna capilar (30 m de longitud; 0,32 mm de diámetro interno; 0,20 µm de espesor de película) de sílice fundida (Supelco, Bellafonte, PA, EE.UU.) y un detector de ionización de llama según el método 2.302 de la Unión Internacional de Química Pura y Aplicada (IUPAC) (IUPAC, 1992). Las mediciones se realizaron por triplicado.

Composición Mineral

Se analizaron calcio (Ca), hierro (Fe) y zinc (Zn), utilizando un espectrómetro de absorción de llama del Servicio de Análisis de Suelos, Plantas y Aguas del Instituto de Ciencias Agrícolas de Madrid, España. Previamente, las muestras (0,5 g) se colocaron en un recipiente de perfluoroalcoxi de teflón y se digirieron por irradiaron a 800 W (15 min a 180 °C) en un sistema de reacción acelerada por microondas (MARS, Charlotte, NC, EE.UU.) en presencia de HNO_3 (4 mL, 14 M) y H_2O_2 (1 mL, 30% v/v). Al final del ciclo de calentamiento las muestras se transvasaron a un tubo de polipropileno y se llevó a volumen con HCl al 5% (12 ml). Las mediciones se realizaron por triplicado (Miranda-Ramos, Millan-Linares y Haros, 2020).

Determinación de ácido fítico

El ácido fítico (InsP_6) presente en las materias primas y en los productos desarrollados se midió como fósforo liberado por las enzimas fitasa y fosfatasa alcalina, mediante un método de cuantificación simple K-PHYT, usando el Kit Magazyme. En primer lugar, los fitatos se extrajeron en solución de HCl 0,5 M con agitación y a temperatura ambiente durante toda la noche. Posteriormente, tras la centrifugación de una alícuota del extracto, la muestra se trató con fitasa (InsP_6 --> InsP_1) para hidrolizar los grupos fosfatos de la molécula del ácido fítico. La liberación del último fosfato del monofosfato de *mio*-inositol (InsP_1) se produjo por acción de la fosfatasa alcalina, debido a que este compuesto no es sustrato de la fitasa. El fosfato liberado se midió por colorimetría según el método 986.11 (AOAC, 2016). Las muestras se analizaron por triplicado.

Análisis de aminoácidos

La composición de aminoácidos totales de las muestras se analizó mediante cromatografía líquida en fase inversa tras la hidrólisis ácida proteica, según el método 994.12 de la AOAC (Asociación de Químicos Analíticos Oficiales) y se derivatizó con etoximetilenmalonato de dietilo para obtener el compuesto de aminoácidos N-(2,2-bis(etoxicarbonilo)) vinilo) (Alaiz et al., 1992). La composición de aminoácidos se expresó en gramos de aminoácidos por 100 g de proteína. Las mediciones se realizaron por triplicado.

Análisis estadístico

Para el análisis estadístico de los resultados se aplicó la comparación de muestras múltiples, con el Statgraphics Plus 5.0. Se utilizó la prueba de diferencia mínima de significación (LSD) de Fisher para describir las medias, a un nivel de significación del 5%.

RESULTADOS Y DISCUSIÓN

Composición proximal de las materias primas

En la Tabla 4.1, se detalla la composición proximal de las harinas utilizadas para la elaboración de pan control y el pan multisemillas prehorneado y almacenado en congelación. El contenido proteico de las harinas integrales de quínoa, amaranto y chía fueron significativamente superiores a la de la harina de trigo. Estos valores se encuentran acorde a los encontrados previamente por otros investigadores (Marineli et al., 2014; Montemurro et al., 2019; Martínez-Villaluenga et al., 2020). Es importante destacar que la lisina es un aminoácido deficiente en cereales (~3%) e inferior a las cantidades encontradas en quínoa, amaranto y chía, 4.6%, 6,1%, 10,9%, respectivamente (Haros & Schoenlechner, 2017; Martínez et al., 2018; Miranda-Ramos et al., 2020), por lo que la inclusión de estos ingredientes en productos panarios, no solo incrementaría el contenido proteico del alimento, sino también su calidad proteica.

La variación en el contenido en lípidos en la semilla de chía en comparación a datos bibliográficos puede atribuirse a la procedencia (Brazil, 32.2% y México, 35.1%) (Grancieri et al., 2019), por factores tales como las condiciones de cultivo y factores ambientales (Haros & Schoenlechner, 2017; Martínez et al., 2018). Dietas con ratios bajos de ω-6/ω-3 presentan impacto positivo en la salud, lo que promovería la prevención de enfermedades coronarias, entre otras (Martínez-Villaluenga et al., 2020; Valdivia-López & Tecante, 2015). En este sentido, la chía destaca entre las materias primas utilizadas en esta investigación, además de presentar el triple de fibra total que los otros dos ingredientes, con un mayor contenido de fibra soluble 6,84% (Tabla 4.1). Esta tendencia también fue observada por otros investigadores (Martínez-Villaluenga et al., 2020; Valdivia-López & Tecante, 2015).

Tabla 4.1

Composición proximal de las harinas utilizadas en la elaboración del pan control y pan multisemillas

Parámetros [a]	Unidades	Trigo [b]	Amaranto [c]	Quínoa [d]	Chía [e]
Humedad	%	13.52 ± 0.23 c	12.56 ± 0.01 b	12.24 ± 0.02 b	7.61 ± 0.04 a
Almidón	% m.s.	73.05 ± 0.37 d	64.57 ± 0.52 b	61.64 ± 0.91 c	n.d.
Proteína	% m.s.	11.73 ± 0.04 a	13.46 ± 0.72 b	16.22 ± 0.12 c	19.11 ± 0.30 d
Lípidos	% m.s.	1.14 ± 0.17 a	6.92 ± 0.14 b	6.96 ± 0.74 b	34.29 ± 0.46 c
Cenizas	% m.s.	0.56 ± 0.03 a	2.49 ± 0.06 b	2.94 ± 0.06 c	4.56 ± 0.00 d
Fibra Dietética Total	% m.s.	4.08 ± 0.19 a	13.93 ± 0.37 b	13.80 ± 0.14 b	40.20 ± 0.08 c
Fibra Dietética Soluble	% m.s.	1.68 ± 0.43 a	2.79 ± 0.24 a	5.31 ± 0.17 b	6.84 ± 0.94 c
Fibra Dietética Insoluble	% m.s.	2.39 ± 0.24 a	11.14 ± 0.60 c	8.49 ± 0.03 b	33.36 ± 1.02 d

[a] Media ± DS. n=3. Valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas a un nivel de confianza del 95%. Códigos: m.s., materia seca, [b]*Triticum aestivum*, [c]*Amaranthus caudatus,* [d]*Chenopodium quínoa Willd,* [e]*Salvia hispánica L.*

Efectos de las propiedades térmicas de almidón en los panes WB y MB

En la Tabla 4.2, se observa que las formulaciones panarias presentaron un pico en el termograma, correspondiente al proceso de gelatinización del almidón, siendo para el pan multisemilla prehorneado (MB), entre 61.69°C y 73.01°C (ΔH_G: 0,33 J/g), mientras que la formulación de trigo (WB), presentó valores más altos de temperaturas de inicio y final (63,3 y 81,79 ºC, respectivamente) con un valor de entalpía de 1,42 J/g, lo que sugiere que se requiere menor energía para gelatinizar la formulación multisemillas de la formulación control. La misma tendencia fue observada con la sustitución del 50% de harina por harina integral de amaranto, obteniendose una reducción de la entalpía de 1,3 J/g a 1,6 J/g (Miranda-Ramos et al., 2019).

Por otro lado, los intervalos de temperatura de la entalpía de retrogradación de la amilopectina no fueron alterados por la formulación. Conforme se incrementó el tiempo de almacenamiento del producto final a 25ºC del (MB) se incrementó la entalpía de retrogradación de la amilopectina. Además, la entalpía de retrogradación de la muestra multisemillas fue ligeramente inferior al pan control, lo que podría atribuirse al alto contenido de lípidos y fibra de la primera formulación, lo que dificulta la recristalización de la amilopectina, y por ende, un retraso en el envejecimiento del pan (Mondragón et al., 2006).

Efectos de los parámetros tecnológicos en los panes CWB, WB, CMB, MB

Las diferencias tecnológicas encontradas entre el pan de trigo y el pan multisemillas fueron descritas en el capítulo anterior. En la Tabla 4.3, se muestran los efectos del almacenamiento en congelación en las dos formulaciones de pan en estudio. Se observó que no hubo diferencias significativas en el contenido de humedad y volumen específico de la pieza panaria (Figura 4.1A), solo una ligera disminución de la relación de aspecto en los productos CMB y MB (Figura 4.1B).

Tabla 4.2
Efecto de la formulación en las propiedades térmicas del almidón de productos finales tras el precocido, congelado, almacenado en congelación, descongelado, cocido.

Muestras	**Unidades**	**Pan de Trigo (WB)**	**Pan Multisemilla (MB)**
Gelatinización del Almidón [a]			
Temperatura de inicio	°C	63.30 ± 0.67 b	61.69 ± 0.84 a
Temperatura pico	°C	71.50 ± 1.72 b	67.50 ± 0.74 a
Temperatura final	°C	81.79 ± 2.46 b	73.01 ± 1.99 a
Entalpia de Gelatinización, ΔH_G	J/g	1.42 ± 0.44 b	0.33 ± 0.15 a
Retrogradación de Amilopectina, 2 días [b]			
Temperatura de inicio	°C	54.70 ± 0.69 a	58.61 ± 0.35 b
Temperatura pico	°C	62.79 ± 0.31 a	62.37 ± 0.23 a
Temperatura final	°C	73.45 ± 1.69 b	66.21 ± 0.49 a
Entalpia de Retrogradación, ΔH_{R2}	J/g	0.21 ± 0.01 b	0.05 ± 0.01 a
Retrogradación de Amilopectina, 4 días [b]			
Temperatura de inicio	°C	55.59 ± 0.05 b	51.76 ± 0.43 a
Temperatura pico	°C	62.55 ± 0.39 a	61.52 ± 0.68 a
Temperatura final	°C	73.30 ± 2.01 a	71.28 ± 0.64 a
Entalpia de Retrogradación, ΔH_{R4}	J/g	0.41 ± 0.01 b	0.16 ± 0.03 a
Retrogradación de Amilopectina, 7 días [b]			
Temperatura de inicio	°C	56.45 ± 0.19 b	53.41± 0.24 a
Temperatura pico	°C	63.51 ± 0.39 a	61.28 ± 0.82 a
Temperatura final	°C	69.79 ± 0.19 a	70.80 ± 0.59 a
Entalpia de Retrogradación, ΔH_{R7}	J/g	0.58 ± 0.05 a	0.55 ± 0.06 a

[a]Media ± DS n=12, [b]n=3, a, b, valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas a un nivel de confianza del 95%.

En cuanto a la diferencia de color (ΔE*) entre la miga del pan control precocido, congelado y no almacenado (CWB) y la miga de la formulación precocida y almacenada congelada (WB) fue superior a 5, lo que indica que un cambio de color podría ser percibido por el consumidor. La diferencia del color podría ser debida al proceso de deshidratación durante el almacenamiento en congelación, lo que es coincidente con lo informado por Rosell, Santos, Sanz Penella, and Haros (2009) en cuanto que el proceso de congelamiento sin almacenamiento afecta en menor grado las características organolépticas de un producto panario. Por otro lado, la formulación multisemillas no presentó diferencias significativas de color entre el producto almacenado en congelación (MB) y el producto no almacenado (CMB). Este resultado podría indicar que en los panes con una mayor cantidad de fibra, el efecto del almacenamiento en congelación no produce cambios significativos de color en la corteza, principalmente porque el agua está más ligada lo que inhibiría la producción de cristales (Arthey, 2001).

Por otro lado, el almacenamiento en congelación de los productos BM y WB condujo a la formación de cristales de hielo, que dañan o debilitan la estructura de la red de gluten; produciendo pérdida de agua con facilidad, después del descongelacmiento y durante la etapa de la cocción final (Rosell, 2015), lo que ha provocado un aumento significativo de la dureza de la miga en comparación a la miga de los productos sin almacenamiento en congelación, CWB y CMB (Tabla 4.3).

La elasticidad y la cohesión de la miga no se afectó con los procesos de almacenamiento en congelación. Sin embargo, la masticabilidad y la gomosidad tuvieron una ligera disminución en los productos almacenados en congelación (MB y WB) en comparación a los productos sin almacenamiento (CMB y CWB). Esto podría deberse al doble proceso de horneado y posterior almacenamiento; atribuido a la formación de cristales de hielo en el interior de la miga, la ruptura de las paredes alveolares y la ligera disminución del número de alveolos/cm^2, sin afectar el área total de los alveolos que no presentaron diferencias siginificativas (Figura 4.1 C).

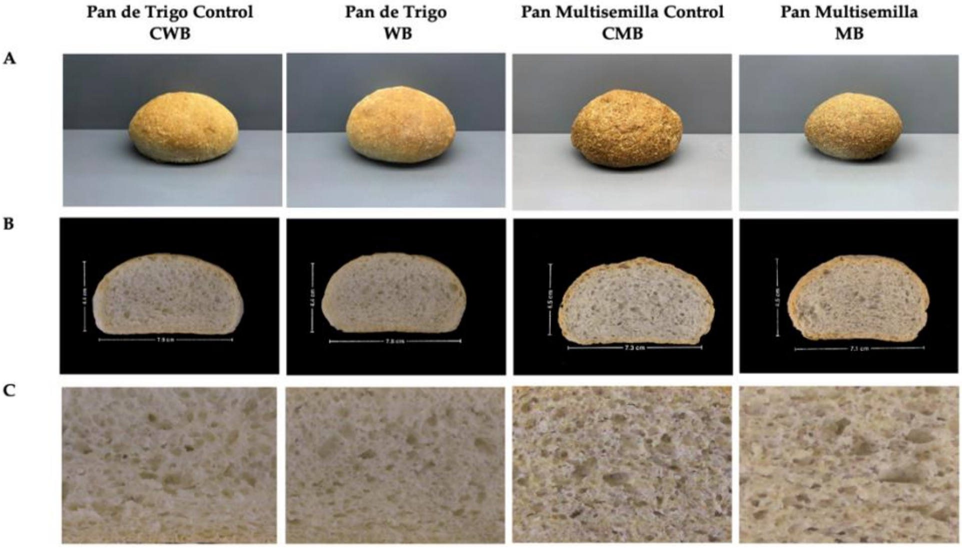

Figura 4.1. **A.** Apariencia de las piezas panarias, **B.** Dimensiones de la rebanada central, **C.** Detalle de la estructura alveolar de la miga.

Por otro lado, se observó una ligera disminución en el volumen específico de las piezas panarias WB y MB, con respecto a los panes control CWB y CMB; esto puede deberse a la deshidratación durante el proceso de almacenamiento en congelación, produciendo un debilitamiento en la red de gluten, con una baja capacidad para retener el CO_2, lo que podrujo una mayor compactación de las piezas panarias con disminución del número de alveolos. En este sentido, el contenido de alveolos/cm^2 de la miga de las formulaciones del pan de trigo (CMB) y el pan multisemilla (CWB) sin almacenamiento (576 aveolos/cm^2 y 507 alveólos/cm^2, respectivamente) fue significativamente superior al número de las muestras almacenadas en congelación MB (532 alveólos/cm^2) y WB (495 alveólos/cm^2), tal como se muestra en las imágenes de la Figura 4.1 C.

Tabla 4.3

Efecto del prehorneado, congelado y almacenado en congelación en la calidad de productos panarios

Parámetros Tecnológicos[a]	**Unidad**	**Formulaciones**			
		Pan de trigo control CWB	**Pan de trigo WB**	**Pan Multisemilla control CMB**	**Pan Multisemilla MB**
Humedad	%	37.4 ± 1.0 a	36.8 ± 0.3 a	36.9 ± 0.6 a	37.0 ± 1.1 a
Volumen específico	mL/g	2.5 ± 0.0 a	2.3 ± 0.5 a	2.5 ± 0.1 a	2.3 ± 0.5 a
Relación de forma	cm/cm	1.8 ± 0.1 b	1.8 ± 0.1 b	1.6 ± 0.1 a	1.6 ± 0.1 a
Color de la corteza[b]					
Luminosidad, L*		68.7 ± 1.2 c	65.0 ± 1.8 b	50.7 ± 0.5 a	52.3 ± 1.4 a
Saturación, C*		33.2 ± 1.6 b	38.00 ± 2.0 b	31.4 ± 0.3 ab	30.1 ± 0.3 a
Tono, h_{ab}		76.6 ± 1.3 c	72.9 ± 1.9 b	65.9 ± 0.1 a	66.8 ± 0.6 a
ΔE*		-----	4.9 ± 0.7 b	-----	1.9 ± 0.6 a
Color de la miga[b]					
*Luminosidad, L**		62.7 ± 1.2 c	67.6 ± 0.8 d	52.8 ± 1.1 a	55.6 ± 0.3 b
*Saturación, C**		14.4 ± 0.5 a	13.8 ± 0.4 a	15.6 ± 0.2 a	17.7 ± 1.8 b
Tono, h_{ab}		91.4 ± 1.0 b	93.9 ± 0.5 c	80.6 ± 2.0 a	80.6 ± 0.8 a
*ΔE**		------	5.1 ± 0.7 b	------	3.1 ± 0.5 a
Textura de la miga [b]					
Firmeza	N	0.6 ± 0.1 a	0.7 ± 0.1 b	0.9 ± 0.1 c	1.03 ± 0.0 d
Elasticidad		2.0 ± 0.0 a	2.1 ± 0.0 a	1.9 ±0.1 a	1.4 ± 0.5 a
Cohesión		0.8 ± 0.0 b	0.9 ±0.0 ab	0.8 ± 0.1 ab	0.7 ± 0.0 a
Masticabilidad		1.3 ± 0.2 ab	1.0 ± 0.0 a	1.7 ± 0.0 b	1.0 ± 0.4 a
Gomosidad	N	0.6 ± 0.1 b	0.5 ± 0.0 a	1.0 ± 0.1 c	0.8 ± 0.0 b
Estructura Alveolar					
Área de alveolo total	cm^2/cm^2	0.42 ± 0.01 a	0.45 ± 0.01 a	0.45 ± 0.02 a	0.45 ± 0.01 a
Pared alveolar total	cm^2/cm^2	0.58 ± 0.01 a	0.55 ± 0.01 a	0.55 ± 0.02 a	0.55 ± 0.01 a
Alveolos/cm^2		507 ± 59 a	495 ± 53 a	576± 35 a	532 ± 45 a

Pan trigo control (CWB) y pan multisemilla control (CMB) fueron prehorneados, congelados, pero no almacenados en congelación. Y el pan de trigo (WB) y pan multisemilla (MB) fueron prehorneados congelados y almacenados en congelación. Media ± DS, n =3; valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas, a un nivel de confianza del 95%.

Perfil aminoacídico de materias primas y productos desarrollados

La calidad proteica de un producto alimenticio está directamente relacionada con su perfil aminoacídico (Agostoni et al., 2010; FAO, 2007). En la tabla 4.4 se muestra el contenido de cada aminoácido presente en las materias primas y en los productos desarrollados. Destacaron los aminoácidos esenciales (AE) lisina, valina y leucina en las harinas integrales de cultivos ancestrales en comparación con la harina de trigo, y la misma tendencia en los productos panarios. También, se observa que el tiempo de almacenamiento en congelación, no afectó significativamente el contenido de los aminoácidos en comparación a la muestra sin almacenar.

Así mismo, el consumo de 100 g de pan multisemillas (MB) aporta el 19% de histidina, 9% de lisina y 17% de treonina, con respecto el valor referencial declarado por la FAO (2008), siendo mayor, que el aporte por el consumo de la misma proporción de pan control. Si bien el contenido de aminoácidos esenciales presentes en el pan multisemilla con almacenamiento en congelación disminuye ligeramente con respecto al pan multisemilla sin almacenamiento, siendo significativo con la valina y la leucina (Tabla 4.4). Esto podría deberse a la formación de cristales de hielo, los cuales aumentan de tamaño durante el almacenamiento, formados con el agua ligada a las proteínas. Este proceso desorganiza la estructura, la cual no se recupera durante la descongelación y las sustancias hidrosolubles son arrastradas con el agua (Zeece, 2020). A pesar de este fenómeno, el valor nutricional del producto multisemillas no se ve alterado en cuanto a su aporte y calidad proteica.

Contenido de Ca, Fe y Zn de materias primas y productos desarrollados. Predicción de la biodisponibilidad mineral

En la tabla 4.5, se muestra el contenido de calcio, hierro y zinc de las materias primas y los productos desarrollados, así como la relación molar fitato/mineral y el aporte a la ingesta diaria de referencia tras la ingesta de una ración de 100 g de los productos desarrollados. Las harinas integrales propuestas en este trabajo, constan de un alto contenido de minerales, así como de fitatos (InsP_6), el cual inhibe la absorción de minerales di y trivalentes en el intestino humano y de animales monogástricos (Ma et al., 2005). El proceso de panificación promueve la disminución del ácido fítico debido a la acción de la fitasa endógena del trigo y/o ingredientes, por lo que se debe evaluar la presencia del antinutriente en el producto terminado para conocer el aporte real de los minerales tras su consumo (Sanz Penella, Laparra, & Haros, 2014).

Tabla 4.4.

Contenido proteico (% m.s.)[a] y perfil aminoácidico (g/100g proteína) de las materias primas y los productos desarrollados

Aminoácido	Referencia Valor de FAO[b]	Materias Primas				Productos Panarios			
		Trigo	Quínoa	Amaranto	Chía	CWB	WB	CMB	MB
Proteína, % m.s.		11.73 ± 0.04 a	16.22 ± 0.12 c	13.46 ± 0.72 b	19.11 ± 0.30 d	19.26 ± 0.01 a	18.91 ± 0.74 a	21.96 ± 0.12 b	21.49 ± 0.19 b
Aspargina		0.42 ± 0.01 a	1.17 ± 0.01 c	0.98 ± 0.01 b	1.53 ± 0.01 d	0.46 ± 0.01 a	0.48 ± 0.01 a	0.73 ± 0.00 b	0.73 ± 0.02 b
Glutamina		3.65 ± 0.02 c	1.99 ± 0.01 a	1.97 ± 0.01 a	3.12 ± 0.04 b	3.95 ± 0.01 c	3.84 ± 0.01 b	3.46 ± 0.00 a	3.44 ± 0.01 a
Serina		0.50 ± 0.00 a	0.61 ± 0.00 b	0.74 ± 0.00 c	1.00 ± 0.01 d	0.56 ± 0.01 a	0.56 ± 0.03 a	0.68 ± 0.01 b	0.66 ± 0.01 b
Glicina		0.38 ± 0.00 b	0.78 ± 0.00 c	0.00 ± 0.00 a	0.89 ± 0.01 d	0.43 ± 0.00 a	0.42 ± 0.01 a	0.62 ± 0.00 b	0.61 ± 0.01 b
Arginina		0.42 ± 0.03 a	1.17 ± 0.01 c	1.06 ± 0.01 b	1.76 ± 0.06 d	0.46 ± 0.02 a	0.43 ± 0.01 a	0.74 ± 0.00 b	0.71 ± 0.01 b
Alanina		0.32 ± 0.00 a	0.64 ± 0.01 c	0.45 ± 0.00 b	0.91 ± 0.00 d	0.36 ± 0.00 a	0.36 ± 0.00 a	0.47 ± 0.00 c	0.46 ± 0.01 b
Prolina		1.24 ± 0.01 d	0.53 ± 0.02 b	0.00 ± 0.00 a	0.68 ± 0.07 c	1.33 ± 0.04 b	1.27 ± 0.01 b	1.06 ± 0.04 a	1.07 ± 0.01 a
Aminoácidos Esenciales (AE)									
Histidina	1.5	0.22 ± 0.00 a	0.41 ± 0.01 c	0.31 ± 0.02 b	0.49 ± 0.00 d	0.24 ± 0.00 a	0.24 ± 0.01 a	0.29 ± 0.01 b	0.29 ± 0.01 b
Valina	3.9	0.46 ± 0.00 a	0.66 ± 0.00 c	0.51 ± 0.00 b	0.85 ± 0.00 d	0.49 ± 0.00 a	0.49 ± 0.00 a	0.58 ± 0.01 c	0.54 ± 0.00 b
Isoleucina	3.0	0.35 ± 0.01 a	0.51 ± 0.00 c	0.42 ± 0.01 b	0.64 ± 0.00 d	0.39 ± 0.01 a	0.40 ± 0.01 a	0.45 ± 0.02 b	0.43 ± 0.01 b
Leucina	5.9	0.74 ± 0.00 b	0.90 ± 0.00 c	0.67 ± 0.00 a	1.22 ± 0.01 d	0.81 ± 0.00 b	0.79 ± 0.01 a	0.86 ± 0.01 d	0.83 ± 0.01 c
Fenilalanina		0.52 ± 0.02 b	0.58 ± 0.01 c	0.49 ± 0.00 a	0.89 ± 0.02 d	0.58 ± 0.01 a	0.57 ± 0.00 a	0.61 ± 0.01 b	0.59 ± 0.00 ab
Tirosina	3.8*	0.30 ± 0.00 a	0.41 ± 0.01 b	0.39 ± 0.00 b	0.57 ± 0.01 c	0.32 ± 0.00 a	0.32 ± 0.00 a	0.38 ± 0.01 b	0.37 ± 0.01 b
Lisina	4.5	0.25 ± 0.00 a	0.82 ± 0.00 c	0.75 ± 0.00 b	0.91 ± 0.01 d	0.25 ± 0.00 a	0.26 ± 0.02 a	0.42 ± 0.03 b	0.39 ± 0.02 b
Treonina	2.3	0.28 ± 0.00 a	0.52 ± 0.00 c	0.43 ± 0.00 b	0.65 ± 0.00 d	0.31 ± 0.00 a	0.32 ± 0.02 a	0.41 ± 0.01 b	0.39 ± 0.00 b

Pan trigo control (CWB) y pan multisemillas control (CMB) fueron congelados, pero no almacenados; mientras que el pan de trigo (WB) y pan multisemillas (MB) fueron congelados y almacenados en congelación. Los valores se expresan como Media ± DS. (n = 3); valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas, a un nivel de confianza del 95%. [a]m.s. Materia seca, [b]Patrón de aminoácidos sugeridos por la FAO, para adultos (g/100 g de proteína). *composición sugerida para los aminoácidos aromáticos Fenilalanina + Triptófano (FAO, 2008). FAO, Organización de las Naciones Unidas para la Agricultura y la Alimentación.

Por cada 100 g diarios de ingesta del pan multisemillas (CMB/MB) aporta aproximadamente cuatro veces más calcio que el pan de trigo (CWB/WB), el cual será totalmente biodisponible de acuerdo a la predicción del ratio molar InsP_6/Ca, inferior a 0.24. Por otro lado, el zinc es altamente biodisponible en el pan multisemillas (MB), con un 22% y 16% de aporte a la ingesta diaria para hombre y mujer, respectivamente. Esta predicción es debida a que la relación molar InsP_6/Zn es inferior a 15 (Tabla 4.5). Sin embargo, el hierro presente en los panes multisemilla, si bien cubre totalmente la ingesta diara para el hombre, y en parte la de la mujer, la relación molar InsP_6/Fe es superior a 1, lo que predice una escasa biodisponibilidad del mineral. Cabe resaltar que el proceso de almacenamiento en congelación no afectó el contenido de minerales en el producto final, y por ende la predicción de biodisponibilidad, destacándose (CMB/MB) por el alto contenido de calcio, zinc y hierro. Los resultados también indicaron que el almacenamiento en congelación no permitió la acción de la fitasa endógena de las materias primas, siendo el contenido de fitatos similar (p<0,05) antes y después del amacenamiento en congelación (Tabla 4.5).

Composición de ácidos grasos y fibra dietética en materias primas y productos desarrollados

Como se observa en la tabla 4.6, tanto en las harinas integrales de quínoa, amaranto y chía, así como el pan multigrano (CMB/MB), muestran un mayor contenido de ácidos grasos poliinsaturados (PUFA) respecto al pan de trigo

(CWB/CMB), por lo que el producto multisemillas se puede considerar un alimento más saludable (WHO/FAO, 2010).

Tabla 4.5.

Contenido de minerales en las materias primas y productos panarios, relación molar fitato/mineral y contribución a la Ingesta Diaria Recomendada (IDR).

Parámetros[a]	**Unidades**	**Materias Primas**			
		Trigo	**Quínoa**	**Amaranto**	**Chía**
Ca	mg 100g dm^{-1}	5.54 ± 0.78 a	24.22 ± 0.50 b	73.60 ± 1.73 c	306.66 ± 5.88 d
Fe	mg 100g dm^{-1}	1.22 ± 0.05 a	4.74 ± 0.07 b	5.78 ± 0.18 bc	7.19 ± 1.06 d
Zn	mg 100g dm^{-1}	0.34 ± 0.02 a	1.33 ± 0.03 c	1.07 ± 0.02 b	1.81 ± 0.01 d
InsP_6	mg 100g dm^{-1}	n.d.	10.06 ± 0.03 a	8.60 ± 0.09 a	13.93 ± 1.20 b

	Unidades	**Productos Panarios**			
		CWB	**WB**	**CMB**	**MB**
Cenizas	mg 100g dm^{-1}	0.98 ± 0.03 a	0.99 ± 0.01 a	1.76 ± 0.10 b	1.67 ± 0.04 b
Ca	mg 100g dm^{-1}	7.96 ± 0.90 a	10.05 ± 1.17 a	45.02 ± 5.20 b	47.67 ± 9.92 b
Fe	mg 100g dm^{-1}	0.94 ± 0.05 a	1.12 ± 0.01 a	2.65 ± 0.05 b	2.69 ± 0.19 b
Zn	mg 100g dm^{-1}	0.40 ± 0.00 a	0.37 ± 0.00 a	0.66 ± 0.02 b	0.70 ± 0.06 b
InsP_6	mg 100g dm^{-1}	1.15 ± 0.03 a	1.41 ± 0.32 a	3.40 ± 0.38 b	3.68 ± 0.06 b

	% de Contribución a la Ingesta Diaria Recomendad/IDR					
	Valor de referencia (Hombre/Mujer), mg/día	**Unidades**	**CWB**	**WB**	**CMB**	**MB**
Ca FAO[b]	1000	%	1	1	4	4
Fe FAO[b]	14/29	%	6/3	7/4	18/9	18/9
Zn FAO[b] Alta biodisp	4.2/3	%	9/13	8/12	15/20	16/22
Zn FAO[b] Media biodisp	7/4.9	%	5/4	5/4	8/7	9/7
Zn FAO[b] Baja biodisp	14/9.8	%	3/4	2/4	4/6	5/7
InsP_6/Ca[c] < 0.24	< 0.24	mol/mol	0.16	0.15	0.08	0.08
InsP_6/Fe[c] < 1.0	< 1.0	mol/mol	1.32	1.36	1.37	1.48
InsP_6/Zn[c] < 15.0	< 15.0	mol/mol	3.06	4.06	5.54	5.66

Pan trigo control (CWB) y pan multisemillas control (CMB) fueron congelados, pero no almacenados; mientras que el pan de trigo (WB) y pan multisemillas (MB) fueron congelados y almacenados en congelación. [a]Los valores se expresan como Media ± DE (n = 3). Valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas, a un nivel de confianza del 95%. [b]IDR (Ingesta Diaria Recomendada) expresada en mg por día para hombres/mujeres ≥18. Contribución a la IDR (%) tras el consumo de 100 g de pan. FAO-OMS: Organización de las Naciones Unidas para la Agricultura y la Alimentación. La FAO considera tres niveles de biodisponibilidad del zinc, dependiendo del contenido de fitatos (InsP_6) en la dieta (FAO alto; medio, y baja biodisponibilidad) FAO/WHO (2001). [c]Relaciones de umbral (InsP_6/Mineral) para la inhibición de la disponibilidad de minerales Ma et al. (2005); InsP_6, hexakisfosfato de *mio*-inositol.

El alto contenido de PUFAs corresponde fundamentalmente al elevado contenido de ácido alfa linolenico (ALA), que se encuentra mayormente presente en la harina integral de chía, y otras materias primas, produjo el incremento de la relación de los ácidos grasos polinsaturados y ácidos grasos saturados (PUFA:SFA).

Se puede observar que la relación ω-6/ω-3, en la formulación optimizada es de 1:1, ratio que se considera adecuada según las autoridades sanitarias y que ayudaría a prevenir enfermedades cardiovasculares (WHO/FAO, 2010; EFSA, 2010). Esta relación corresponde principalmente al elevado contenido de ALA (22.14%) y ácido linoleico LA (6.27%) presentes en la chía. De esta manera, con la ingesta de 100 g de pan multigrano se cubriría los requerimientos diarios recomendados en ese sentido.

En cuanto a la relación de fibra soluble/insoluble, Jaime et al. (2001) sostiene que debería mantenerse una relación 1:2 g/g, para que la acción fisiológica sea más efectiva tras la ingesta de un alimento con fibra. De acuerdo con esto, el producto multisemillas (MB), presentó un valor cercano al indicado, por lo que 100 g de este producto aportaría un 42% del total de la ingesta diaria adecuada de fibra en adultos (25 g/día) Tabla 4.7 (EFSA, 2017).

Tabla 4.6 *Composición de ácidos grasos, de harinas y productos panarios, así como la contribución a la ingesta adecuada*

Parámetros		**Unidad**	**Materias Primas**				**Productos Panarios**			
			Trigo	**Quínoa**	**Amaranto**	**Chía**	**CWB**	**WB**	**CMB**	**MB**
Lípidos		% m.s.	1.14 ± 0.17 a	6.96 ± 0.74 b	6.92 ± 0.14 b	34.61 ± 0.04 c	0.17 ± 0.06 a	0.22 ± 0.02 a	3.91 ± 0.16c	3.42 ± 0.19b
Ácido Palmítico	C16:0	g 100g dm^{-1}	0.22 ± 0.00 a	1.27 ± 0.01 b	0.73 ± 0.01 c	2.57 ± 0.03 d	0.03 ± 0.00 a	0.04 ± 0.00 a	0.46 ± 0.00b	0.41 ± 0.01b
Ácido Esteárico	C18:0	g 100g dm^{-1}	0.03 ± 0.00 a	0.05 ± 0.00 a	0.26 ± 0.00 b	1.18 ± 0.01 c	0.00 ± 0.00 a	0.00± 0.00 a	0.12 ± 0.00b	0.10 ± 0.00b
Ácido Cis Vaccénico	C18:1n7c	g 100g dm^{-1}	0.01 ± 0.00 a	0.07 ± 0.00 b	0.07 ± 0.00 b	0.28 ± 0.00 c	0.00 ± 0.00 a	0.00± 0.00 a	0.04 ± 0.00b	0.03 ± 0.00b
Ácido Oleico	C18:1n9c	g 100g dm^{-1}	0.14 ± 0.01 a	1.59 ± 0.00 b	1.91 ± 0.03 c	2.07 ± 0.02 d	0.02 ± 0.00 a	0.03 ± 0.00 a	0.47 ± 0.00b	0.43 ± 0.03b
Ácido Linolénico	C18:2n6	g 100g dm^{-1}	0.38 ± 0.02 a	4.03 ± 0.03 c	3.29 ± 0.01 b	6.27 ± 0.02 d	0.10 ± 0.00 a	0.13 ± 0.00 a	1.26 ± 0.00b	1.15 ± 0.07b
Ácido α-Linolénico	C18:3n3	g 100g dm^{-1}	0.43 ± 0.03 c	0.26 ± 0.00 b	0.06 ± 0.00 a	22.14 ± 0.07 d	0.01 ± 0.00 a	0.01 ± 0.00 a	1.55 ± 0.01c	1.29 ± 0.10b

% de Contribución de ingesta adecuada (IA)[a]

	Valor Referencia		**CWB**	**WB**	**CMB**	**MB**
[b]PUFA: SFA ratio			3.15	3.08	4.84	4.70
% de Contribución de AI_{LA} E% para ácido Linoleico	2.5-9%E	%	2	2	19	21
% de Contribución de AI_{ALA} E% para ácido α-Linolénico	>0.5%E	%	1	1	117	98
Ratio ω-6/ω-3	5:1		10:1	13:1	1:1	1:1

Pan trigo control (CWB) y pan multisemilla control (CMB) fueron congelados, pero no almacenados congelados. Y el pan de trigo (WB) y pan multisemilla (MB) fueron congelados y almacenados en congelación. a-c Los valores son expresados como media ± DE (n = 3). Valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas, a un nivel de confianza del 95%. [a]IA (Ingesta adecuada) porcentaje de contribución para la ingesta promedio de 100 g de pan; AI_{LA} o AIA_{LA} E% (porcentaje de ingesta energética) para LA (ácido linoleico) o ALA (ácido α-linolénico) por adulto ≥ 18, respectivamente, E = (Kcal proteína + Kcal lípidos + Kcal carbohidratos) en 100 g de pan (EFSA, 2017). [b]PUFA: Total ácidos grasos poliinsaturados, SFA: Total ácidos grasos saturados.

Tabla 4.7

Composición de fibra dietética de los productos panarios y su contribución a la ingesta adecuada

Parámetros	Valor referencial[a]	Unidad	Productos panarios			
			CWB	WB	CMB	MB
Fibra Soluble	--	g 100g dm^{-1}	1.74 ± 0.22 ab	1.52 ± 0.49 a	2.46 ± 0.26 bc	2.98 ± 0.08 c
Fibra Insoluble	--	g 100g dm^{-1}	4.83 ± 0.94 a	4.84 ± 0.21 a	7.82 ± 0.23 b	7.63 ± 0.12 b
Fibra Total Dietética	--	g 100g dm^{-1}	6.57 ± 0.72 a	6.35 ± 0.28 a	10.28 ± 0.02 b	10.62 ± 0.04 b
Ratio Fibra Dietética Soluble/Insoluble	1:2	g/g	1:2.8	1:3.2	1:3.2	1:2.6
Contribución de la ingesta diaria (AI)	25 g/d	%	26	25	41	42

Pan trigo control (CWB) y pan multisemilla control (CMB) fueron congelados, pero no almacenados congelados. Y el pan de trigo (WB) y pan multisemilla (MB) fueron congelados y almacenados en congelación. a-c Los valores son expresados como media ± DE (n = 3). a, b, c, d valores seguidos de la misma letra en una misma línea indican que no presentan diferencias significativas, a un nivel de confianza del 95%. a Ratio de Fibra dietética soluble/insoluble, 1:2 g/g (Jaime et al., 2002), AI (ingesta adecuada) contribución (%) para una ingesta media diaria de 100 g de pan. AI en adultos ≥18 es de 25 g/d (EFSA, 2017).

CONCLUSIÓN

El proceso de precocido/congelado/almacenamiento en congelación no mermó las características tecnológicas del producto multisemillas, a excepción del ligero aumento en la firmeza de la miga y menor masticabilidad. Sin embargo, estos cambios pueden no ser percibidos por el consumidor, posiblemente por la alta proporción de fibra de la formulación multisemillas que evitó el exceso de deshidratación en las etapas de congelamiento/descongelamiento. Además, este alto contenido de fibra podría cubrir el 42% del total de la ingesta diaria adecuada de fibra en adultos tras un consumo de 100 g del alimento, con una relación apropiada de fibra soluble e insoluble, lo que podría contribuir al correcto funcionamiento intestinal. La formulación propuesta aportó un mayor contenido de lisina y minerales biodisponibles como el calcio y el zinc, mientras que el hierro se encuentra comprometido por el mayor contenido de fitatos. Adicionalmente, esta formulación podría contribuir postivamente a la prevención de desarrollar trastornos cardiovasculares por presentar una ratio ω-6/ω-3 cercano a 1:1. En síntesis, el proceso de doble horneado y el almacenamiento en congelación durante tres meses no disminuyó de forma significativa las propiedades tecnológicas de los productos panarios ni su valor nutricional.

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IV. DISCUSIÓN GENERAL

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La incorporación de harinas sustitutas como harinas integrales de cereales, semillas oleaginosas y pseudocereales es la tendencia de hoy en día (Ballester-Sánchez et al., 2019; Iglesias-Puig & Haros, 2013; Iglesias-Puig et al., 2015).

Estudios epidemiológicos y experimentales han demostrado que la ingesta regular de productos integrales, ricos en fibra, como panes elaborados con semillas o harinas integrales de pseudocereales/cereales; previene el desarrollo de enfermedades metabólicas, cardiovasculares y crónicas como la diabetes tipo 2 (Capitani et al., 2012). De ahí el creciente interés del consumidor. Diversos estudios se enfocan en encontrar fórmulas que tengan mayor valor proteíco, así como menor digestibilidad de almidón (Liu et al., 2017). Sin embargo, a pesar de ser alimentos nutritivos, la aceptabilidad está condicionada a sus aspectos sensoriales y tecnológicos (Parenti et al., 2020).

En ese sentido, el propósito de la presente investigación fue revalorizar la calidad nutricional, tecnológica y sensorial, de las materias primas andinas cultivadas en Ecuador, como la quínoa, el amaranto y la semilla de chía, consiguiendo demostrar su valor para ser utilizadas como ingredientes con potencial funcional en productos panarios de alta calidad. Por otro lado, se obtuvo una formulación panaria con óptimas características nutricionales y organolépticas, que por la naturaleza de sus ingredientes; mantuvieron su valor nutricional y calidad tecnológica después del precocido, congelado y almacenamiento en congelación durante tres meses, seguido de cocción final.

Revalorización de materias primas utilizadas como ingredientes con potencial funcional

Las formulaciones panarias con el reemplazo de harina por el 25 y 50% de harina integral de amaranto (*Amaranthus spinosus* (WAs-25 y WAh-50) y *Amaranthus hypochondriacus* (WAh-25 y WAh-50)) incrementaron significativamente el contenido de cenizas, proteínas, lípidos y fibra dietética comparado con el producto control. Solo la muestra WAh-50, mostró un contenido mayor de fibra dietética insoluble, con su correspondiente incremento de humedad como fue descrito por otros investigadores (Sanz-Penella et al., 2013).

En cuanto a la relación del contenido de fibra soluble/insoluble 1:2, la formulación WAs-50, fue la más cercana a esta relación. Además, se pudo determinar que la fibra en las formulaciones sustituidas al 50% pueden cubrir más de la mitad de los requerimientos de una ingesta diaria para adultos. El contenido de minerales (Ca, Fe y Zn) se incrementó hasta 4 veces en las formulaciones sustituidas al 50% con las harinas integrales de amaranto. En este sentido, la ingesta dietética de Zn y Fe podrían estar cubiertas en un 50% y 70% tras la ingesta de 100g de pan, respectivamente. Este resultado es válido siempre que los inhibidores de la absorción de minerales estuvieran ausentes, según lo indicado por la Alimentación y Consejo de Nutrición del Instituto de Medicina, Academia Nacional de Ciencias (2005). Si bien, el mayor perfil nutricional se encontró en las muestras sustituidas al 50%, la evaluación sensorial fue superior en las muestras sustituidas al 25%, siendo la variedad *A. hypochondriacus* la de mayor aceptabilidad por parte de los consumidores, quienes indicaron un “nuevo sabor”, probablemente relacionado con la presencia de saponinas (Haros & Schoenlechner, 2017).

También se evaluó el efecto de la inclusión de harina integral de amaranto en las propiedades térmicas del almidón, encontrándose que la entalpía de gelatinización dismuye conforme se incrementa el porcentaje de amaranto en la formulación, independientemente de la especie estudiada. Esta disminución puede relacionarse con la mayor cantidad de agua que absorbe los gránulos de almidón de amaranto, con mayor hinchamiento y solubilidad, comparado con el almidón de trigo. Además, la inclusión de la harina de amaranto en las formulaciones retarda el envejecimiento en términos de retrogradación de amilopectina, especialmente en las formulaciones con un mayor porcentaje de sustitución. Esta tendencia también fue encontrada por otros investigadores (Perez-Rea & Antezana-Gomez, 2018; Repo-Carrasco-Valencia et al., 2009).

Por otro lado, las semillas de chía (CWS), la harina integral de chía/semillas molidas (CWF) y sus coproductos tras la extracción del aceite [harina semidesgrasada (CSDF) y harina baja en grasa (CLFF)] fueron utilizados como ingredientes panarios en sustitución de harina al 5% y 10%. La ingesta de 100 g de pan con 10% de ingredientes de chía podría cubrir la cuarta parte de los requerimientos diarios de fibra dietética total en adultos. En cuanto al aporte proteico de los ingredientes de chía, estos mostraron mayor contenido que la harina (W) (CLFF> CSDF> CWF> CWS> W) y de mejor perfil aminoacídico. Los ingredientes CSDF y CLFF mostraron valores más altos de tirosina (Tyr), histidina (His), metionina (Met), triptófano (Trp), lisina (Lys) y cisteína (Cys) comparados con las semillas de chía y la harina integral de chía. Sin embargo, en el pan de semilla de chía el contenido de lisina fue superior que el presente en las demás formulaciones.

Con la ingesta diaria de 15 g de semillas o 3 g de proteína de chía considerando un adulto de 70 kg, se cubre parcialmente las necesidades de aminoácidos esenciales. Además, la ingesta diaria de 12 g de harinas o 2 g de proteína de chía desgrasadas (CSDF/CLFF) proporcionaría el 6% de la cantidad diaria recomendada en adultos de Lys y Val, el 13% de la dosis diaria de Met + Cys y el 19% de Phe + Tyr.

Por otro lado, es importante destacar que los procesos de horneado pueden provocar rancidez oxidativa en los ácidos grasos insaturados, disminuyendo así el contenido total de los mismos. Este fenómeno sucedió con todas las formulaciones que involucraron harina integral de chía o harinas desengrasadas de chía. La excepción (o la menor disminución de PUFAs) se observó en las muestras formuladas con semillas de chía, a pesar de poseer mayor proporción de ácidos α-linolénico (ALA) y linoleico (LA) que las muestras desengrasadas. Esto es debido a la protección que ejerce la integridad de la semilla frente al contacto con el oxígeno, minimizando las reacciones de oxidación lipídica fomentadas por las altas temperaturas de horneado.

Las recomendaciones dietéticas en cuanto a la ingesta adecuada (IA) de ácidos grasos poliinsaturados, se expresan en % de la energía dietética total (E%), y los productos con semillas de chía aportan una mayor contribución ella, resultados similares fueron encontrados por otros investigadores (Coelho & Salas-Mellado, 2015; Wąsowicz et al., 2004).

Las formulaciones sustituidas en un 10% de harina por LFF y SDF, presentaron valores calóricos más bajos, esto puede atribuirse a que estas harinas se enriquecieron en mucílagos y proteínas tras la extracción del aceite (Fernandes & Salas-Mellado, 2017). En cuanto a las IDR de Ca y Fe, la formulación adicionada con la harina desengrasada de chía (LFF) fue las del mayor aporte, mientras que en la formulación al 5% predice una mayor biodisponibilidad de Zn comparado con las formulaciones de harina integral chía al 10%, básicamente por su mayor proporción de fitatos de estas últimas.

Características físico – químicas, nutricionales y tecnológicos de un pan multisemillas (quínoa, chía y amaranto) fresco, prehorneado y almacenado en congelación

Utilizando un diseño factorial 3^3, se formuló un pan multisemillas por sustitución de la harina de trigo por harina integral de chía en tres niveles (0, 10 y 20%), harina integral de quínoa en tres niveles (0, 20 y 40%); y harina integral de amaranto en tres niveles (0, 20 y 40%). Teniendo en cuenta que las materias primas empleadas cuentan con un alto porcentaje de proteína, fibra dietética, ácidos grasos insaturados y minerales se esperan formulaciones con alto valor nutricional, sobre todo por la inclusión de la harina de chía que aporta altas concentraciones de lisina y ácido alfa-linolénico. El valor calórico, al estar relacionado principalmente con el contenido de lípidos, aumenta al incrementar la cantidad de harina de chía, seguido de la harina de amaranto y por último por la harina de quínoa. Desde un punto de vista tecnológico, durante la optimización se observó que, al incrementar el grado de sustitución, con cualquiera de las combinaciones de harinas sustitutas, disminuyó el volumen específico de las piezas panarias. Esto se debió fundamentalmente por dilución del gluten en la formulación dbido a que los ingredientes sustitutos están libres de gluten. Sin embargo, la formulación de harina de chía al 10% mostró valores superiores a la muestra control a pesar de la dilución del gluten. Esto puede ser explicado por la presencia del mucílago de la chía que forma complejos hidrofílicos entre sus grupos iónicos y las proteínas del gluten, conservando así la red tridimensional que da estructura a la masa panaria durante la fermentación (Iglesias-Puig & Haros, 2013). En cuanto a la firmeza de la miga, esta se incrementó con el aumento de harina de chía, lo contrario ocurrió cuando se aumentó la sustitución con harina de quínoa y amaranto. Las piezas panarias se oscurecen y se tornan rojizas con cambios significativos en los parámetros de color cuando se incrementó el contenido de harina de chía, pese a la interacción entre las harinas integrales de quínoa y amaranto, que condujeron a la reducción de las diferencias de color de la corteza.

Para la optimización de una formulación panaria multisemillas se asignaron condiciones para cada respuesta, es decir, en cuanto al *Valor nutricional*: se maximizaron el contenido de lípidos, proteínas y cenizas y se minimizaron el valor calórico del producto; en cuanto a la *Calidad tecnológica*: se maximizó el volumen específico de la pieza panaria, y se minimizó la relación de aspecto de la rebanada central, la firmeza de la miga, y la diferencia de los parámetros de color de la corteza y miga en comparación a la muestra control; y por último, el *Análisis sensorial*: se maximizó la puntuación en los atributos de apariencia, textura, sabor y aceptabilidad global del producto desarrollado. La formulación óptima que respondió a todas estas consignas fue la formulación $Ch_{10}Q_4A_{19}$ (10% chía, 4% de quínoa y 19% de amaranto), un producto panario con 33% de sustitución de harina. El mismo presentó cantidades altas de fibra soluble e insoluble y un valor calórico inferior al pan de trigo integral, sin diferencias significativas en cuanto a la aceptabilidad global. Sin embargo, la diferencia de color de la corteza y de la miga comparados con la muestra control fue percibida por los consumidores, como se comentó anteriormente. Una ración diaria de 100 g del pan multisemillas optimizado, puede contribuir a una ingesta adecuada de calcio (FAO/WHO, 2001), tres veces superior que la proporcionada por el pan control, siendo totalmente biodisponible de acuerdo a la predicción que indica la ratio molar InsP_6/Ca, inferior a 0.24. Además, la formulación optimizada destacó por el contenido de ácidos grasos poliinsaturados, principalmente el ácido α-linolénico, siendo superior comparado con formulaciones de pan de trigo sustituidos al 10% con harina integral de chía. En este sentido, este alimento presentó una ratio LA/ALA de 0,6:1, por lo que la ingesta de este producto podría revertir el desequilibrio actualmente existente en las dietas occidentales en cuanto a la ratio de omega--6/omega-3. Adicionalmente, una ingesta diaria de 100 g del pan multisemillas optimizado podría cubrir las necesidades de ácido α –linolénico en adultos (ALA, >0.5%E).

La digestibilidad de las proteínas *in vitro* (IVPD), fue inferior que la encontrada en el pan control y similar a la digestibilidad de cereales listos para comer (Gilani, Cockell, & Sepehr, 2005). En relación al contenido de fibra, la ratio fibra soluble/insoluble encontrada en el pan multisemillas optimizado fue cercano al recomendado de 1:2. Por otro lado, el consumo de 100 g de pan contribuirá con el 60% del total de ingesta diaria adecuada recomendada de fibra (EFSA, 2017; Laura Jaime et al., 2002)**.** El pan optimizado obtuvo un menor indice glicémico comparado con el del pan de trigo, lo que puede deberse al contenido de fibra soluble, debido a que la fibra regula la respuesta del vaciado gástrico (Schuchardt et al., 2016).

Una vez obtenida la formulación óptima se estudió el efecto del proceso de precocción, congelación/almacenamiento en congelación (3 meses) y cocción final en la calidad nutricional, tecnológica y sensorial del producto final. Para ello, se utilizaron la formulación control (WB) y la formulación optimizada multisemillas (MB) y se compararon con su contraparte sin almacenamiento en congelación (CWB y CMB, respectivamente).

Se observó que el almacenamiento en congelación afectó los parámetros tecnológicos disminuyendo el volumen específico de la pieza panaria, la masticabilidad, gomosidad y un ligero aumento de la firmeza, y los parámetros de color (Martínez, Oliete, & Gómez, 2013). Además, la formulación multisemillas presentó una entalpía de retrogradación de la amilopectina ligeramente inferior a la formulación control, debido fundamentalmente al mayor contenido en lípidos y fibra de la primera formulación, lo que dificulta la recristalización de la amilopectina, y por ende, un retraso en el envejecimiento del pan (Mondragón, Mendoza-Martínez, Bello-Pérez, & Peña, 2006).

El almacenamiento en congelación no afectó el perfil aminoacídico de ambas formulaciones. La formación de cristales de hielo, los cuales aumentan de tamaño durante el almacenamiento, se forman con el agua ligada a las proteínas, desorganiza la estructura, la cual no se recupera tras la descongelación y las sustancias hidrosolubles son arrastradas con el agua (Zeece, 2020). A pesar de este fenómeno, el valor nutricional del producto multisemillas no se ve alterado en cuanto a su aporte y calidad proteica. Así mismo, el consumo de 100 g de pan multisemillas (MB) aporta el 19% de histidina, 9% de lisina y 17% de treonina, comparado con el valor de referencia declarado por la FAO/WHO/UNU (2007), siendo mayor que el aporte del producto elaborado con harina. Si bien, el almacenamiento en congelación no afecta el perfil de aminoácidos, el doble proceso de horneado y otros factores como el pH o el tiempo de fermentación, puede modificar este perfil (Oğur, 2014; Turfani, Narducci, Durazzo, Galli, & Carcea, 2017; WHO/FAO, 2010).

El contenido de minerales en las harinas y los panes, así como la relación molar mineral/fitato y la contribución promedio requerida, así como el contenido en fitatos (InsP_6) son prámetros que definen el aporte y biodisponibilidad de minerales porlo que se han utilizado en este estudio (Ma et al., 2005). El zinc es altamente biodisponible en el pan multisemillas (MB) debida a que la relación molar InsP_6/Zn es inferior a 15, con un 22% y 16% de aporte a la ingesta diaria para hombre y mujer, respectivamente, tras la ingesta de 100 g de pan. La relación molar InsP_6/Fe es superior a 1, lo que predice una escasa o nula biodisponibilidad de este mineral. Para incrementar la biodisponibilidad de Fe es necesario prolongar la fermentación panaria y/o incluir masas agrias para activar la fitasa endógena de los ingredientes, o bien, incluir un iniciador panario productor de fitasa (Sanz-Penella et al., 2009; 2012).

La combinación de las harinas integrales estudiadas, con alto contenido de PUFAs, produjo un incremento de la relación de los ácidos grasos poliinsaturados y ácidos grasos saturados PUFA:SFA. Además, la relación omega-6/omega-3 en el producto optimizado precocido y almacenado en congelación es de 1:1, valor recomendado por WHO/FAO (2008). Así mismo, en cuanto a la relación de fibra soluble/insoluble, se sostiene que debe mantenerse la relación 1:2 g/g, para que la acción fisiológica sea más efectiva, siendo el pan multisemillas con almancenamiento en congelación el que presentó el valor más cercano al indicado y aportaría el 42 % del total de la ingesta diaria adecuada de fibra en adultos, 25 g/día, tras la ingesta de 100 g de producto (EFSA, 2017; Jaime et al., 2001).

Las harinas sustitutas alternativas propuestas en esta investigación podrían incrementar sustancialmente el valor nutricional y funcional de alimentos a base de cereales a bajos porcentajes de sustitución y sin modificar sus características nutricionales por la utilización de metodologías de precocción y almacenamiento en congelación, con una ligera merma de la calidad tecnológica y sensorial del producto.

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V. CONCLUSIONES

Tras la realización de la presente investigación, las conclusiones alcanzadas fueron las siguientes:

1. Los productos panarios elaborados con harinas de amaranto (*A. spinosus* y *A. hypochondriacus*), presentaron proporciones de fibra soluble/insoluble cercanas a 1:2, lo que presenta un equilibrio adecuado para la acción fisiológica de la misma en el organismo. Los niveles de lípidos, minerales y fosfatos de *mio*-inositol también aumentaron significativamente con la incorporación de este pseudocereal.
2. Los panes de amaranto sustituidos al 50%, aportaron un alto porcentaje de la ingesta diaria recomendada de zinc y hierro en adultos, pero también incrementaron el contenido de fitatos que pueden afectar la biodisponibilidad de estos minerales.
3. Los catadores mostraron mayor aceptación del producto reemplazado al 25% con harina integral de *A. hypochondriacus* que con harina integral de *A. spinosus*, por lo que podría estudiarse la aplicación de la harina de *A. spinosus* en formulaciones con menor sustitución.
4. La caracterización química de los subproductos de la semilla de chía, obtenidos tras la extracción del aceite por prensado en frío o extracción supercrítica, presentaron alta concentración de fibra total, proteínas con aminoácidos esenciales como la lisina, tirosina, histidina, metionina, y ácidos grasos esenciales, principalmente ácido α-linolenico. En este sentido, los subproductos de chía tras la extracción de su aceite, podrían ser incluidos en formulaciones de alimentos por su alta calidad nutricional.

5. Los productos panarios sustituidos al 10% con chía, harina integral de chía y subproductos de chía tras la extracción del aceite, mostraron índices glucémicos inferiores a los productos con menor nivel de sustitución y muestra control, debido fundamentalmente al mayor contenido de fibra total; principalmente por la presencia de mucílago. También, aportaron un alto contenido de calcio, zinc y acidos grasos omega-3.
6. El modelo superficie respuesta utilizado para la optimización de una formulación panaria sustituída con harinas integrales de amaranto, quínoa y chía, fue validado teniendo en cuenta parámetros nutricionales y tecnológicos, obteniéndose una formulación panaria con 33% de sustitución de harina por amaranto, quínoa y chía en las proporciones 19, 4, y 10 % en base a harina, respectivamente.
7. La formulación optimizada presentó mejores características tecnológicas, nutricionales y sensoriales que un producto integral de trigo. En cuanto a la ratio PUFAs/SFAs, contenido de fibra dietética y aporte de fibra soluble y minerales fueron superiores que la muestra control, con una estimación inferior del índice glucémico. Los parámetros de calidad tecnológicos fueron superiores comparados con el producto integral de trigo, en cuanto a la firmeza de la miga, el volumen específico de la pieza panaria, la relación de aspecto de la rebanada central, con excelentes características sensoriales similares a la muestra control.
8. Los efectos del proceso del doble horneado y el almacenamiento en congelación durante tres meses en las propiedades tecnológicas se reflejaron en la disminución del volumen específico de la pieza panaria,

ligero aumento de la firmeza de la miga, y cambios de color, sin embargo, esto último podría no ser percibido por el consumidor posiblemente por el alto contenido de fibra que no permitió un exceso de deshidratación debido al ciclo de congelamiento y descongelamiento.

9. La formulación propuesta aportó un mayor contenido de lisina y minerales biodisponibles como el calcio y el zinc, mientras que el Fe se encuentra comprometido por el mayor contenido de fitatos. Además, presentó una ratio omega-6/omega-3 cercano a 1:1. La ingesta de 100 g del pan optimizado, podría cubrir el 42% de la ingesta adecuada para adultos, con una relación apropiada de fibra soluble e insoluble, lo que podría contribuir al correcto funcionamiento intestinal. Por sus características, el producto optimizado podría contribuir tras su ingesta diaria, a la prevención del desarrollo de enfermedades precedidas por el síndrome metabólico.
10. Las harinas sustitutas alternativas al trigo propuestas en esta investigación podrían incrementar sustancialmente el valor nutricional y funcional de alimentos a base de cereales, sin modificar sus características nutricionales por la utilización de procesos de precocción y almacenamiento en congelación, con una ligera merma de la calidad tecnológica y sensorial del producto.

VI. ANEXOS

La presente tesis doctoral ha dado lugar a 3 publicaciones científicas en Revistas de alimentos:

Miranda-Ramos, K., Sanz-Ponce, N., & Haros, C. M. (2019). Evaluation of technological and nutritional quality of bread enriched with amaranth flour. LWT-Food Science and Technology, 114, 8. doi:10.1016/j.lwt.2019.108418. Factor de impacto (JCR 2018): 3,714

Miranda-Ramos, K.C, Millán-Linares, M. C., & Haros, C. M. (2020). Effect of Chia as Breadmaking Ingredient on Nutritional Quality, Mineral Availability, and Glycemic Index of Bread. Foods, 9(5), 663. doi:doi.org/10.3390/foods9050663. Factor de impacto (JCR 2019): 4,092

Miranda-Ramos, K. C., & Haros, C. M. (2020). Combined Effect of Chia, Quinoa and Amaranth Incorporation on the Physico-Chemical, Nutritional and Functional Quality of Fresh Bread. Foods, 9(12), 1859. doi:10.3390/foods9121859. Factor de impacto (JCR 2019): 4,092

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Evaluation of technological and nutritional quality of bread enriched with amaranth flour

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ABSTRACT

The objective of this investigation was to develop bread, with high nutritional and technological quality, using whole flour of *Amaranthus spinosus* and *Amaranthus hypochondriacus*. Bread quality was analyzed in terms of chemical composition, loaf specific volume, width/height ratio of the central slice, crust/crumb color, crumb structure and firmness, and sensory analysis. Starch thermal properties were studied in terms of enthalpies of starch gelatinization during baking and amylopectin retrogradation during storage. Incorporation of amaranth flour significantly increased protein, lipid, fiber, ash, and *myo*-inositol phosphate contents. Bread with amaranth flours exhibited soluble/insoluble fiber ratios close to 1:2, which presents the most effective physiological action. Intake of products with high substitution of amaranth could cover the protein requirement in adults, and could contribute substantially to intake of dietary fiber, Fe, and Zn according to daily recommendations. Bread with *A. hypochondriacus* showed higher acceptability than formulations with *A. spinosus*. Inclusion of amaranth allowed delaying and decreasing crumb staling in terms of amylopectin retrogradation. The inclusion of amaranth could be limited to a maximum of 25 g/100 g, with considerable nutritional improvement and acceptable sensory and technological quality, even during the staling process.

1. Introduction

Wheat bread is a popular food in the category of bakery products. Refined wheat flour is the most used raw material for this product but it has a low level of essential amino acids such as lysine and threonine, and reduced fiber content compared to whole flour bread. In fact, many studies have been aimed at improving wheat flour nutritional characteristics by the incorporation of new functional ingredients and at developing safe, healthy, nutritious products (Dewettinck et al., 2008). An alternative could be bakery products enriched with a high amount of dietary fiber, amino acids and bioactive compounds from whole flours or entire grain of cereal/pseudocereal that help to prevent diseases associated with the metabolic syndrome such as cardiovascular diseases, arteriosclerosis, and colon cancer (Motta et al., 2019; Salas-Salvadó, Bulló, Pérez-Heras, & Ros, 2006). The health benefits of pseudocereals are well known, and one of the most important of them is amaranth (*Amaranthus* spp.), which is a native plant found in South and Central America, with the exception of some species that belong to tropical and subtropical regions of India, such as *Amaranthus spinosus* (Reguera & Haros, 2017). From a nutritional point of view, pseudocereals are considered better than cereals such as wheat, barley, or rice because of their content/composition of starch, oil, dietary fiber, vitamins (A, K, B_6, C, E, and B), and minerals such as calcium, magnesium, phosphorus, iron, potassium, zinc, copper, and manganese (Alvarez-Jubete, Arendt, & Gallagher, 2009; Reguera & Haros, 2017). Although many species are considered opportunistic weeds, only three of them are used for human consumption: *A. caudatus* (or *A. paniculatus*, *kiwicha*, o *quihuicha*), *A. cruentus* (or *A. flavus*, or *A. leucocarpus*), *A. hypochondriacus* (or *A. edulis*, or *A. mantegacianus*) (Gamel, Linssen, Mesallam, Damir, & Shekib, 2006; Reguera & Haros, 2017; Sangameswaran & Jayakar, 2008). However, studies have established that the seeds or plant parts of *A. spinosus* possess various biological activities such as analgesic and anti-inflammatory, anti-diabetic, anti-hyperlipidemic and spermatogenic effects (Jhade, Ahirwar, Jain, Sharma, & Gupta, 2009; Rjeibi, Ben Saada, & Hfaiedh, 2016; Sangameswaran & Jayakar, 2008). Amaranth has managed to capture growing interest as a functional ingredient because of its high-quality nutritional and technological properties, especially in baking processes, and because it is very versatile for processing and industrialization (Sanz-Penella, Wronkowska, Soral-Smietana, & Haros, 2013).

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Article

Effect of Chia as Breadmaking Ingredient on Nutritional Quality, Mineral Availability, and Glycemic Index of Bread

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Abstract: Chia seeds and chia flour could be used as ingredients to enrich foods owing to their high amount of nutrients. The goal of this investigation was to provide further information about how replacing wheat flour with chia ingredients (seeds, whole flour, semi-defatted, and low-fat flours) affects the nutritional and functional value of bread. The higher levels of proteins, lipids and minerals determined in raw chia flours directly affected the increase of these nutrients. High levels of phytates were found in chia ingredients (5.1–6.6 µmol/g d.b.), which affect Zn and Fe bioavailability, as predicted by phytate/mineral molar ratios. Bread with chia had a high amount of linoleic acid, especially in bread with chia seeds, owing to protection of seed integrity during baking. Chia ingredients did not show limiting essential amino acids such as lysine, which is deficient in cereals. Glycemic index was lower in bread with chia ingredients compared to control. The beneficial effects on glucose metabolism together with the nutritional and functional characteristics could be clinically important for prevention of metabolic diseases.

Keywords: *Salvia hispanica*; chia ingredients; breadmaking products; fatty acid profile; essential amino acid profile; minerals; PRI/RDA/AI (Popular Reference Intake/Recommended Dietary Allowance/Adequate Intake)

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1. Introduction

The increasing consumer demand for nutritious, healthy food has made the food industries examine their own resources to discover and take advantage of functional ingredients. Nowadays, with regard to bakery goods, wheat bread has been enriched with new food ingredients [1,2]. Numerous epidemiological and experimental studies suggest that changes in the diet are decisive in the prevention of various metabolic disorders included in the so-called metabolic syndrome, such as type 2 diabetes, insulin resistance, hypertension, obesity, and cardiovascular disease [3]. Furthermore, intake of prebiotic foods, wholegrain, high fiber, seed breads, or high amounts of omega-3 leads to lower blood cholesterol and consequently reduces the risk of cardiovascular disease [4].

The seeds of *Salvia hispanica* (chia) have high nutritional and functional characteristics. Its oil has predominantly unsaturated fatty acids, such as α-linolenic acid (64.4%) and linoleic acid (21.5%), and less than 10% of saturated fatty acids [5]. Chia seed oil has a low n-6/n-3 ratio, therefore intake of it could help to get the ratio between 5:1 and 9:1, in accordance with WHO/FAO (World Health Organization/Food and Agriculture Organization) [6] and EFSA (European Food Safety Authority Food) Panel on Dietetic Products and Allergies [7] recommendations to prevent the development

foods

MDPI

Article

Combined Effect of Chia, Quinoa and Amaranth Incorporation on the Physico-Chemical, Nutritional and Functional Quality of Fresh Bread

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Abstract: With regard to constant technological innovations in the bakery sector in order to increase bread nutritional value without affecting its technological and sensory characteristics, we applied pseudocereals/oilseeds to obtain an optimal formulation. A factorial design 3^3 was used and the independent factors were chia flour (levels: 0, 10, 20% flour basis), quinoa flour (levels: 0, 20, 40% flour basis), and amaranth flour (levels: 0, 20, 40% flour basis). Their effects and interactions were studied through the response surface methodology to optimise the bread formulation from a holistic viewpoint, which included the nutritional, technological and sensory characteristics. The optimum formulation with the highest quality was the blend made with 10, 4, and 20% of chia, quinoa, and amaranth, respectively. The results showed a significant increase in protein amount, ash, lipids, and crumb firmness compared to wheat bread. The calorie value of the control sample and the optimised formula were significantly similar, bearing in mind the high lipid amounts present in raw materials. Loaf-specific volume slightly decreased in comparison to control bread, as expected in formulations with gluten-free raw materials and a large amount of fibre. The optimised formula presented nutritionally/functionally higher indexes and similar overall acceptability to the control bread ($p < 0.05$).

Keywords: bread; *Salvia hispanica* L.; *Chenopodium quinoa* Willd; *Amaranthus caudatus*; technological characteristics; nutritional value

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1. Introduction

In the last few years, scientific studies have demonstrated that the regular intake of wholemeal or whole grain products prevents certain chronic diseases from developing, such as cardiovascular diseases, type 2 diabetes, and certain cancer types. Hence, consumer interest in such products has grown, although consumer acceptability is conditioned by their sensorial aspects despite being nutritional food with biological functionality in our organism [1,2]. Thanks to efforts to develop healthy and appealing bread products to supply nutritional, technological, and sensorial quality requirements, researchers have studied different strategies in order to develop products that use wholemeal flours with coadjuvants/additives that cover these requirements. These include adding baking enhancers, such as enzymes and/or chemical compounds [1,3,4], using wholemeal flours with different granulometries to increase sensorial quality [5–7], and/or partially replacing flour with more nutritional and healthy ingredients such as legumes [8–10], oilseeds [11–13], and pseudocereals [14–16]. The use of wholemeal flours of legumes, oilseeds, cereals, and pseudocereals increases their mineral content, but this increase comes with higher levels of phytic acid (InsP_6), forming insoluble compounds