FRACCIONAMIENTO DE QUINOA POR MOLIENDA SECA Y HÚMEDA PARA LA FORMULACIÓN DE ALIMENTOS CON ALTO VALOR AÑADIDO: ESTUDIO NUTRICIONAL Y EVALUACIÓN DE PROPIEDADES SALUDABLES

TESIS DOCTORAL

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CERTIFICAN QUE:

El Graduado en Ciencia y Tecnología de los Alimentos, D. Jaime Ballester Sánchez ha realizado el trabajo que tiene por título **"Fraccionamiento de quinoa por molienda seca y húmeda para la formulación de alimentos con alto valor añadido: Estudio nutricional y evaluación de propiedades saludables"**, que reúne los requisitos necesarios para optar al Grado de Doctor por la Universidad de Valencia y que ha dado lugar a un compendio de publicaciones que se detallan a continuación:

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- Development of Healthy, Nutritious Bakery Products by Incorporation of Quinoa. Foods, 2019, 8(9), 379.
- Effect of Incorporating White, Red or Black Quinoa Flours on Free and Bound Polyphenol Content, Antioxidant Activity and Colour of Bread. Plant Foods for Human Nutrition, 2019, 74, 185-191.
- Quinoa wet-milling: Effect of steeping conditions on starch recovery and quality. Food Hydrocolloids, 2019, 89, 837-843.
- Isolation of red quinoa fibre by wet and dry milling and application as a potential functional bakery ingredient. Food Hydrocolloids, 2020, 101, 105513. doi.org/10.1016/j.foodhyd.2019.105513

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"Hoy mismo, cuando Inglaterra ha querido hacer un gran diccionario de su lengua, no ha esperado a que naciese un Littré para consagrar su vida a esa labor. Ha llamado en su ayuda a los voluntarios, y mil personas se han ofrecido espontánea y gratuitamente para registrar las bibliotecas y terminar en pocos años un trabajo para el cual no habría bastado la vida entera de un hombre. En todas las ramas de la actividad inteligente aparece la misma tendencia, y sería preciso conocer muy poco la humanidad para no adivinar que el porvenir se anuncia en esas tentativas de trabajo colectivo en vez del trabajo individual. "

Piotr Kropotkin

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RESUMEN

La quinoa (*Chenopodium quinoa*) se considera un alimento completo por sus propiedades nutricionales y saludables atribuidas a su contenido en proteínas de alto valor biológico con una equilibrada composición en aminoácidos esenciales, aceite rico en omega-3, fibra dietética, minerales, vitaminas y compuestos bioactivos. Se ha relacionado la ingesta de alimentos ricos en estos compuestos con una menor incidencia del síndrome metabólico y por ende de patologías asociadas con este como la enfermedad cardiovascular ateroesclerótica y la diabetes tipo II. Por todo ello, existe la tendencia actual de impulsar la inclusión de la quinoa en la dieta a través de su consumo directo o para la formulación de alimentos. Hasta ahora, el uso de la quinoa se ha basado en la aplicación de granos enteros o molidos, sin embargo, no existen precedentes de la aplicación de sus co-productos a modo de ingredientes alimentarios debido a que todavía no existe un proceso tecnológico satisfactorio para su obtención. Por tanto, el aislamiento de los componentes individuales de la quinoa está todavía por explorar y supone una alternativa interesante para la industria alimentaria.

El objetivo principal de esta investigación fue la revalorización del grano de quinoa mediante su aprovechamiento integral a través de la aplicación de sus granos molidos o co-productos en el desarrollo de productos panarios sensorialmente aceptados y con impacto en la salud. Por una parte, se estudió desde el punto de vista tecno-funcional, nutricional y antioxidante el potencial de la harina integral de quinoa para tal fin y se caracterizaron los productos derivados de su inclusión. La sustitución de harina de trigo refinado por harina de Quinoa Real boliviana de variedades blanca, roja y negra generó ligeras mermas tecnológicas en los productos desarrollados, como la reducción del volumen de las piezas panarias y el correspondiente aumento de la firmeza de sus migas. Sin embargo, una mejora significativa en el perfil nutricional, contenido polifenólico y capacidad antioxidante, junto con una aceptación general por parte de los consumidores, fue concluyente para proponer el reemplazo parcial de harina por

quinoa como estrategia para la mejora nutricional en la fabricación de productos de panadería.

Además, se abordó el fraccionamiento de las diferentes partes anatómicas y/o componentes del grano de quinoa que pudieran ser utilizados como ingredientes con valor añadido en la industria alimentaria. Para ello, se adaptaron los procesos actualmente utilizados en la industria de primera transformación de los cereales, en concreto la industria de la molienda seca de trigo o la molienda húmeda de maíz. El proceso de molienda húmeda desarrollado permitió el aislamiento de los componentes principales del grano de quinoa. Se centró el interés en la fracción amilácea, por sus múltiples aplicaciones en la industria alimentaria, y también en la fracción de fibra por su clara asociación con la salud y porque su empleo en la industria alimentaria todavía supone un desafío tecnológico y sensorial. La caracterización físicoquímica de la fracción rica en almidón obtenida mostró un elevado porcentaje de recuperación y una destacable ausencia de lípidos que indicaron una eficiente separación del germen durante la molienda húmeda. A su vez, los estudios de tecno-funcionalidad en términos de propiedades térmicas y propiedades de pegado mostraron características diferentes a las descritas en la bibliografía para otros almidones y ofreciendo, por tanto, nuevas posibilidades de uso. En cuanto a la fracción de fibra obtenida, sus propiedades se compararon con las de la fracción resultante del proceso de molienda seca. La molienda húmeda mostró un mayor rendimiento y recuperación así como una fracción de fibra más pura. Ambas fracciones de fibra destacaron por su contenido en polifenoles y actividad antioxidante siendo ambos parámetros superiores en la fibra resultante de la molienda en seco. Se estudió el potencial de las fibras como ingrediente panario,los productos resultantes de su inclusión presentaron un enriquecimento en nutrientes, fibra dietética, contenido en polifenoles y capacidad antioxidante con respecto al pan control de trigo. Sin embargo, la molienda en húmedo generó una fracción de fibra con un tamaño de partícula inferior al de la molienda seca lo que dio lugar a productos con menor calidad. Aunque la presente investigación se ha centrado en la mejora de productos

panarios, los ingredientes obtenidos presentaron características que les confieren aplicabilidad en otras matrices alimentarias.

RESUM

La quinoa (*Chenopodium quinoa*) es considera un aliment complet per les seues propietats nutricionals i saludables atribuïdes al seu contingut en proteïnes d'alt valor biològic amb una equilibrada composició en aminoàcids essencials, oli ric en omega-3, fibra dietètica, minerals, vitamines i compostos bioactius. S'ha relacionat la ingesta d'aliments rics en aquests compostos amb una menor incidència de la síndrome metabòlica i per tant de patologies associades amb aquest com la malaltia cardiovascular ateroesclerótica i la diabetis tipus II. Per tot això, hi ha la tendència actual d'impulsar la inclusió de la quinoa en la dieta mitjançant el seu consum directe o per a la formulació d'aliments. Fins ara, l'ús de la quinoa s'ha basat en l'aplicació de grans sencers o mòlts, però, no existeixen precedents de l'aplicació dels seus co-productes com ingredients alimentaris a causa de que encara no hi ha un procés tecnològic satisfactori per la seua obtenció. Per tant, l'aïllament dels components individuals de la quinoa està encara per explorar i suposa una alternativa interessant per a la indústria alimentària.

L'objectiu principal d'aquesta investigació va ser la revaloració del gra de quinoa mitjançant el seu aprofitament integral a través de l'aplicació dels seus grans mòlts o co-productes en el desenvolupament de productes panaris sensorialment acceptats i amb impacte en la salut. D'una banda, es va estudiar des del punt de vista tecno-funcional, nutricional i antioxidant el potencial de la farina integral de quinoa per a tal fi i es van caracteritzar els productes derivats de la seua inclusió. La substitució de farina de blat refinat per farina de Quinoa Real boliviana de varietats blanca, vermella i negra va generar lleugeres minves tecnològiques en els productes desenvolupats, com la reducció del volum dels pans i el seu corresponent augment de la fermesa de les seues molles. No obstant això, una millora significativa en el perfil nutricional, contingut polifenòlic i capacitat antioxidant, juntament amb una acceptació general per part dels consumidors, va ser concloent per proposar la substitució parcial de

farina per quinoa com a estratègia per a la millora nutricional en la fabricació de productes de fleca.

A més, es va abordar el fraccionament de les diferents parts anatòmiques i / o components del gra de quinoa que puguin resultar ingredients amb valor afegit en la indústria alimentària. Per a això, es van adaptar els processos actualment utilitzats en la indústria de primera transformació dels cereals, en concret la indústria de la mòlta seca de blat o la mòlta humida de dacsa. El procés de mòlta humida desenvolupat va permetre l'aïllament dels components principals del gra de quinoa. Es va centrar l'interès en la fracció amilácea, per les seues múltiples aplicacions en la indústria alimentària, i també en la fracció de fibra per la seua clara associació amb la salut i perquè la seua ocupació en la indústria alimentària encara suposa un desafiament tecnològic i sensorial. La caracterització fisicoquímica de la fracció rica en midó obtinguda va mostrar un elevat percentatge de recuperació i una destacable absència de lípids que van indicar una eficient separació del germen durant la mòlta humida. Alhora, els estudis de tecno-funcionalitat en termes de propietats tèrmiques i propietats d'enganxat van mostrar característiques diferents a les descrites en la bibliografia per a altres midons i oferint, per tant, noves possibilitats d'ús. Pel que fa a la fracció de fibra obtinguda, les seues propietats es van comparar amb les de la fracció resultant del procés de mòlta seca. La mòlta humida va mostrar un major rendiment i recuperació així com una fracció de fibra més pura. Les dues fraccions de fibra van destacar pel seu contingut en polifenols i activitat antioxidant; ambdós paràmetres superiors a la fibra resultant de la mòlta en sec i es va estudiar el potencial com a ingredient per al pà. En els dos casos, els productes resultants van presentar un enriquiment en nutrients, fibra dietètica, contingut en polifenols i capacitat antioxidant pel que fa al pa control de blat. No obstant això, la mòlta en humit va generar una fracció de fibra amb una mida de partícula inferior al de la mòlta seca el que va produir productes amb menys qualitat. Tot i que la present recerca s'ha centrat en la millora de productes panaris, els ingredients obtinguts van presentar característiques que els confereixen aplicabilitat en altres matrius alimentàries.

ABSTRACT

Quinoa (*Chenopodium quinoa*) is considered a complete food because of its nutritional and healthy properties attributed to its content in proteins of high biological value with a balanced composition in essential amino acids, oil rich in omega-3, dietary fiber, minerals, vitamins and bioactive compounds. The intake of foods rich in these compounds has been associated with a lower incidence of metabolic syndrome and hence associated diseases such as atherosclerotic cardiovascular disease and type II diabetes. Therefore, there is a current trend to promote the inclusion of quinoa in the diet through direct consumption or for food formulation. Until now, the use of quinoa has been based on the application of whole or milled grains, but nevertheless, there are no precedents for the application of its co-products as food ingredients because there is still no satisfactory technological process for its obtention. Therefore, the isolation of the individual components of quinoa is still to be explored and is an interesting alternative for food industry.

The main objective of this research was the revaluation of the quinoa grain through its integral exploitation using its ground grains or co-products in the development of sensorially accepted bakery products with an impact on health. On the one hand, the potential of whole quinoa flour was studied from the techno-functional, nutritional and antioxidant point of view and the food products derived from its inclusion were characterized. The replacement of refined wheat flour by white, red and black varieties of bolivian Royal Quinoa generated slight technological losses in the developed products, such as the reduction of the volume of the bread pieces and the corresponding increase in the firmness of their crumbs. However, a significant improvement in the nutritional profile, polyphenolic content and antioxidant capacity, added to a general acceptance by consumers, was conclusive to propose the partial replacement of flour by quinoa as a strategy for nutritional improvement of bakery products manufacturing.

Moreover, the fractioning of the different anatomical parts and / or components of quinoa grain that could be ingredients with added value in food industry was address. For this, the processes currently used in the first cereal processing industry were adapted, in particular the wheat dry milling or maize wet milling industry. The developed wet milling process allowed the isolation of the main components of the quinoa grain. The focus was on the starch fraction, for its multiple applications in food industry, and also in the fibre fraction for its clear association with health and because its use in the food industry still means a technological and sensory challenge. The physicochemical characterization obtained from the starch-rich fraction showed a high percentage of recovery and a remarkable absence of lipids that indicated an efficient separation of the germ during wet milling. At the same time, techno-functionality studies in terms of thermal and pasting properties showed different characteristics to those described in the literature for other starches and, therefore, offering new use possibilities. Regarding the fibre fraction obtained, its properties were compared with those of the fraction resulting from the dry milling process. Wet milling showed greater yield and recovery as well as a purer fibre fraction. Both fibre fractions stood out for their content in polyphenol and antioxidant activity, both parameters being higher in the fibre resulting from dry milling. The potential of the fibres as a bakery ingredient was studied, the products resulting from its inclusion presented a nutrient enrichment, dietary fiber, polyphenol content and antioxidant capacity with respect to wheat control bread. However, wet milling generated a fraction of fiber with a smaller particle size than the one of dry milling, what produced products with lower quality. Although this research has focused on the improvement of bread products, the ingredients obtained presented characteristics that confer applicability in other food matrices.

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I. INTRODUCCIÓN

1. LA QUINOA

La quinoa (*Chenopodium quinoa)*, al igual que el amaranto (*Amaranthus* spp.) y el trigo sarraceno (*Fagopyrum* spp.), es un pseudocereal que se incluye en el grupo de los cereales debido a su elevado contenido en almidón y a la similitud de la composición química de sus granos con la de estos, aunque pertenecen a familias diferentes. La diferenciación entre estos dos grupos de plantas es clara en base al número de cotiledones, los cereales como el trigo (*Triticum* spp) o el arroz (*Oryza sativa*) son monocotiledóneas mientras que los pseudocereales son plantas dicotiledóneas. A pesar de ello, dada la similitud en composión recién mencionada entre los granos de pseudocereales y cereales, los usos de ambos son similares (Haros y Schoenlechner, 2017). Debido al alto valor nutritivo de sus semillas, su diversidad genética y su excelente adaptabilidad a diferentes ambientes, los pseudocereales se consideran cultivos que podrían contribuir a la seguridad alimentaria en todo el mundo. Una de sus características más destacadas es su composición química, que incluye un alto contenido en hidratos de carbono complejos, proteínas, lípidos, vitaminas, minerales y polifenoles, algunos de ellos con propiedades bioactivas (Haros y Schoenlechner, 2017).

1.1 Origen y situación actual de la producción de quinoa

La quinoa fue un grano ampliamente cultivado y consumido por las culturas precolombinas de las regiones andinas de Sudamérica (Bazile y col., 2014). Con la llegada de las colonizaciones europeas, el cultivo quedó relegado a poco más que un cultivo de subsistencia (Bazile y col., 2014). No fue hasta la década de los años

80, debido a la demanda de productos de origen vegetal, sin gluten y ricos en proteínas, mayoritariamente por parte de consumidores de América del Norte y Europa, cuando el cultivo resurge en los países tradicionalmente productores (Chevarria-Lazo y col., 2015). Posteriormente, la Asamblea General de las Naciones Unidas declaró el año 2013 como el "Año Internacional de la Quinoa", en reconocimiento a la tradición de los pueblos andinos por conservar el cultivo de este excepcional alimento para generaciones presentes y futuras (Bazile y Baudron, 2015). Y con el propósito de facilitar la generación de proyectos para el desarrollo sostenible del cultivo y consumo de la quinoa en el mundo, debido principalmente a su alta calidad nutricional y su capacidad de adaptabilidad al medio agroecológico (Bazile y Baudron, 2015). El notable incremento en la producción de quinoa en los principales países productores (Bolivia, Perú y Ecuador), llegando a una producción de más de 145 mil toneladas en 2017, se ha ido reduciendo debido a la extensión del cultivo a nivel mundial (FAOSTAT, 2017). Actualmente, la quinoa se produce en más de setenta países, entre ellos, Francia, Inglaterra, Suecia, Dinamarca, Holanda, Italia, Kenia, India, Estados Unidos y España (Jacobsen, 2001; Bazile y col., 2014; 2015).

En España, la mayor superficie de cultivo de quinoa se concentra principalmente en Andalucía, donde se están cultivando más de 2.000 ha anuales con un rendimiento medio entre 1.000 y 2.200 kg/ha en secano, pudiendo alcanzar los 4.000 kg/ha en regadío (Peláez, 2017). También se ha informado del cultivo de quinoa en Castilla y León con un rendimiento medio de 1.500 kg/ha (Calleja, 2017) y en Aragón comienza a producirse debido al creciente interés de los agricultores (V. M., 2018).

1.2 Estructura del grano

La quinoa produce semillas pequeñas, de forma circular, con diámetros que varían entre 1,0 y 2,6 mm y entre 250 y 500 semillas por gramo (Valencia-Chamorro, 2003; Vilche y col., 2003), con diversas coloraciones que varían desde el blanco al negro pasando por el amarillo, naranja, rojo y marrón, debido a su amplia diversidad genética (Saturni y col., 2010). Esta diversidad genética, también genera grandes diferencias en la composición química del grano (Miranda y col., 2012a; Vidueiros y col., 2015). Estructuralmente, las semillas de quinoa están compuestas por tres partes principales, que desde el interior a la parte externa de la semilla incluyen el perispermo, el embrión y la cubierta de la semilla (Fig. 1). El perispermo es la fracción mayoritaria en el grano de quinoa, cuyo principal componente es el almidón. Además, esta estructura contiene en menor proporción proteínas, lípidos, minerales y fibra (Ando y col., 2002; Czekus y col., 2019). El germen o embrión de la semilla es la parte reproductiva de la planta, está formado por dos cotiledones y la radícula, y constituye alrededor del 30% del volumen total de la semilla (Prego y col., 1998). Este está compuesto mayoritariamente por lípidos y proteínas (Ando y col., 2002; Czekus y col., 2019).
El pericarpio en la semilla de quinoa está compuesto mayoritariamente de fibra dietética y es la principal fuente de minerales como el K y el Ca. Además, diversos estudios han indicado un importante contenido de compuestos fenólicos en esta fracción (Gómez-Caravaca y col., 2014; Hemalatha y col., 2016); principalmente aquellos ligados a la fibra, conocidos como polifenoles no extraíbles o insolubles (Saura-Calixto, 2012).

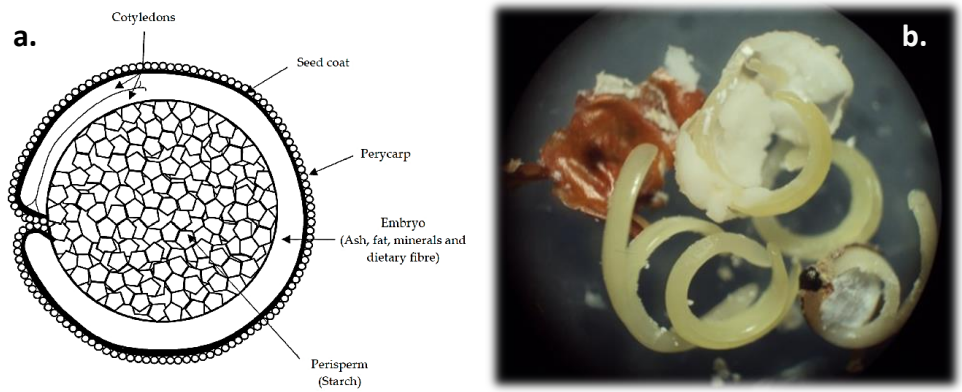

Fig. 1. a. Sección longitudinal de la semilla de quinoa. b. Semilla disgregada. Adaptado de Alonso-Miravalles y O'Mahony (2018).

El pericarpio es también la reserva de saponinas, causantes del sabor amargo presente en algunas variedades de quinoa (Ando y col., 2002; Abugoch, 2009).

1.3 Composición química de la quinoa

La composición química y nutricional de la quinoa es conocida (Vega-Gálvez y col., 2010). Las características generales de los principales componentes se detallan a continuación.

1.3.1 Proteínas

La quinoa tiene un alto contenido de proteínas en comparación con la mayoría de los cereales, pero es inferior que en las semillas oleaginosas y las leguminosas (Guzmán-Maldonado y Paredes-López, 1998). La proteína de la quinoa tiene una calidad similar a la de la caseína de la leche, debido a su excelente composición de aminoácidos (D´Amico y col., 2017). Es particularmente rica en histidina,

isoleucina, metionina y lisina en comparación con los cereales comunes, que generalmente son limitantes en lisina (Haros y Sanz-Penella, 2017). El elevado contenido de lisina se debe a la alta proporción de albuminas y globulinas, las cuales son las proteínas mayoritarias, unidas por puentes disulfuro (D´Amico y col., 2017; Czekus y col., 2019). De entre estas, las más abundantes son las del tipo 11S, también llamadas globular chenopodin, con un tamaño molecular de 50-60 kDa (Brinegar y Goundan, 1993), seguidas de las de tipo 2S albumin, las cuales son polipéptidos con un tamaño relativamente pequeño, sobre 9-10 kDa (Brinegar y col., 1996; Hager y col., 2012). El predominio de globulinas y albúminas en quinoa es tecnológicamente significativo ya que presentan propiedades espumantes, emulgentes y gelificantes, en algunos casos similares a las propiedades tecnofuncionales de las proteínas de soja o las caseínas (Janssen y col., 2017). El contenido de prolaminas en la semilla de quinoa es muy bajo (0.5-7.0%), libre de gluten, lo que lo convierte en un alimento o ingrediente idóneo para el diseño de alimentos para pacientes celíacos (Coulter y Lorenz, 1990; Javaid, 1997; Morita y col., 2001).

1.3.2 Fibra dietética

El contenido de fibra dietética de quinoa de variedades comerciales es similar al del grano entero de los cereales comunes. Sin embargo, se ha descrito una gran variabilidad en el contenido de fibra dietética entre los distintos cultivos de quinoa. Esto se debe no solo a las diferencias varietales, sino también a las diferencias en el proceso de eliminación de las saponinas y/o a las condiciones agroecológicas en su cultivo (Alvarez-Jubete y col., 2009; Reguera y col., 2018).

Del contenido total de fibra, casi el 80% corresponde a la fracción de fibra insoluble. Esta está compuesta principalmente por ácido galacturónico, arabinosa, galactosa, xilosa y glucosa (Lamothe y col., 2015). El otro 20% corresponde a la fibra soluble, valor ligeramente superior al informado en otros cereales como el trigo o el maíz (~15%) (Lamothe y col., 2015). La fibra soluble está compuesta principalmente de glucosa, ácido galacturónico, arabinosa y xilosa, composición que se asemeja más a la fibra de frutas y verduras que a la de los cereales (Lamothe y col., 2015). Otro de los carbohidratos presentes en la quinoa, y considerado parte de la fibra dietética es el almidón resistente. Por lo general el contenido de almidón resistente en el grano de quinoa es inferior (0,18%) que el reportado para trigo (0,39%) u otros pseudocereales como el amaranto (1,29%) (Linsberger-Martin y col., 2012). Sin embargo, el método de aislamiento del almidón asi como el tratamiento del grano puede modificar su contenido (Linsberger-Martin y col., 2012).

1.3.3 Minerales

Los minerales en el grano de quinoa se encuentran en concentraciones mayores a las observadas para la mayoría de los cultivos de cereales (Vega-Gálvez y col., 2010). Sin embargo, estos pueden variar de un cultivo a otro de la misma especie debido a diferencias en las condiciones agroecológicas, como el tipo de suelo y/o la aplicación de fertilizantes entre otras variables (Vega-Gálvez y col., 2010; Reguera y col., 2018). En concreto, en la bibliografía se encuentran valores variables en el contenido de Ca (32,9 - 148,7 mg/100g), Mg (207 - 250 mg/100g), Zn (1,8 – 4,4 mg/100g) y Fe (5,5 – 13,2 mg/100g), para semillas de quinoa

cultivadas en diferentes regiones (Reguera y Haros, 2017). Con respecto a la ubicación de los minerales en el grano de quinoa, mientras minerales como P, S y Mg se encuentran mayoritariamente en el embrión asociados o formando parte del ácido fítico, el K y Ca se distribuyen mayoritariamente en el pericarpio, asociados a la pectina de la pared celular (Konishi y col., 2004; Vega-Gálvez y col., 2010).

1.3.4 Almidón

En la quinoa, el contenido de almidón oscila entre 55,2 y 69,2% de la materia seca (Repo-Carrasco-Valencia y Arana, 2017). En general, el almidón presente en semillas tiene una estructura granular que presenta diversas formas y tamaños en función del origen (Valcárcel-Yamani y col., 2012). Los gránulos de almidón de quinoa tienen una forma poligonal con un diámetro de entre 1,5 a 3,0 µm, más pequeños que los gránulos de los cereales comunes (Koziol, 1992; Vega-Gálvez y col., 2010). El almidón es un polímero de moléculas de glucosa, que están unidas entre sí por enlaces α-1,4 y se ramifican a través de enlaces α-1,6 para formar amilosa y amilopectina. La amilosa es principalmente lineal con muy pocas ramificaciones, mientras que la amilopectina está altamente ramificada. En general, las ramificaciones de la amilopectina no ocurren al azar; están dispuestas en grupos, lo que permite la formación de dobles hélices. Estas hélices pueden empaquetarse juntas en láminas cristalinas organizadas, que están separadas por regiones amorfas, compuestas principalmente por amilosa. Esta organización de amilopectina y amilosa es la base de la estructura semicristalina del gránulo de almidón (Ball y col., 1998). El almidón de la mayoría

de plantas contiene entre 20-30% de amilosa, mientras que el contenido de amilosa informado para el almidón de quinoa se encuentra entre 7 y 27% (Repo-Carrasco y Arana, 2017). Esta variabilidad en el contenido de amilosa genera propiedades térmicas dispares, ya que existe una correlación positiva entre el contenido de amilosa y la temperatura de gelatinización. Así, se ha informado de temperaturas de gelatinización de almidón de quinoa relativamente bajas (57-71 °C) en comparación con otros almidones como los de maíz (85 °C) y similares al de trigo (65,0-67,5 °C) o patata (56,7-62,5 °C) (Ahamed y col., 1996; Lindeboom y col., 2005). Aunque el almidón de quinoa se gelatiniza a temperaturas similares al almidón de trigo, su comportamiento reológico es considerablemente diferente. A concentraciones iguales de almidón, el almidón de quinoa exhibe una mayor viscosidad en un amilógrafo Brabender (Atwell y col., 1983). Estas características, junto con el reducido tamaño de los gránulos de almidón de quinoa podrían tener aplicaciones interesantes en la industria alimentaria, como es el reemplazo de grasa, la formación de películas y/o recubrimientos biodegradables, así como también para otras industrias como la cosmética o farmacéutica (Ahamed y col., 1996; Ando y col., 2002).

Además de las características tecnológicas, el almidón de quinoa tiene interesantes propiedades desde el punto de vista de la salud, debido a que presenta bajo índice glucémico (Chaturvedi y col., 1997; Wolter y col., 2014; Fuentes y Paredes, 2015; Pereira y col., 2019). Esta característica hace de su harina un ingrediente potencialmente interesante para formular alimentos para personas con trastornos en el metabolismo de la glucosa, regímenes especiales e incluso en la dieta de individuos sanos (James y col., 2016). Sin embargo, se debe

tener en cuenta, que el proceso de obtención, las características y composición de los alimentos condicionan la respuesta glucémica más que el propio almidón (Atkinson y col., 2008; Reyes-Pérez y col., 2013). Además, debido a la gran diversidad genotípica, las variedades de quinoa son muy heterogéneas en cuanto a tamaño, forma y composición nutricional, lo cual podría traducirse en respuestas glucémicas particulares dependiendo de la especie e incluso condiciones agroecológicas.

1.3.5 Lípidos

Alrededor del 50% del contenido total de lípidos se ubican en el embrión y una menor cantidad se localiza en el perispermo (Ando y col., 2002). La quinoa contiene entre 4,4 y 8,8% de lípidos, con un alto grado de insaturación (Valcárcel-Yamani y col., 2012; Pereira y col., 2019). Esta variabilidad en el contenido de lípidos, al igual que en otros componentes del grano, se debe a diferencias de genotipo y condiciones agroecológicas (Miranda y col., 2012b). Del total de ácidos grasos presentes en la quinoa, solo entre un 11 y un 17% son ácidos grasos saturados, con predominio del ácido palmítico [16:0], seguido de pequeñas cantidades de ácido mirístico (14:0), esteárico (18:0), behénico [22:0] y lignocérico [24:0] (Valencia-Chamorro y col., 2017). Sin embargo, entre el 82,7 y 85,0% de los ácidos grasos presentes en el grano son insaturados, los principales son los ácidos oleico [18:1(9)], linoleico [18:2(9,12)] y α-linolénico [18:3(9, 12,15)], lo que sitúa a la quinoa como fuente de ácidos grasos esenciales (Vidueiros y col., 2015; Pereira y col., 2019). Si bien el contenido de lípidos puede variar entre los genotipos o cultivares de quinoa, no se han observado cambios

significativos en la composición de ácidos grasos entre diferentes variedades de quinoa (Tang y col., 2015; Valencia-Chamorro y col., 2017; Pereira y col., 2019). Los constituyentes principales de los lípidos neutros en la semilla de quinoa son triacilglicéridos (73,7%), seguidos de diglicéridos (20,5%) y monoglicéridos (3,1%). Los lípidos polares mayoritarios son el lisofosfatidiletanolamina seguido del fosfatidiletanolamina (Valencia-Chamorro y col., 2017).

1.3.6 Vitaminas

La quinoa es una buena fuente de vitaminas, especialmente de vitamina E, siendo el γ-tocoferol el vitámero más abundante (4,7-5,3 mg/100g) seguido del α-tocoferol (1,7-2,6 mg/100g) (Tang y col., 2015; Pereira y col., 2019). También contiene cantidades significativas de vitamina C y vitaminas del complejo B, en comparación con otros granos (Reguera y Haros, 2017). No obstante, su contenido en tiamina (vitamina B_1) (0,29-0,40 mg/100g) es similar al de otros granos como el arroz, la cebada, o el trigo y el de niacina (vitamina B_3) (1,24-1,60 mg/100g) es inferior (Reguera y Haros, 2017). Hay que tener en cuenta que los datos de concentración de vitaminas en quinoa podrían ser engañosos debido a que la quinoa es lavada o sometida a pulido (pelado abrasivo) para reducir su contenido en saponinas. Esta acción modifica el contenido de vitaminas presentes en el grano (Reguera y Haros, 2017).

1.3.7 Polifenoles

La quinoa contiene cantidades significativas de polifenoles (Hung, 2016). Los polifenoles son metabolitos secundarios de las plantas caracterizados por poseer una potente actividad antioxidante (Stevenson y Hurst, 2007). Diferentes estudios han probado la capacidad antioxidante y el contenido fenólico de la semilla de quinoa (Abderrahim y col., 2015; Tang y Tsao, 2017; Pellegrini y col., 2018; Lin y col., 2019). En la quinoa, al igual que en el resto de alimentos de origen vegetal, los polifenoles existen como formas solubles e insolubles (Pérez-Jiménez y Saura-Calixto, 2005; Pérez-Jiménez y col., 2013; Acosta-Estrada y col., 2014). Las formas solubles, también llamadas extraíbles, son compuestos de bajo o medio peso molecular que pueden extraerse empleando solventes acuosos o mezclas acuoso-orgánicas. En la quinoa, entre los polifenoles extraíbles identificados como mayoritarios se encuentran flavonoles, quercetina y kaemferol, todos ellos pertenecientes al grupo de los flavonoides, y ácidos fenólicos, principalmente ácido vanílico, ferúlico y sus derivados (Tang y Tsao, 2017). Por otro lado, las formas insolubles o no extraíbles, son compuestos de alto peso molecular (taninos condensados) o formas unidas a macromoléculas de la pared celular (polifenoles hidrolizables). En la quinoa se han identificado formas no hidrolizables siendo el ácido ferúlico y sus derivados las formas predominantes (Tang y Tsao, 2017), mientras que los taninos condensados no están presentes (Pérez-Jiménez y Saura-Calixto, 2005). Las formas no hidrolizables permanecen en los residuos de las extracciones acuosas y acuoso-orgánicas desde donde pueden liberarse mediante hidrólisis ácida o alcalina. La mayoría de los trabajos sobre polifenoles en la quinoa, hacen referencia a las

formas extraíbles ignorándose en estos casos cantidades significativas de polifenoles potencialmente bioactivos (Tang y Tsao, 2017). Este proceder es generalizado y se debe a que tradicionalmente se han menospreciado las formas no extraíbles ante la creencia de su baja o nula implicación en la salud y a pesar de que su contribución al contenido de polifenoles totales es habitualmente superior a la de las formas solubles (Pérez-Jiménez y col., 2013). Puesto que el consumo de alimentos incluye la ingesta tanto de las formas extraíbles como la de las no extraíbles, es importante considerar también a estos últimos y proporcionar datos globales que permitan estimar de forma más realista el potencial saludable de los mismos. Esto es importante ya que existen estudios que indican el papel beneficioso de los polifenoles no extraíbles en la salud (Acosta-Estrada y col., 2014). El contenido en polifenoles totales así como el tipo y concentración de compuestos específicos, difieren entre los diferentes ecotipos de la quinoa (Tang y col., 2015).

1.3.8 Ácido fítico

El ácido fítico (o hexakisfosfato de *mio*-inositol) es un ácido orgánico formado por un polialcohol cíclico de seis átomos de carbono que recibe el nombre de *mio*-inositol, donde cada residuo alcohol está fosforilado. Este compuesto cumple la función fisiológica de almacén de fósforo y cationes en plantas (Reddy y col., 1989). El contenido de fitatos en quinoa informado en la bibliografía varía ampliamente (9,3-20,3 µmoles de ácido fítico/g) debido fundamentalmente a factores agroecológicos como el tipo de suelo, fertilización, entre otros (Bohn y col., 2008; Reguera y col., 2018). La afinidad de los grupos fosfato de esta

molécula, cargados negativamente a pH fisiológico, por cationes metálicos como Ca, Mg, Zn, Cu y Fe, forma complejos insolubles que inhiben su biodisponibilidad en el tracto digestivo (Sanz-Penella y col., 2012; Tang y Tsao, 2017). Debido a esto, esta considerado un factor antinutricional, sin embargo, también se le ha atribuido efectos beneficiosos para la salud, actuando positivamente sobre enfermedades como la diabetes, ateroesclerosis y enfermedades coronarias (Schlemmer y col., 2009; Terán y col., 2015).

1.3.9 Otros antinutrientes

Uno de los factores que limitan la utilización de la quinoa es el sabor amargo causado por la presencia de saponinas (Ruales y Nair, 1993). Estos compuestos se han considerado antinutricionales, debido a que se ha demostrado que dañan las células de la mucosa intestinal, alterando la permeabilidad de la membrana celular e interfiriendo en el transporte activo (Schoenlechner y col., 2008). Sin embargo, existe un interés farmacológico en ellos debido a su capacidad para ayudar en la absorción de ciertos medicamentos (Vega-Gálvez y col., 2010) y a sus efectos hipocolesterolémicos (Schoenlechner y col., 2008). Las saponinas se ubican principalmente en las capas externas de la semilla de quinoa, lo que permite su eliminación a través de diferentes procesos como el lavado o perlado (Farfan y col., 1978; Taylor y Parker, 2002). Sin embargo, estos tratamientos podrían modificar la composición del grano, reduciendo el contenido de fibra dietética y/o minerales como se ha mencionado anteriormente (Alvarez-Jubete y col., 2009; Vidueiros y col., 2015).

Otros compuestos considerados antinutrientes, al igual que las saponinas y el ácido fítico, son los oxalatos y los inhibidores de proteasas (Lopes y col., 2009). Sin embargo, en nutrición humana, estos compuestos tienen pocas consecuencias, ya que son termolábiles y generalmente se destruyen en las condiciones normales de preparación de los alimentos (Khattab y Arntfield, 2009).

1.4 Efectos beneficiosos de la quinoa sobre la salud

La quinoa reúne excelentes propiedades que la convierten en un alimento con impacto beneficioso en la salud. La comprensión del papel de sus componentes principales en la salud así como la existencia de evidencias científicas que recomiendan su consumo han sido, y lo siguen siendo, fundamentales para impulsar su inclusión en la dieta. Así mismo, los avances científicos suponen un importante aliado para la industria alimentaria a la hora de conseguir la aceptación por parte del consumidor de productos innovadores a base de quinoa o elaborados con ingredientes de quinoa y sus derivados.

Como se acaba de comentar, la quinoa es rica en fibra, ácidos grasos poliinsaturados y antioxidantes. Se ha asociado la ingesta de alimentos ricos en estos compuestos con una menor prevalencia del síndrome metabólico (SM) (Sahyoun y col., 2006; Amiot y col., 2016). El SM es una alteración que combina una serie de factores de riesgo en una misma persona, en concreto la obesidad, la hipertensión, la hipercolesterolemia y desórdenes en el metabolismo de la glucosa (Panchal y Brown, 2011; McCraken y col., 2017). Tiene como consecuencias clínicas principales la enfermedad cardiovascular ateroesclerótica

(ECV) y la diabetes tipo II, pero también puede intervenir en el desarrollo de otras patologías como el hígado graso, la litiasis biliar, algunas formas de cáncer e incluso se ha llegado a asociar como factor de riesgo de procesos neurodegenerativos (Vanhanen y col., 2006; Razay y col., 2007; Pasinetti y Eberstein, 2008; Panchal y Brown, 2011; Luque-Contreras y col., 2014; McCracken y col., 2018). El SM representa un problema de salud pública a nivel mundial por su elevada prevalencia entre la población. Aunque existen aproximaciones terapéuticas para su tratamiento, las políticas sanitarias se inclinan cada vez más por aplicar estrategias preventivas, basadas en la actividad física y la dieta, para disminuir el impacto sobre los recursos sanitarios. La dieta juega un papel importante en el desarrollo del SM, sobre todo cuando se produce un desequilibrio energético derivado del aporte de grasas predominantemente animales y carbohidratos simples pobres en nutrientes, fibra y micronutrientes. En este sentido, existen evidencias científicas de los beneficiosos derivados de la ingesta de granos enteros en base a los niveles de biomarcadores de salud detectados en individuos con SM (Sahyoun y col., 2006). En este contexto, en el año 2005 la Unión Europea creó el programa HEALTHGRAIN, destinado a reducir la incidencia de enfermedades relacionadas con el SM y cuyo objetivo era incrementar la ingesta de granos enteros (Poutanen y col., 2008; 2010). Entendiéndose como grano entero de cereal y/o pseudocereal "el grano intacto, molido, partido, en copos o procesado de otra manera después de la eliminación de las partes no comestibles, como la cáscara. Todos los componentes anatómicos, incluidos el endospermo, el germen y el salvado, deben estar presentes en las mismas proporciones relativas que en el grano intacto" (AACC,

1999; HEALTHGRAIN, 2010; van der Kamp y col., 2014). La *American Association of Cereal Chemistry* (AACC), en el marco de la *Whole Grain Global Summit* (Newcastle, UK), en el año 2009, planteó la estrategia de incorporación del grano entero/harinas integrales en la dieta de los consumidores en proporciones paulatinas en productos formulados a base de cereales para una adaptación progresiva a los cambios sensoriales (Miller Jones, 2009).

En el caso concreto de la quinoa, existen datos científicos de efectos saludables derivados de su consumo y que apoyan las bondades que desde hace tiempo se le atribuyen en base a su composición. Las observaciones se han basado en estudios *in vitro*, preclínicos y clínicos (Simnadis y col., 2015; Graf y col., 2015) y en resumen indican el efecto de la quinoa sobre las disminución del estrés oxidativo, mejora del perfil de lípidos séricos, mejor control del peso corporal y sobre la glucosa sérica, ayudando a disminuir el riesgo de desarrollar una enfermedad cardiovascular o diabetes tipo 2 (Arneja y col., 2015; Bastidas y col., 2016).

La quinoa ejerce su papel beneficioso en la salud a través de la fibra, antioxidantes, ácidos grasos insaturados y otros compuestos. Se sabe que la ingesta de fibra dietética, está asociado a la reducción del riesgo del desarrollo de SM (Jensen y col., 2004; Santos-Marcos y col., 2019). La fibra dietética promueve beneficios que incluyen efectos laxantes y atenuación de los niveles del colesterol y/o glucosa en la sangre (Zarzuelo y Galisteo, 2007). La administración de una dieta suplementada con un 3% de pericarpio de quinoa (fracción rica en fibra) alivió la hipercolesterolemia inducida en ratones (Konishi y col., 2000).

En la quinoa se encuentran compuestos polifenólicos caracterizados por su alta actividad antioxidante y por tanto con capacidad para contrarrestar el estrés oxidativo producido cuando se altera el balance entre la producción de radicales libres y los mecanismos antioxidantes de autodefensa que dispone el organismo (Báez-Duarte y col., 2016). Se ha visto una elevación del estrés oxidativo en procesos, como la obesidad o la resistencia a la insulina, relacionados con el desencadenamiento del SM (Skyler y col., 2017; McCracken y col., 2018) y podría ser en parte la causa de su desarrollo (Skyler y col., 2017). Por tanto, cualquier acción que suponga una disminución del estatus oxidativo es interesante. En el caso de la quinoa, los estudios *in vivo* de sus efectos antioxidantes en modelos experimentales se han basado en la detección del incremento de la actividad de enzimas antioxidantes (glutatión peroxidasa, catalasa o superóxido dismutasa), marcadores de daño oxidativo (malondialdehído), así como en la reducción de la peroxidación lipídica en plasma y órganos (Matsuo, 2005; Pasko y col., 2010). Se ha descrito que los polifenoles de la quinoa poseen, además de propiedades antioxidantes, propiedades antiinflamatorias en células Caco-2 y capacidad de reducir la ganancia de peso en un modelo murino de obesidad (Noratto y col., 2015).

La quinoa también puede ejercer efectos beneficiosos a través de los ácidos grasos mono y poliinsaturados presentes en su composición (Belitz y Grosh, 1997; Haros y Schoenlechner, 2017). Se ha descrito que la sustitución de ácidos grasos saturados (SFA) por ácidos grasos poliinsaturados (PUFA) y/o ácidos grasos monoinsaturados (MUFA) en la ingesta contribuye a disminuir la concentración del colesterol LDL y la relación colesterol total/colesterol HDL, y

por tanto el riesgo de padecer alguna enfermedad cardiovascular (FAO, 2008; Field, 2003; Elmadfa y Kornsteiner, 2009). La incorporación de quinoa en la dieta de animales de experimentación tuvo un claro efecto hipocolesterolémico e hipolipidémico (Pasko, 2010; Takao y col., 2005). Este efecto podría deberse a los ácidos grasos insaturados presentes en la quinoa, como se ha descrito para otros granos (Ryan y col., 2007), pero también parecen intervenir otros compuestos como proteínas (Takao y col., 2005) o fibra como se ha comentado anteriormente.

2 PROCESAMIENTO DE QUINOA PARA LA OBTENCIÓN DE INGREDIENTES Y ALIMENTOS

El grano de quinoa, con alta calidad nutricional y fuente de compuestos bioactivos, puede ser utilizado de igual forma que los cereales. Sin embargo, algunos de estos nutrientes y compuestos bioactivos de la quinoa se encuentran concentrados en partes anatómicas específicas del grano. El estudio del fraccionamiento del grano de quinoa se podría abordar en principio con la adaptación de los procesos actualmente utilizados en la industria de primera transformación de los cereales, en concreto la industria de la molienda seca de trigo o la molienda húmeda de maíz. Posteriormente, los productos intermedios de la primera transformación de quinoa se podrían utilizar para la elaboración de productos de la industria de la segunda transformación.

2.1 Industria de primera transformación del grano de quinoa

Las principales industrias de primera transformación de los cereales son: la industria harinera, cuya principal materia prima es el trigo blando, la industria semolera, que usa trigo duro, y en menor medida la industria molinera de otros cereales. La industria de molienda húmeda es otra industria de primera transformación de gran importancia, que utiliza principalmente maíz para la obtención de almidón como materia prima para la producción de almidones modificados, jarabes de glucosa, etanol, entre otros. Otras industrias como la arrocera o maltera también pertenecen a la industria de primera transformación de cereales, sin embargo el principal objetivo de estas no es el fraccionamiento del grano ni la reducción de su tamaño.

Los pseudocereales tienen una composición similar a los cereales por lo que permiten ser procesados de forma similar a estos. Sin embargo, las proporciones relativas de los tres componentes del grano (embrión, endospermo/perispermo, pericarpio) varían entre los diferentes cereales y pseudocereales (Baltensperger, 2003). El objetivo de la molienda es obtener productos intermedios que puedan utilizarse posteriormente en la fabricación de productos a base de cereales o pseudocereales tales como harinas, sémolas, semolinas, salvado, germen, entre otros. Los dos principales métodos de fraccionamiento de cereales son la molienda seca y la molienda húmeda. El objetivo de la molienda en seco es separar las partes anatómicas del grano (endospermo, germen y pericarpio), mientras que el de la molienda húmeda es separar los componentes químicos (almidón, proteína, fibra y lípidos) (Haros y Wronkowska, 2017). Por tanto, conocer cómo se distribuyen los componentes en las distintas estructuras del

grano, así como las características de estos, es fundamental para un fraccionamiento efectivo durante el proceso de molienda. Existen diferentes métodos por los cuales la quinoa ha sido fraccionada, sin embargo, todavía es necesaria una investigación intensa sobre la aplicación de los procesos de molienda seca y húmeda en busca de un fraccionamiento con alta recuperación y pureza de sus partes anatómicas o componentes químicos, respectivamente (Haros y Schoenlechner, 2017).

2.1.1 Molienda seca

El trigo es el principal cereal que se fracciona por molienda seca, y en menor medida el centeno, la espelta o el maíz. Previo a la molturación, y tras la recepción y limpieza del grano de trigo, es conveniente realizar un acondicionado del mismo. Por lo general, el acondicionado consiste en adicionar una determinada cantidad de agua al cereal en función de la temperatura y humedad inicial con el fin de mejorar su comportamiento tecnológico en la fase de molienda. Después del mojado del cereal y tras ser homogeneizado, este deberá permanecer un tiempo en reposo. La cantidad de agua que se añade, así como el tiempo de reposo posterior, varían dependiendo del contenido en humedad de partida y de la dureza del grano (McKevith, 2004, Kweon y col., 2009).

De esta manera la cubierta del grano se hace más flexible y facilita su separación en grandes trozos, obteniéndose harinas más puras (McKevith, 2004, Kweon y col., 2009). Además, al incrementar la humedad del endospermo se mejora la trituración y la compresión de la sémola, facilitando el cernido y aumentando el rendimiento en harina con una disminución del gasto de energía necesaria para

el proceso. La molturación del trigo se realiza con dos tipos de molinos de rodillos: en molinos de trituración o estriados, y molinos de reducción de partículas o lisos. La separación y clasificación de las partículas se realiza con tamizadores y purificadores. El propósito de la molienda es la separación de los componentes del endospermo del embrión y la cubierta de la semilla, además de la reducción del tamaño del endospermo en harina (*Triticum aestivum*) o sémola (*Triticum durum*). La molienda seca también se utiliza para la producción de harinas integrales a partir de los granos enteros. En el caso de la quinoa, en los países de origen, se han utilizado tradicionalmente molinos de piedra y de martillos a nivel rural, así como molinos de disco a nivel industrial, para la producción de harina integral (Meyhuay, 2013). Sin embargo, debido al reducido tamaño del grano de quinoa y a las diferencias morfológicas y estructurales con los cereales, los sistemas de fraccionamiento y/o separación típicamente utilizados en trigo, maíz o arroz son poco eficientes (Haros y Wronkowska, 2017).

Harina integral de quinoa

Se denomina harina integral de quinoa al producto resultante del proceso de trituración de los granos de quinoa enteros, debiendo contener todos los componentes de la semilla de origen. La harina integral de quinoa se puede emplear casi en todos los productos de la industria de segunda transformación (pan, galletas, pasta, bebidas, etc.). Sin embargo, la sustitución de harina de trigo por harina integral de quinoa provoca desafíos tecnológicos y sensoriales en los productos elaborados. Así, se ha investigado la adición de hasta 40 % de harina de quinoa en formulaciones de pan, 40 % en pastas, 60 % en bizcochos y 70 % en

galletas (Mujica y Jacobsen, 2006). Sin embargo, estudios realizados más recientemente, sugieren un mejor comportamiento tecnológico y sensorial de productos elaborados con 20% de harina de quinoa y 80% de harina de trigo (Reynaga y col., 2013).

La posibilidad de usar la quinoa para obtener harinas integrales o fracciones de sus componentes ha generado interés en el ámbito de la investigación y la industria debido a su valor nutricional/funcional y sus interesantes propiedades tecnológicas.

Fraccionamiento en seco

Diversas metodologías han sido utilizadas para el fraccionamiento en seco de granos de quinoa, sin embargo, el uso de un molino de rodillos se ha informado como el método idóneo (Taylor y Parker, 2002). Chauhan y col. (1992) usaron un molino de rodillos de laboratorio para reducir la quinoa en fracciones de salvado y harina (Fig. 2).

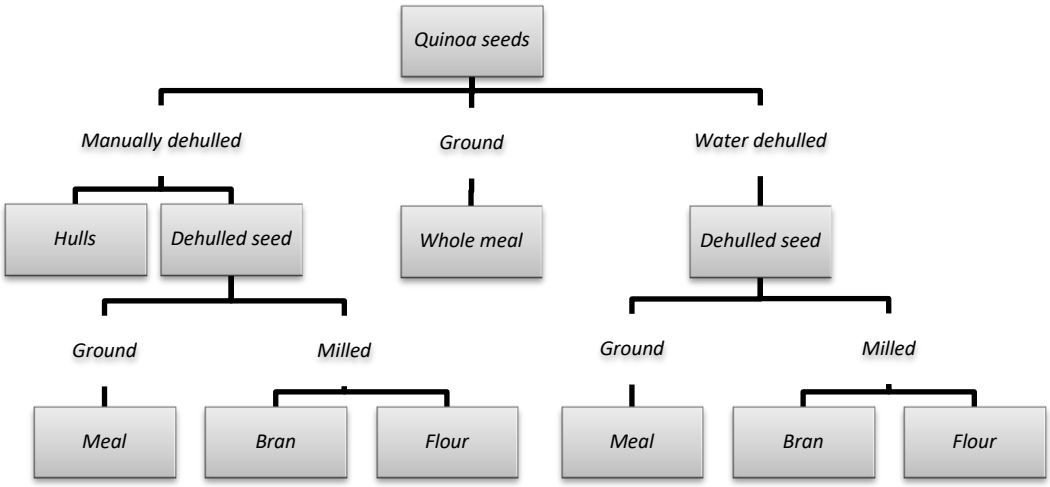

Fig. 2. Separación de las fracciones del grano de quinoa mediante molino de rodillos a escala laboratorio. Adaptado de Chautan y col. (1992).

En este estudio, la fracción de salvado presentó una alta proporción de proteínas (20,0–24,3%) y también de lípidos (11,0–13,2%), mientras que la fracción de harina resultó rica en almidón (73,8%) y pobre en proteínas (6,5%). Opazo-Naverrete y col. (2018) sometieron a molienda y posteriormente a tamizado, semillas de quinoa utilizando diferentes tamaños de tamices para obtener fracciones enriquecidas en proteínas y almidón con una pureza de ∼32% y 86–89% respectivamente. El uso de molinos de abrasión también ha sido utilizado para el fraccionamiento de granos de quinoa en sus partes anatómicas: salvado, embrión y perispermo (Ando y col., 2002), así como para el estudio de la distribución de los nutrientes en el grano (D´Amico y col., 2019). Sin embargo, la presencia de otros componentes del grano contaminando las diferentes fracciones, mostró un deficiente grado de pureza de estas. No obstante, como se

ha explicado con anterioridad, si la pretensión es la obtención de los componentes del grano con la mayor pureza posible, la principal vía utilizada es la molienda húmeda.

2.1.2 Molienda húmeda

El proceso de molienda húmeda comenzó en el siglo XIX, y fue diseñado para el aislamiento de almidón de maíz, debido a su bajo precio y a su adaptabilidad a diferentes procesos industriales, desde la industria alimentaria pasando por la industria farmacéutica o papelera (Haros y Wronkowska, 2017). Hoy en día, el maíz continúa siendo uno de los cereales más utilizados para ser fraccionado por molienda húmeda, pero esta se ha adaptado a otros granos como el trigo o el arroz.

La molienda en húmedo es un proceso más complejo que la molienda en seco y es fuente de una gran variedad de productos. Aunque el producto principal de la molienda húmeda es el almidón, otros subproductos de interés son las fracciones ricas en fibra y la fracción rica en proteínas, ambas utilizadas para alimentación animal y reciben el nombre de *gluten-feed* y *gluten-meal*, respectivamente, a pesar de la carencia de gluten de este cereal (Haros y Wronkowska, 2017). También se separa el germen, del cual se extrae el aceite (Haros y Wronkowska, 2017).

Los métodos para simular la molienda húmeda de maíz consisten en primer lugar en la maceración del grano en presencia de una solución acuosa de SO_2 o álcali, tal como se realiza a nivel industrial (Fig. 3). Durante la maceración, la solución acuosa difunde hacia el interior del grano, lo que provoca un ablandamiento tal,

que permite la desintegración del grano simplemente frotando con los dedos. Posteriormente, se realiza el paso por el molino de discos estriados, que permitirá la separación del germen por flotación, debido a su menor densidad por alto contenido lipídico. La suspensión resultante se vuelve a moler para pulverizar las partículas de endospermo mientras se deja intacto el material fibroso. La suspensión se filtra a través de una serie de tamices con tamaño de malla decreciente, lo que permite que pasen la suspensión de almidón y proteína, reteniéndose la fracción de fibra. Los métodos para separar almidón y proteína se basan generalmente en la diferencia en su densidad o en su granulometría diferente. Se usan métodos de centrifugación para separarlos, porque la densidad de los gránulos de almidón es superior al de la fracción de proteína. Debido a que el proceso de molienda en húmedo es relativamente complejo, el rendimiento y la calidad de las fracciones se ven afectados por diversos factores, como son el efecto de la variedad empleada, el tratamiento postcosecha (secado y almacenamiento) y las condiciones de maceración (temperatura, tiempo, concentración de SO_2 o álcali, pH, agitación, entre otras variables operativas) (Haros y Wronkowska, 2017). También existen otros criterios para valorar la adecuación del grano a la molienda húmeda, como son los criterios tecnológicos: comportamiento del grano durante el macerado, la susceptibilidad a la rotura (almidón dañado), la degerminación eficiente y, sobre todo, la buena separación de proteínas y almidón (Singh y Eckhoff, 1996).

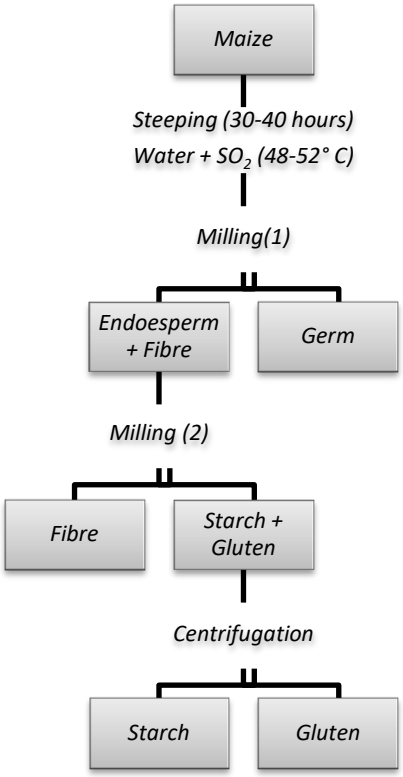

Fig. 3. Diagrama de flujo de la molienda húmeda para la obtención de almidón de maíz. Adaptado de Daniel y Whistler (2000).

El proceso de molienda húmeda se diseñó originalmente para producir almidón para uso industrial y para alimentos, hoy en día, el objetivo es lograr una separación máxima de cada fracción del grano con la máxima pureza y eficiencia. La tendencia en las últimas décadas se ha centrado en la búsqueda de fuentes no convencionales como alternativa para obtener almidones que presenten diversas propiedades fisicoquímicas, estructurales y funcionales que amplíen las posibilidades de uso en la industria en general (Hernández-Medina y col., 2008). En este sentido, la quinoa podría ser utilizada como nueva fuente de este

polímero. Además, la calidad de su fracción proteica, así como el saludable perfil de ácidos grasos de sus lípidos localizados principalmente en el germen, genera un valor añadido de estas fracciones, permitiendo su uso más allá de la alimentación animal, como ocurre con el maíz. Diversas son las investigaciones que han conseguido el aislamiento del almidón de quinoa, y en general, a escala de laboratorio. Atwell y col. (1983) y Lorenz (1990) aislaron el almidón de la quinoa mediante molienda en húmedo tras macerar las semillas en tampón de acetato a pH 6,5 y separando la proteína por centrifugación. Metodologías similares, pero con maceraciones en solución de hidróxido de sodio, fueron estudiadas por varios investigadores (Qian y Kuhn, 1999; Wright y col., 2002; Jan y col., 2017). Ligarda Samanez y col. (2012) mostraron que la utilización de soluciones acuosas neutras es más eficiente para separar la fracción de fibra dietética de la quinoa en comparación al método alcalino. La separación de la fracción proteica de quinoa se estudió mediante hidrólisis y precipitación enzimática (Fig. 4) o alcalina (Fig. 5) (Scanlin y col., 2009; Kruger, 2012; Pouvreau y col., 2014).

Fig. 4. Diagrama de flujo de la molienda húmeda de quinoa adaptado de Pouvreau y col. (2014).

Más recientemente, Mufari y col. (2018) propusieron un método en húmedo para la separación del germen del perispermo del grano de quinoa utilizando un molino de rodillos y tamices, obteniendo una fracción enriquecida del germen con una recuperación del 96.5% de las proteínas y del 95.8% de los lípidos.

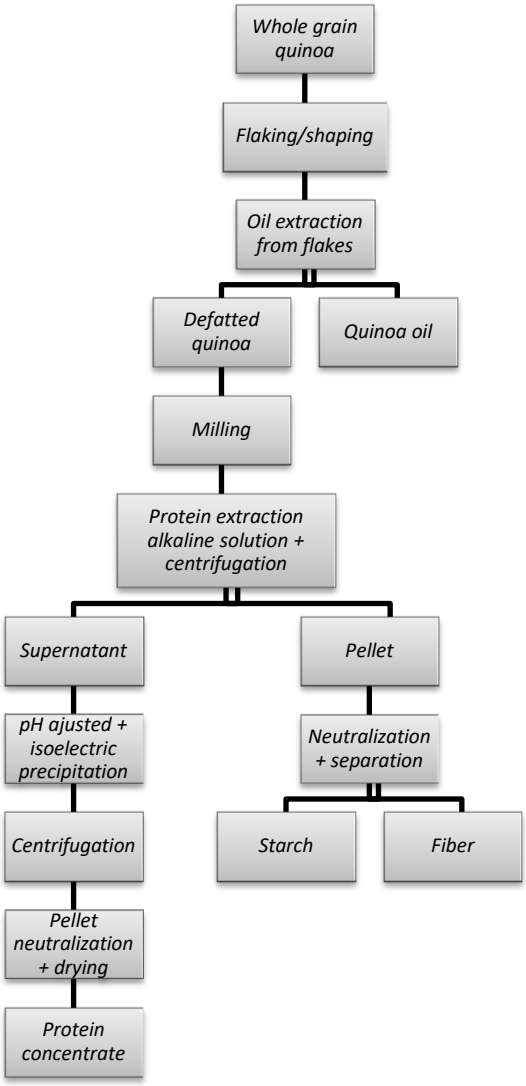

Fig. 5. Diagrama de flujo del proceso de molienda húmeda de quinoa por Scanlin y col. (2009).

Sin embargo, la mayoría de las fracciones aisladas presentes en la bibliografía han sido obtenidas con el objetivo de ser caracterizadas sin tener en cuenta el desarrollo de metodologías que pudieran ser transferibles al sector industrial.

Esto se debe a que algunos métodos de extracción investigados utilizan productos químicos y/o procedimientos de laboratorio que generalmente no son de uso alimentario. Además, las técnicas de investigación que se centran en el aislamiento concreto de un compuesto pueden degradar y/o modificar otros compuestos como el almidón, la fibra y/o el aceite, lo que no sería viable para fines comerciales.

2.1.3 Combinación molienda seca-húmeda

La combinación del fraccionamiento en seco y húmedo fue investigado para obtener fracciones ricas en proteínas de la quinoa (Ávila Ruiz y col., 2016). En este método se molió el grano en seco y se separó el embrión en corriente de aire. El resto del grano se sometió nuevamente a molienda y la harina resultante se resuspendió en solución 0,5 M de NaCl y se ultrafiltró dando como resultado una fracción rica en proteínas con una pureza de 59,4% (Ávila Ruizy col., 2016). Otro estudio hallado en la bibliografía que combinó la molienda seca y húmeda de quinoa fue el descrito por Hemalatha y col. (2016). La molienda de los granos se realizó con un molino abrasivo para el descascarillado de arroz, lo que resultó en tres fracciones: cascarilla, salvado y grano molido (Fig. 6). Sin embargo, el objetivo de este trabajo fue el estudio de la distribución de los compuestos fenólicos, por lo que el rendimiento y pureza de las fracciones pudieran no ser interesantes desde el punto de vista de la industria.

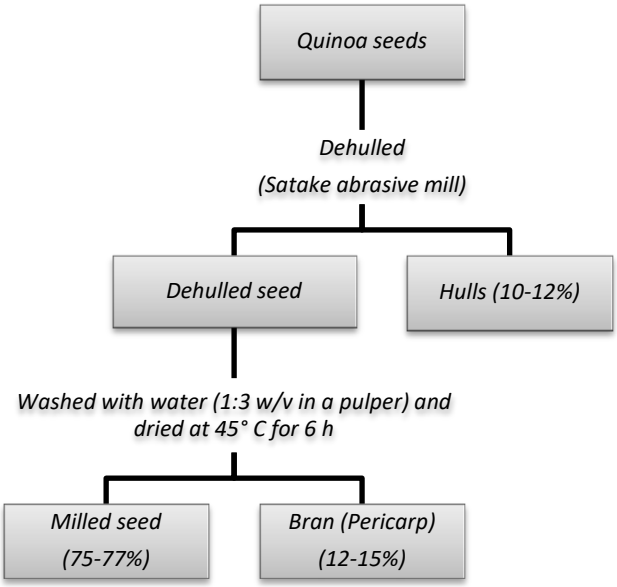

Fig. 6. Diagrama de flujo de la molienda y separación de fracciones de quinoa por Hemalatha y col. (2016).

2.2 Productos de segunda transformación. Usos y aplicaciones industriales de la quinoa y sus derivados.

Dentro de los productos de segunda transformación de los cereales figuran todos los elaborados por la industria de piensos, la aceitera, la industria de preparados para desayuno y aperitivos, la de pastas alimenticias, la industria cervecera y como no, la industria panadera. Como se comentó con anterioridad, la posibilidad de uso de la quinoa es similar a la de los cereales, por lo que cualquier producto de segunda transformación de los cereales es susceptible, en principio, de ser elaborado con quinoa. Tradicionalmente, la quinoa se ha utilizado en una amplia variedad de alimentos. La semilla entera se utiliza en la

elaboración de caldos, sopas y guisos, con un uso similar al del arroz. La harina se ha usado para hacer gachas y en la elaboración de pan. La quinoa también se puede fermentar para hacer una bebida alcohólica llamada chicha (Taylor y Parker, 2002). En la actualidad, los principales usos de la quinoa son para cocinar, en productos alimenticios procesados como el pan, los cereales para el desayuno, pasta, fideos, bebidas y galletas, y también en alimentación animal.
En general, en productos alimenticios, la quinoa se emplea en gran medida como complemento de las harinas de trigo, maíz y arroz debido a su alta calidad de proteínas y a la ausencia de gluten (Haros y Sanz-Penella, 2017).

2.2.1 Productos de panadería

El pan es un alimento básico que se consume en todo el mundo y tiene un papel importante en la nutrición humana. Los métodos actuales de procesamiento de cereales se han optimizado para ofrecer productos elaborados con harinas refinadas. El pan preparado a partir de harina refinada es pobre desde el punto de vista nutricional y no cumple adecuadamente los requisitos nutricionales de muchos macro o micronutrientes (Skrbic y Filipcev, 2008). Debido a esto y sumado a la creciente demanda de la sociedad moderna hacia hábitos dietéticos que incluyan alimentos más sanos y sabrosos, numerosas investigaciones dirigen sus esfuerzos en mejorar el valor nutritivo de los productos de panadería sin afectar su aceptabilidad. Por ejemplo, se ha sugerido incluir salvado, cereales enteros, harinas de pseudocereales, o mezclas de diferentes granos para aumentar el valor nutricional de los productos elaborados con harina de trigo refinada (Sanz-Penella y col., 2008; Miller Jones, 2009; Swieca y col., 2014; Xu y

col., 2019). En este sentido, diversos estudios han utilizado la quinoa en productos panarios como sustituto parcial de la harina de trigo refinada (Lorenz y Coulter, 1991; Swieca y col., 2014; Xu y col., 2019) obteniendo resultados aceptables con o sin adición de enzimas como lipasas (Morita y col., 2001; Park y col., 2005) y/o fitasas exógenas (Iglesias-Puig y col., 2015). Sin embargo, la incorporación de harinas enteras de pseudocereales, especialmente en grandes cantidades, genera desafíos tecnológicos. Se han descrito cambios a nivel tecnológico como: menor tolerancia a la fermentación, menor volumen de la pieza panaria, migas más firmes y menos elásticas y diversos cambios de coloración y sabor, dependiendo del pseudocereal y/o tipo de pan, debido principalmente a la incorporación de fibra y la dilución del gluten (Sanz-Penella y col., 2012; Poutanen y col., 2014; EL-Sohaimy y col., 2019; Xu y col., 2019). Aun así, diversos estudios han demostrado que formulaciones panarias con sustitución de hasta un 20-30% de quinoa reciben buena aceptación global en pruebas de análisis sensorial (Iglesias-Puig y col., 2015; EL-Sohaimy y col., 2019). La incorporación de semillas enteras de quinoa en los productos desarrollados fueron percibidos como más sanos que la muestra control y con buen sabor, por lo que mostraron potencial comercial (Calderelli y col., 2010). Además, algunos de los compuestos bioactivos presentes en la planta y el grano de quinoa permanecen casi inalterados tras los procesos de cocción y horneado, como es el caso del contenido fenólico y su correspondiente capacidad antioxidante (Brend y col., 2012; Gawlik-Dziki y col., 2015).

Conocer las características nutricionales, tecnológicas y funcionales de estos productos, puede ayudar a la mejora en el desarrollo de nuevos alimentos que

contribuyan a alcanzar los requerimientos nutricionales de macro y micronutrientes establecidos y por ende, tengan un mayor impacto sobre la salud del consumidor.

2.2.2 Pastas

Las pastas son productos alimenticios obtenidos del amasado y moldeado de mezclas no fermentadas de harina de trigo con agua (Mujica y Jacobsen, 2006). La pasta italiana tradicional se elabora con harina de sémola obtenida por molienda seca del trigo duro, tal y como se mencionó anteriormente. Los pseudocereales, y entre ellos la quinoa, también se han propuesto como una fuente alternativa para aumentar la calidad nutricional y funcional de la pasta, como en el caso de los productos de panadería. En este sentido, la elaboración de pastas con diferentes porcentajes de sustitución con harina de quinoa ha sido estudiada con resultados muy prometedores en cuanto a textura, sabor y aceptabilidad general (Lorenz y col., 1993; Schoenlechner y col., 2010; Burgos y col., 2019). La quinoa también puede suponer una alternativa válida para la preparación de pastas libres de gluten (Reynaga y col., 2013; Sosa y col., 2019). El alto valor nutricional de sus semillas hace de la quinoa un ingrediente interesante para la generación de productos típicamente italianos con un mayor valor añadido.

2.2.3 Bebidas

Debido a su alto valor nutricional y funcional, la incorporación de quinoa en la elaboración de bebidas resulta atractivo para los consumidores y una respuesta a grupos poblacionales con requerimientos específicos (Casarotti y col., 2014). Pineli y col. (2015) elaboraron una bebida de quinoa no fermentada que proporciona una alternativa novedosa como sustituto de la leche, y que tiene un mayor contenido de proteínas y un índice glucémico bajo en relación a otro tipo de bebidas vegetales basadas en otros granos como el arroz o la soja.

Como se comentó con anterioridad, tradicionalmente, en Sudamérica se elabora una bebida fermentada (Chicha), similar a la cerveza. De hecho, la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) enumera la quinoa como 1 de los 11 cereales/pseudocereales de interés para la industria cervecera (Meussdoerffer y Zarnkow, 2009). En este sentido, diversos trabajos han estudiado el comportamiento de la quinoa con y sin maltear en la elaboración de cerveza (Dezelak y col., 2014; Bogdan y Kordialik-Bogacka, 2017; Kordialik-Bogacka y col., 2018).

2.2.4 Usos del almidón

El uso particular de un almidón va a depender de sus características fisicoquímicas, principalmente en la variabilidad del contenido de amilosa/amilopectina y de su peso molecular, entre otras. Generalmente, las características deseadas en un almidón son la estabilidad a altas temperaturas, estabilidad al esfuerzo de cizalla, claridad de la pasta y la estabilidad al congelado y descongelado (Zobel, 1988; Taggart, 2004). Se ha descrito que el almidón de

quinoa tiene una capacidad de absorción de agua y poder de hinchamiento superior que el almidón de trigo y cebada, por lo que se muestra como un buen espesante para rellenos o salsas y como sustituto de grasas (Lindeboom y col., 2004). También es muy estable al congelado y descongelado, debido a su bajo contenido en amilosa (Coulter y Lorenz, 1990; Ahamed y col., 1996) y podría ofrecer una alternativa interesante para sustituir almidones modificados químicamente (Repo-Carrasco y col., 2003). Además, el reducido tamaño del gránulo de almidón de quinoa permite su aplicación como ingrediente en la producción de películas biodegradables. Las películas de almidón de quinoa muestran propiedades mecánicas mejores que las de almidón de maíz (Ahamed y col., 1996; Lindeboom, 2005). Otra aplicación, debido al pequeño tamaño de su gránulo, es en la industria papelera (Wilhelm y col., 1998; Lindeboom, 2005), como material de soporte en la industria cosmética (Whistler, 1995), así como en la industria textil y fotográfica (Biliaderis y col., 1993; Lindeboom y col., 2004).

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II. OBJETIVOS

El objetivo principal de esta investigación es la revalorización del grano de quinoa mediante la obtención de nuevos ingredientes y su integración en la dieta a través de su inclusión en el desarrollo de nuevos productos sensorialmente aceptados y con impacto en la salud. Para ello, se han abordado nuevas estrategias de molienda para la obtención de productos intermedios ofreciendo una alternativa para la industria alimentaria en general y la industria panadera en particular.

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

1. Desarrollar productos de panadería de alta calidad tecnológica y sensorial con propiedades nutricionales y saludables mejoradas por inclusión de harina integral de quinoa.
   1A. Evaluar el perfil proteico, las características reológicas y las propiedades térmicas de la harina integral de tres variedades de quinoa real como ingrediente panario y estudiar la calidad tecno-funcional y sensorial de los productos desarrollados.
   1B. Investigar el impacto de la incorporación de harina integral de tres variedades de quinoa real en formulaciones panarias sobre el perfil nutricional de los productos desarrollados.
   1C. Estudiar el contenido fenólico y la capacidad antioxidante de la harina integral de tres variedades de quinoa real y su aporte como ingrediente panario en los productos elaborados.
2. Adaptar los métodos de molienda húmeda de maíz y molienda seca de trigo para abordar el fraccionamiento de granos de quinoa.

2A. Desarrollar y optimizar un proceso de molienda húmeda de quinoa teniendo en cuenta las variables operativas utilizadas en la industria de la molienda húmeda de maíz. Investigar cómo afectan las variables de maceración del grano (tiempo y temperatura) al rendimiento y calidad de la fracción rica en almidón y la fracción rica en fibra.

2B. Evaluar la eficiencia de separación del salvado de la harina de quinoa por adaptación del proceso de molienda seca de trigo.

2C. Estudiar las propiedades fisicoquímicas y antioxidantes de la fracción rica en fibra de quinoa real roja obtenida por molienda húmeda y seca, y su potencial para ser utilizadas como ingrediente panario.

2D. Investigar el impacto de la incorporación de las fracciones de fibra obtenidas por molienda húmeda y seca sobre las características tecno-funcionales, perfil nutricional y propiedades antioxidantes de los productos panarios desarrollados.

III. RESULTADOS Y DISCUSIÓN

PARTE 1. Desarrollo de productos de panadería de alta calidad tecnológica y sensorial con propiedades nutricionales y saludables mejoradas por inclusión de harina integral de quinoa.

CAPÍTULO 1

Rheological and thermal properties of royal quinoa and wheat flour blends for breadmaking

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ABSTRACT

The increasing interest in quinoa in Europe has generated a large number of studies with this seed as a partial substitute for refined wheat flour in bakery products as a strategy to improve their nutritional value. However, the wide genetic diversity of this seed offers very different compositions in different varieties, which would lead to different technological behaviours in the breadmaking process. The aim of this work was to make a comparative study of the protein profile and rheological and thermal properties of three varieties of quinoa widely available commercially in Europe to study their technological potential as breadmaking ingredients with 25% replacement of wheat flour by whole quinoa flour. The results obtained during the analysis offered a view of the proteins present in the various quinoas, and of the processes of hydrolysis and generation of new bonds between wheat and quinoa proteins during the breadmaking process. The changes in the thermal and pasting properties of the bread doughs that included whole quinoa flour led to the development of baked products with different physico-chemical and textural properties, producing an increase on crumb staling. However, replacement of 25% of the wheat flour with whole quinoa flour produced only a slight decrease in the technological quality of the products. A significant increase ($p < 0.05$) in dietary fibre, minerals, lipids, and proteins in comparison with a whole wheat product, together with the overall consumer acceptance of the products that were developed, was conclusive for proposing replacement with quinoa flour as a strategy for nutritional improvement in the manufacture of bakery products.

INTRODUCTION

Bread is one of the most common foods made with cereals in the world. However, the main cereal used for breadmaking is flour obtained by dry milling of wheat grain, which removes valuable nutrients and bioactive compounds (Haros and Sanz-Penella, 2017). Whole cereal and pseudocereal flours can be included in bakery products as a strategy to improve their nutritional profile without needing to use whole products completely (Sanz-Penella et al., 2012; Garcia-Mantra et al., 2014; Iglesias-Puig et al., 2015). Among the pseudocereals, quinoa (*Chenopodium quinoa*) is a dicotyledon originally from South America, although, because of its adaptation characteristics and wide genetic diversity, it is now grown in nearly every continent in the world, including Europe (Ruiz, 2013). Because its composition is similar to that of cereals, it has a suitable balance of carbohydrates, proteins, lipids, and minerals, and it can be sold without restrictions in Europe in accordance with Regulation (EU) 2015/2283, which means that a large number of varieties are marketed in countries of the European Union, all of which has created increasing interest in society. Moreover, unlike wheat, which contains gluten-forming proteins (gliadins and glutenins), the main proteins in quinoa are albumins and globulins, bound together by disulfide bridges (D´Amico et al., 2017). The most abundant of these proteins is of type 11S, also known as globular chenopodin, with a molecular size of 50–60 kDa (Brinegar and Goudan, 1993), followed by those of type 2S albumin, which are polypeptides of a relatively small size, about 9–10 kDa (Brinegar et al., 1996; Hager et al., 2012). The predominance of globulins and albumins in quinoa is technologically significant, because they have foaming,

emulsifying, and gelling properties, which in some cases are similar to the techno-functional properties of soya or casein proteins (Janssen et al., 2012).

Various studies show that the incorporation of whole quinoa flour in bread formulations causes technological changes produced by the dilution of gluten, inclusion of fibre, and/or lipids, or its starch characteristics (Park et al., 2005; Haros and Sanz-Penella, 2017). However, marked differences between varieties have been reported in recent years, regarding their chemical composition and physical properties, size of starch granules and amylose/amylopectin ratio, polyphenol content and antioxidant capacity, among other things (Lindeboom et al., 2005; Aluwi et al., 2017; Reguera and Haros, 2017; Ballester-Sánchez et al., 2019).

Accordingly, the aim of this work was to make a comparative study of the protein profile and rheological and thermal properties of three varieties of quinoa widely available commercially in Europe to study their technological potential as breadmaking ingredients with 25% replacement of wheat flour by whole quinoa flour.

MATERIALS AND METHODS

Materials

Three types of commercial Bolivian quinoa seeds (*Chenopodium quinoa*) grown by members of ANAPQUI (La Paz, Bolivia) were purchased from Ekologikoak (Ondarroa-Bizkaia, Spain). Organic “quinoa real” (royal quinoa) (white, red, and black) was used to produce flour in a mill (Aromatic, Taurus, Oliana, Spain). The chemical composition of the white, red, and black quinoa flours according to the labelling was: 12.0, 11.0, and 11.2 g/100 g of moisture; 64.0, 56.7, and

57.2 g/100 g of carbohydrates; 6.0, 5.4, and 5.1 g/100 g of lipids; 4.0, 11.8, and 12.8 g/100 g of fibre; and 14.0, 15.1, and 13.7 g/100 g of proteins, respectively. Dehydrated yeast (*Saccharomyces cerevisiae*, Maizena, Spain) was used as starter for the breadmaking process. Commercial strong wheat flour (Carrefour, Madrid, Spain) was used for the bread formulation. The chemical composition of the wheat flour was: 12.6 g/100 g of moisture; 71 g/100 g of carbohydrates; 1.4 g/100 g of lipids; 3 g/100 g of fibre; and 12 g/100 g of proteins.

Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), dehydrated yeast (1.0 g/100 g flour basis), sodium chloride (1.6 g/100 g flour basis), and distilled water (70.8 g/100 g flour basis). Whole quinoa flour was incorporated in the bread dough formula at 25 g/100 g on flour basis. The breadmaking procedure was performed in a breadmaker (BM 3989, Severin, Germany). The process variables consisted of the following steps: (a) kneading phase and rising phase for 9 min and 20 min, respectively; (b) kneading phase and rising phase for 14 min and 20 min, respectively; short stirring for 30 s; (c) rising phase for 4 min and 30 s; and (d) rising phase for 45 min, and lastly baking for 60 min. The breads obtained were cooled at room temperature for 2 h for subsequent analysis. The breadmaking process was performed in triplicate.

Chemical composition

Moisture content was determined by an official assay procedure (AOAC, 1996). Starch content was measured by an enzymatic procedure according to Method

996.11 (AOAC, 1996). Protein determination was carried out by the Dumas Combustion method (N conversion factor 5.7) according to ISO/TS 16634-2 (2016). Lipid content was extracted with petroleum ether under reflux conditions by the Soxhlet technique (AACC, 1995), whereas ash content was determined in a muffle furnace by incineration at 900 °C (AACC, 1995). The dietary fibre content was measured by an enzymatic and gravimetric method (AOAC, 1996). The analyses were performed in triplicate.

Technological parameters

The technological parameters analysed were as follows: the height of the bread piece (cm) and the texture profile analysis using the TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) with a 35 mm flat-end aluminium compression disc (Gámbaro et al., 2004). Each parameter was measured at least in triplicate in crumb of fresh bread and after 24 and 48 h of storage at room temperature in polyethylene bags. The experiments were conducted in triplicate.

Digital image analysis was used to measure the bread crumb structure. Images were taken at 600 pixels per cm with a scanner (HP Scanjet G2410, Hewlett Packard, USA) supported by HP Photosmart Essential 3.5 software. Data were processed using Fiji Image J (version 1.49q, National Institute of Health, USA) and NIS-Elements (Basic Research version, Nikon Instruments Inc., Amsterdam). The analysis was performed in triplicate.

Preliminary sensory analysis of the fresh breads was performed by a panel of 50 untrained tasters who usually consume bread, using a nine-point hedonic scale

of overall acceptance (9. like extremely; 8. like very much; 7. like moderately; 6. like slightly; 5. neither like nor dislike; 4. dislike slightly; 3. dislike moderately; 2. dislike very much; 1. dislike extremely).

Protein profile

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method was performed, based on the original procedure of Laemmli (1970) modified by Fu and Sapirstein (1996). To obtain equal concentrations of proteins, the quinoa flours, wheat flour, wheat bread, and wheat and quinoa bread samples were weighed on the basis of their dry weight protein contents and mixed with 1 mL of sample buffer solution (pH 6.8) containing 0.063 mol/L Tris–HCl, 2% (w/v) SDS, 20% (w/v) glycerol (Merck, Germany), and 0.01% (w/v) Pyronine Y (Sigma–Aldrich, USA). The reduced samples were prepared using 7% (v/v) β-mercaptoethanol (2-ME, Sigma–Aldrich, USA) included in sample buffer. The blend was vortexed (Reax Top model, Heidolph, Germany) for 1 min every 10 min during 2 h. Extracted and dissolved samples were heated in a dry block heating thermostat (Bio TDB-120 model, BIOSAN, Latvia) for 3 min to denature proteins before analysing, and then applied (10 µL) to the SDS-PAGE, which was carried out in a cooled slab gel unit (Protean II xi Cell, Bio-Rad, CA, USA). The acrylamide concentrations of resolving gel and stacking gel were 12.5% and 5%, respectively. After concluding the electrophoresis, the gels were rinsed in rinsing solution [57% (v/v) water + 33% (v/v) methanol + 10% (v/v) trichloroacetic acid (100% w/v)] overnight to remove excess SDS from the surface of the gels. Then, the gels were stained overnight with Coomassie Brilliant Blue G-250 (Merck,

Darmstadt, Germany) according to Ng and Bushuk (1987). Apparent molecular weights were determined using wide-range molecular weight protein markers (S8445, Sigma, MO, USA) as standards. The determination of the molecular weights of the protein bands in the quinoa flours, wheat flour, wheat bread, and wheat and quinoa breads was carried out using the Bio-Rad Image Lab 5.0 software after scanning from the gel imager (ChemiDoc MP Imaging System, Bio-Rad, USA).

Differential scanning calorimetry (DSC)

The thermal properties of the raw materials and during baking of the fermented dough as well as the amylopectin retrogradation induced during the bread storage were measured on a differential scanning calorimeter (DSC-7, PerkinElmer) according to the methodology described by Iglesias-Puig et al. (2015) with modifications. The calorimeter was calibrated with indium (enthalpy of fusion 28.4 J/g and melting point 156.4 °C). Flours were weighed into DSC pans and mixed with Milli-Q water to obtain a water: flour ratio of 3:1. Samples were scanned at a rate of 10°C/min from 25 °C to 110 °C. Fermented dough samples (30–40 mg) were weighed directly in DSC stainless steel pans (LVC 0319-0218, PerkinElmer). After sealing, the pans were kept at 25 °C for 1 min, scanned at a rate of 10°C/min from 25 °C to 110 °C, kept at this temperature for 5 min, and cooled to 25 °C at 50°C/min. Afterwards, the pans were stored at 4 °C for 24 and 48 h and heated again in the calorimeter from 25 to 130 °C at 10°C/min to analyse amylopectin retrogradation. An empty pan was used as a reference, and three replicates of each sample were analysed. The parameters recorded were

onset (To), peak (Tp), and conclusion (Tc) temperatures of gelatinization and retrogradation transitions. The starch gelatinization and amylopectin retrogradation (ΔHG and ΔHR, respectively) were calculated as the area enclosed between the straight line and the endotherm curve between To and Tc. They were expressed in joules per gram of starch and the experiments were conducted in triplicate.

Rapid visco analyser (RVA)

The pasting properties of samples were measured using a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriewood, Australia) according to AACC Method 76 – 21 (AACC, 1995). Distilled water (25 mL) was added to 3.0–3.5 g of sample placed into the aluminium RVA canister. The suspensions were stirred thoroughly at 160 rpm. The temperature was first maintained at 50 °C for 1 min to obtain a uniform temperature and then raised to 95 °C at a rate of 12°C/min, held at 95 °C for 2.5 min, cooled to 50 °C at a rate of 12°C/min, and finally held at 50 °C for 2 min. Pasting parameters evaluated included: pasting temperature (Ptemp), peak viscosity (PV), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV–HPV), and setback (CPV – HPV). The RVA experiments were conducted in triplicate.

Statistical analysis

The data generated were analysed by ANOVA using SPSS Statistics Version 22 (International Business Machines Corporation, USA). Fisher's least significant

difference (LSD) test was used to determine statistically significant differences ($p < 0.05$) between mean values for different samples, at a 95% confidence level.

RESULTS AND DISCUSSION

SDS-PAGE protein profiles in reduced and unreduced forms

Total extractable proteins of whole quinoa flours, wheat flour, wheat bread, and wheat and quinoa breads in reduced form are shown in Fig. 1. There were a few differences among the protein patterns of the quinoa flours, such as a noticeable protein band with a molecular weight (MW) of 102 kDa in white quinoa flour, whereas red quinoa flour and black quinoa flour did not have this protein band (Lanes 1, 2, and 3); there was also a clear protein band with 38 kDa MW (Lane 3). Otherwise, the protein band profiles of the quinoa flours were generally very similar in reduced form (Fig. 1). The main protein fractions in quinoa grain are albumins and globulins (chenopodin) which are stabilized by disulfide bonds. The globulins, also called chenopodin or 11S-type proteins, consist of two subunits which are acidic subunits (30–40 kDa MW) and basic subunits (20–25 kDa MW). Lower MW (8–11 kDa) proteins of quinoa grain are called 2S-type proteins (Brinegar and Goudan, 1993; Brinegar et al., 1996; Abugoch et al., 2008; Abugoch et al., 2009). These proteins are also indicated in Figs. 1 and 2. The effects of the breadmaking process on quinoa flour proteins were also investigated in reduced form. The composition of individual proteins in the quinoa flours was significantly modified during both fermentation and baking processes.

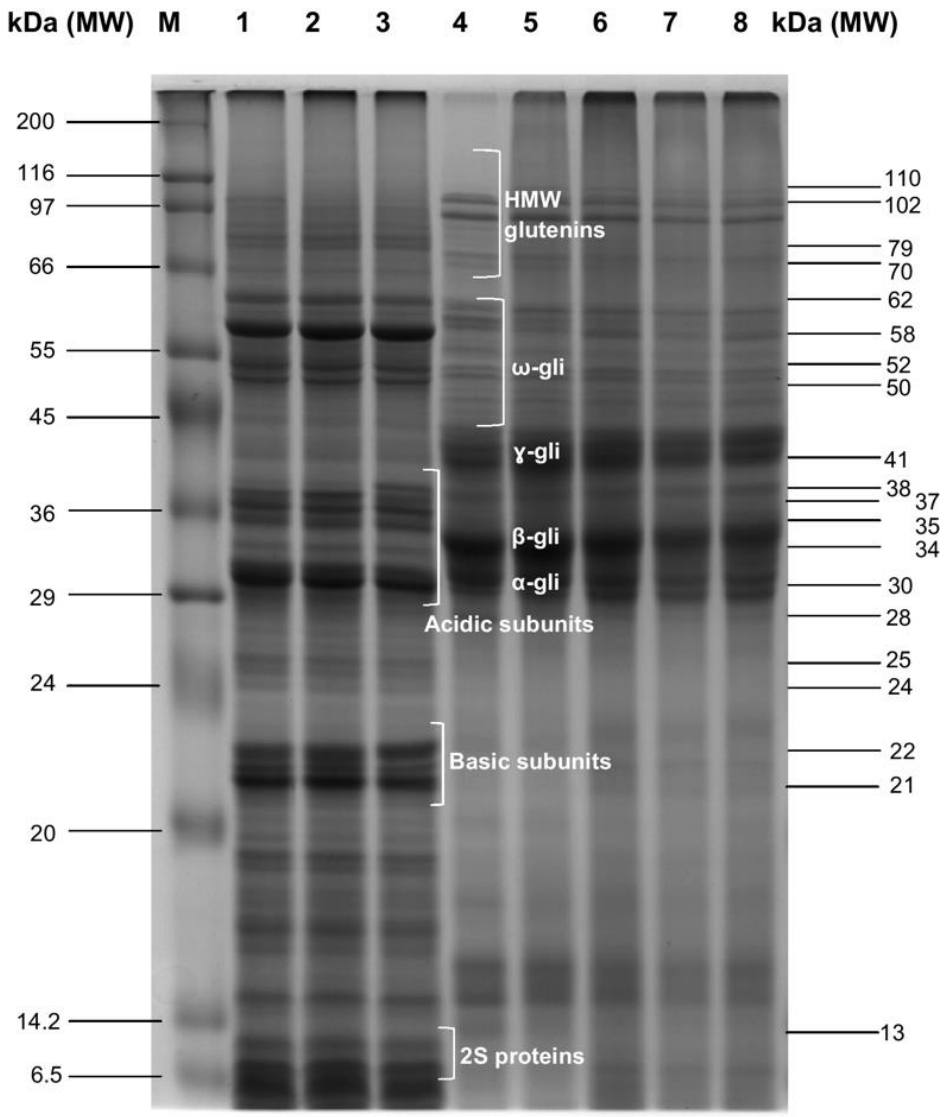

Fig. 1. SDS-PAGE patterns of the total extractable proteins of quinoa flours and wheat and quinoa bread samples. All samples were reduced with 7% β-mercaptoethanol. Lane M wide-range protein markers (Sigma S8445). Lane 1 white quinoa flour. 2 Red quinoa flour. 3 Black quinoa flour. 4 Wheat flour. 5 Wheat bread. 6 Wheat bread with white quinoa. 7 Wheat bread with red quinoa. 8 Wheat bread with black quinoa. MW molecular weight

It was found that, during the breadmaking process, the mixing, fermentation, and baking processes caused some changes in quinoa flour proteins, such as protein hydrolysis by proteases that caused breaking of proteins (Horszwald et al., 2009) or disulfide formation through oxidation causing polymerization of proteins which could not enter into the gel. These changes are mainly responsible for the flavour during the fermentation and baking stages (Martínez-Anaya, 1996; Hansen and Schieberle, 2005). Ingredients notably influence aromatic compounds, and flours usually have distinct aromatic characteristics (Di Renzo et al., 2018). In contrast, a small number of protein bands were observed in wheat and quinoa bread samples when compared with those found in the corresponding flours. In all the quinoa flours, a double protein band around 79 kDa MW seemed to be hydrolysed and then smaller fragments may have been polymerized with other wheat proteins (Lanes 1, 2, and 3; Lanes 6, 7, and 8). The intensities of the protein bands with MW of 50, 52, 58, and 62 kDa decreased considerably after the breadmaking process (Lanes 1, 2, and 3; Lanes 6, 7, and 8). These protein bands might be hydrolysed and then polymerized with wheat proteins, and conclusively an intense protein band around 41 kDa MW appeared in wheat and quinoa bread samples (Lanes 6, 7, and 8). Similarly, the protein bands at 35 and 37 kDa in the quinoa flours were hydrolysed via protease attack and then accumulated as a protein band at 34 kDa that appeared very intensely on gel. In addition, the intensity of the binary protein band around 30 kDa in the quinoa flours (Lanes 1, 2, and 3) decreased substantially after the breadmaking process (Lanes 6, 7, and 8). The protein bands located below 25 kDa MW in all the quinoa flours also did not appear after the breadmaking

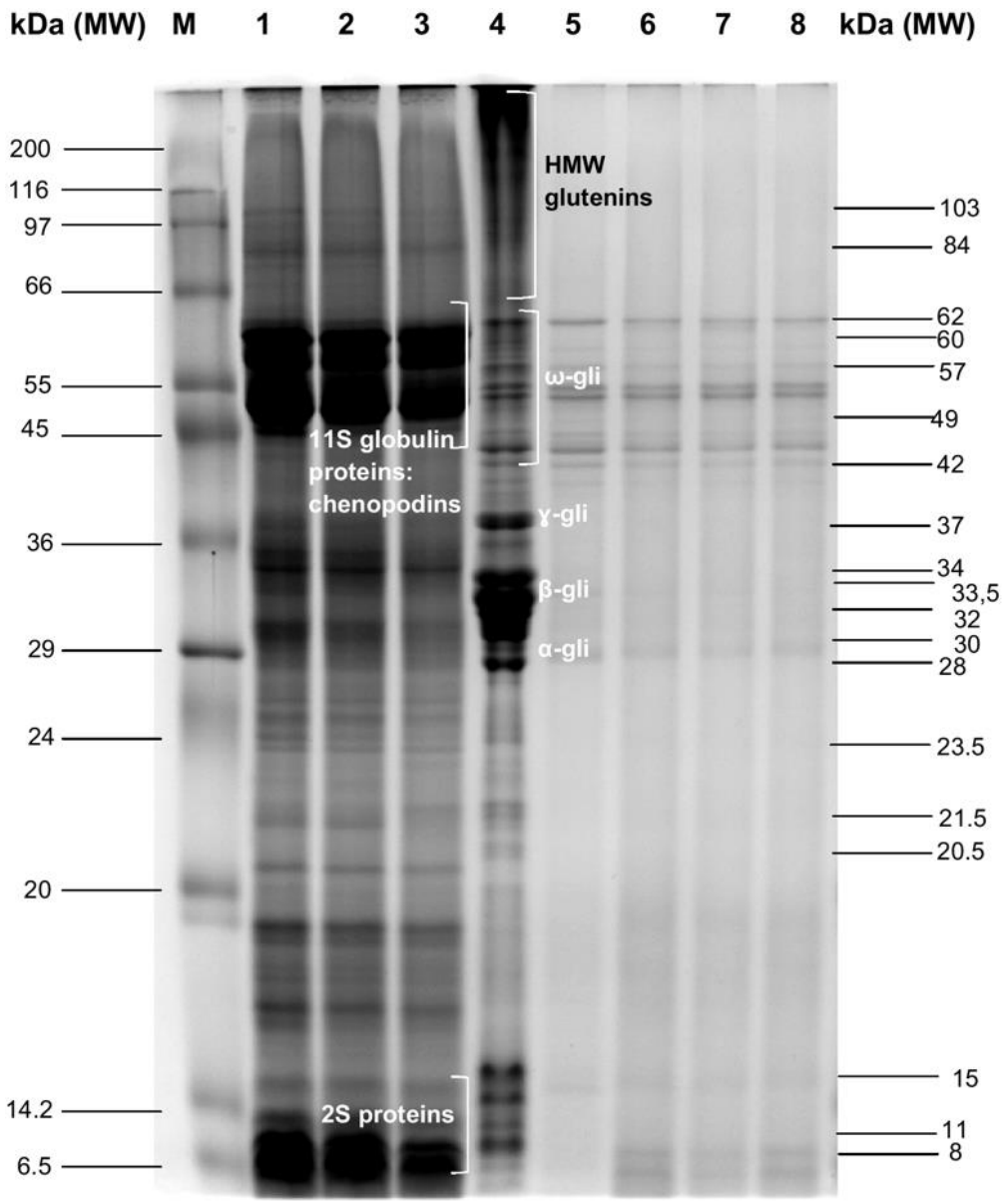

Fig. 2. SDS-PAGE patterns of the total extractable proteins of quinoa flours and wheat and quinoa bread samples prepared without using any reducing agent. Lane M wide-range protein markers (Sigma S8445). Lane 1 white quinoa flour. 2 Red quinoa flour. 3 Black quinoa flour. 4 Wheat flour. 5 Wheat bread. 6 Wheat bread with white quinoa. 7 Wheat bread with red quinoa. 8 Wheat bread with black quinoa. MW molecular weight

process, owing to protein hydrolysis or polymerization with higher MW wheat proteins.A protein band that did not appear in the protein profile of wheat flour (Lane 4) was detected at 110 kDa MW in the profile of wheat bread (Lane 5). The protein bands detected in wheat flour at 13, 28, and 58 kDa did not appear after breadmaking owing to protein hydrolysis and subsequent polymerization with other wheat proteins by formation of cross-linking via disulfide linkages.

Total extractable proteins of quinoa flours, wheat flour, wheat bread, and wheat and quinoa bread samples were investigated without using reducing agent (2-ME), and the SDS-PAGE results of the unreduced samples are shown in Fig. 2. The protein patterns of the quinoa flours in unreduced form were generally found to be similar (Lanes 1, 2, and 3). However, some changes were observed that were due to varietal differences in the quinoa flours. For example, white quinoa flour and red quinoa flour had a thin protein band at 103 kDa MW, whereas the black quinoa flour did not have this protein band in the unreduced form (Lanes 1, 2, and 3). Similarly, intense protein bands between 34 and 37 kDa MW were observed in the white quinoa flour and red quinoa flour, but these protein bands were not detected in the black quinoa flour. Furthermore, protein bands around 21.5 kDa and 30 kDa were detected in the white and red quinoa flours, but were not detected in the black quinoa flour. Double protein bands around 84 kDa in the white quinoa flour were also not detected in the red and black quinoa flours in unreduced form (Fig. 2).

After the breadmaking process, a few faint bands of proteins were detected in the wheat bread and wheat and quinoa breads in unreduced form (Fig. 2). The higher MW protein bands above 49 kDa in the quinoa flours did not appear in

unreduced form, probably owing to protein polymerization, because they could not enter into the gel. The intense protein bands at 49, 57, and 60 kDa MW were probably hydrolysed by proteases or may have been polymerized with other proteins, and finally, they did not appear on gel after breadmaking. Similarly, the protein bands between 30 and 37 kDa MW and the protein bands lower than 29 kDa MW did not appear on gel in unreduced form after breadmaking (Lanes 6, 7, and 8; Fig. 2). When the protein profiles of the wheat flour and its bread were examined (Lanes 4 and 5 in Fig. 2), it was seen that the intensities of the protein bands between 42 and 62 kDa decreased after breadmaking. In addition, the intensity of the protein band at 28 kDa decreased in unreduced form as well (Lanes 4 and 5).

The results presented in Fig. 1 indicated that during thermal processing, owing to Maillard and protein cross-linking reactions, the structure of the dough proteins might have changed. This could cause formation of aggregates or protein cross-linking through the formation of disulfide bonds, resulting in the creation of high MW insoluble proteins. Since MWs higher than 200 kDa could not enter into the gel, they could not be detected on the gel. Similar findings have been reported previously in several studies (Singh, 2005; Miller and Gerrard, 2005; Horszwald et al., 2009). Singh (2005) explained that a low degree of protein extraction from bread samples was due to differences in rate of temperature change and in moisture content in different parts of the bread, and disulfide bonds were the major cross-links formed in bread crusts during baking and they were responsible for protein insolubility.

Thermal properties

The thermal properties of the raw materials, analysed in the differential scanning calorimeter (DSC), are shown in Table 1. These properties are influenced by the protein and lipid contents, the granule structure (amorphous/crystalline structure relationship), and the molecular structure of the amylopectin, such as its branching, chain length and molecular weight, among other things (Tester, 1997). The starch gelatinization onset temperature (To) of the quinoa flours presented lower values than those of the wheat flour, and this difference was significantly lower ($p < 0.05$) in black quinoa. In addition, lower peak temperature (Tp) values were observed in the white quinoa flour than in the wheat flour ($p < 0.05$). Lower gelatinization temperatures indicate shorter amylopectin chains, because they need lower temperatures to dissociate completely (Yaminet al., 1999; Jane et al., 1999). The conclusion temperature (Tc) and gelatinization enthalpy (ΔHG) were significantly higher ($p < 0.05$) in the red and black quinoa flours than in the wheat and white quinoa flours, owing to the high crystallinity of the starch granules in the quinoa (Steffolani et al., 2013).

In varieties from Peru, Repo-Carrasco-Valencia and Valdez-Arana (2017) reported ΔHG values similar to those observed in the present work, but the gelatinization temperatures were slightly higher. These differences are basically due to the variability between cultivars.

The thermal properties of the bread doughs during the simulation of baking are shown in Table 1. With regard to gelatinization, a general increase in the To and Tp temperatures was observed in the formulations with quinoa in comparison with the control sample, but this increase was only significant ($p < 0.05$) in the

formulations with white or black quinoa. Furthermore, there was a general decrease in the Tc and ΔHG values in comparison with the control dough, and they were significantly lower ($p < 0.05$) in the doughs with white quinoa. This behaviour is due to the inclusion of fibre from the whole quinoa flour. During the cooking stage, when the gelatinization of the starch takes place, the water is less available in the formulations with quinoa, basically because of the presence of fibre, so the ungelatinized granules would need higher temperatures and less energy to gelatinize, producing increases in To and Tp and decreases in Tc and ΔHG (Santos et al., 2008).

A significant increase ($p < 0.05$) in the enthalpy of the amylopectin retrogradation (ΔHR) was observed during storage in all the formulations (Fig. 4a), as reported by other authors in studies on retrogradation kinetics (Haros et al., 2002; Ribotta et al., 2003]. No significant changes in ΔHR were observed during the first 24 h of storage. However, the incorporation of quinoa in the doughs produced a significant reduction ($p < 0.05$) of this parameter with respect to the control after 48 h. The replacement of wheat flour with red or black quinoa caused a significant increase ($p < 0.05$) in the retrogradation temperatures with respect to the control and the formulation with white quinoa during storage (data not shown).

Table 1. Thermal properties of raw materials and doughs

Parameter[a]		Flours				Doughs			
Starch gelatinization	Units	Control	White	Red	Black	Control	White	Red	Black
T_o	°C	56.7±0.6b	56.7±0.6b	55.4±0.8a,b	53.9±0.7a	62.3±0.6a	64.4±0.8b	62.5±0.9a	63.9±0.7b
T_p	°C	62.9±0.1b	61.8±0.3a	62.6±0.1a,b	62.0±0.7a,b	69.5±0.8a	70.3±0.8a,b	69.8±0.7a	71.1±0.6b
T_c	°C	69.7±0.4a	69.8±0.5a	71.8±0.2b	73±1b	80.5±0.7b	77.3±0.4a	79.8±0.4b	80.2±0.4b
ΔH_G	J/g of starch	8.1±0.1a	8.20±0.08a	9.28±0.02c	8.57±0.06b	0.67±0.05b	0.42±0.08a	0.9±0.1c	0.6±0.4b

Mean ± Standard Deviation, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. DSC: Differential Scanning Calorimetry; T_o. onset temperature; T_p. peak temperature; T_c. conclusion temperature; ΔH_G. enthalpy of gelatinization.

Pasting properties

The pasting properties of the raw materials and the bread mixtures were analysed (Table 2). The pasting temperature (Ptemp) of the quinoa flours was significantly higher ($p < 0.05$) than that of the control flour, which might lead to poor cooking characteristics (Hoseney, 1984), although the inclusion of 25% of whole quinoa flour did not alter this parameter significantly. The quinoa flours presented significantly higher ($p < 0.05$) peak time (Ptime) values than the control (Table 2). However, the inclusion of these flours in the formulation produced a significant decrease ($p < 0.05$) in the time needed for peak formation, denoting a non-additive behaviour and suggesting the appearance of physico-chemical interactions between the components of the flours. The differences in size and structure of the starch granules cause unequal distribution of moisture during heating, and therefore, the behaviour of the doughs is different from that of the individual flours (Waterschoot et al., 2014). On the other hand, it is worth noting that the peak viscosity (PV) and breakdown values were significantly lower ($p < 0.05$) in the quinoa flours than in the wheat flour, which caused a corresponding decrease in these parameters in the analysis of the breadmaking mixtures. Hot paste viscosity (HPV) is related to the final volume of the loaf after baking, owing to its effect on the incorporation and capacity of movement of CO_2 in the dough (Bath et al., 1992; Kim et al., 2001). This might indicate that the lower HPV shown by the quinoa flours with respect to the wheat flour might lead to an increase in the volume of the final product (Lee et al., 2006; Onyango et al., 2010). However, the incorporation of quinoa flours in the breadmaking mixtures led to a general increase in HPV, which was significant ($p < 0.05$) in the mixtures

with white or red quinoa. Setback is the stage in which there is a regrouping and/or reordering of starch molecules and it is associated with the texture of bakery products (Michiyo et al., 2004). The analysis of the raw materials showed significantly lower ($p < 0.05$) setback values in the quinoa flours than in the control sample. However, the only significant reduction ($p < 0.05$) in the breadmaking mixtures was in the one with black quinoa. In general, the values of the pasting properties of the quinoa flours were lower than those of the wheat flour. In general, the values of the pasting properties of the quinoa flours were lower than those of the wheat flour. This can be explained by the characteristics of the starch granules of the various raw materials with regard to their degree of crystallinity and amylopectin chain length and by the higher fibre content in the quinoa flours, reducing the availability of water in the breadmaking mixtures and consequently affecting the pasting properties (Bulut-Solak et al., 2016). In general, the results obtained for the royal quinoa flours in the present study fit within the results reported by Wu et al. (2014) after analysing 13 varieties of quinoa.

Table 2. Pasting properties of raw materials and quinoa/wheat blends

Sample	Units	Flours				Quinoa/wheat blends		
		Control	White	Red	Black	White	Red	Black
P_{temp}	°C	68.0±0.6a	84.4±0.5c	81.42±0.03b	80.3±0.6b	68.47±0.03a	68.4±0.1a	68.1±0.6a
P_{time}	min	5.87±0.00b	7.00±0.00c	7.00±0.00c	7.00±0.00c	5.67±0.09a	5.73±0.00a	5.73±0.00a
PV	cP	2271±21d	909±3a	1084±24b	942±2a	2062±81c	2086±37c	2001±11c
HPV	cP	1320±7c	782±23a	1018±6b	811±4a	1382±40d	1381±30d	1325±13d
CPV	cP	2725±14c,d	1467±4a	1706±16b	1666±38b	2743±85c,d	2805±56d	2663±13c
Breakdown	cP	951±14d	127±19b	66±18a	131±2b	680±41c	705±7c	676±1c
Setback	cP	1405±7d,e	685±27a	687±9a	855±35b	1361±45c,d	1424±25e	1338±1c

Mean ± Standard Deviation, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level. RVA: Rapid Visco Analyser; P_{temp}. pasting temperature; P_{time}. peak time; PV. peak viscosity; HPV. hot paste viscosity; CPV. final or cool paste viscosity; Breakdown: PV – HPV; Setback: CPV – HPV; cP. centipoises.

Effect of incorporation of quinoa on bread performance

The physico-chemical parameters of the wheat bread and the bakery products incorporating whole quinoa flour are shown in Table 3. A significant decrease ($p < 0.05$) in loaf height was observed in the breads made with black quinoa in comparison with the control sample (~ 6.5%). Although the incorporation of white or red quinoa did not lead to significant differences with respect to the control, the value of this parameter tended to decrease. The reduction in loaf height was similar to the loss of volume reported by other authors (Park et al., 2005; Wang et al., 2015), basically affected by the dilution of gluten and the higher fibre concentration in the quinoa flours. However, there were no significant changes in loaf weight between the breads that incorporated quinoa and the control bread (Table 3). The moisture content of the samples with quinoa, except the one with red quinoa, increased significantly ($p < 0.05$), basically owing to the use of whole quinoa flours. The protein content tended to increase, and this increase was statistically significant ($p < 0.05$) in the formulations with white and red quinoa. It is worth noting that the replacement of wheat flour with whole quinoa flour not only increases the protein content but also produces an improvement in the biological value of the proteins in these formulations, because quinoa proteins are more digestible than wheat proteins and they provide essential amino acids that are limiting in wheat flours (Vega-Gálvez et al., 2010; Repo-Carrasco-Valencia and Arana, 2017).

Table 3. Effect of whole quinoa flour on bread performance

Sample	Units d.m.	Control	Quinoa		
			White	Red	Black
Physico-chemical parameters[a]					
Moisture (w.m.)	%	36.6±0.1b	38.6±0.1c	35.6±0.1a	38.49±0.01c
Loaf weight	g	638±1a	641±3a	647±17a	639±3a
Loaf height	cm	12.4±0.3b	12.3±0.4b	12.0±0.2a,b	11.6±0.3a
Starch	%	60±3b	60±1b	59±1b	56±1a
Proteins	%	11.00±0.06a	11.5±0.1b	11.5±0.2b	11.16±0.05a
Total dietary fibre	%	5.9±0.5a	8.51±0.01b	9±1b	10.66±0.00b,c
Lipids	%	0.25±0.03a	0.7±0.1b	0.79±0.02c	0.78±0.05c
Ash	%	1.06±0.04a	1.48±0.02b	1.50±0.03b	1.61±0.01c
Textural Parameters					
Firmness	N	0.70±0.04a	1.08±0.07b	1.03±0.09a,b	1.3±0.4b
Springiness		1.72±0.08a	1.70±0.05a	1.73±0.02a	1.7±0.1a
Cohesiveness		0.93±0.02b	0.87±0.01a	0.87±0.01a	0.87±0.08a
Gumminess	N	0.65±0.04a	0.97±0.03b	0.90±0.09b	1.5±0.3c
Chewiness	N	1.12±0.02a	1.66±0.00b	1.6±0.2b	2.5±0.2c
Resilience		0.49±0.01a,b	0.47±0.01a	0.48±0.01a,b	1.20±0.04b
Crumb grain					
Cell area/total area	cm^2/cm^2	0.45±0.00a	0.44±0.00a	0.46±0.01b	0.47±0.00b
Wall area/total area	cm^2/cm^2	0.55±0.00b	0.56±0.00b	0.54±0.01a	0.53±0.01a
Cells/cm^2		17.6±0.8a	18±2a	17.85±0.05a	16.8±0.5a
Median cell area	mm^2	0.67±0.02d	0.57±0.01c	0.38±0.01b	0.31±0.01a
Maximum cell area	mm^2	73±9a	75±7ab	98±9b	176±8c
Sensory Analysis[b]					
Overall acceptability		7.1 ±1.3a	7.4 ±1.1a	6.9 ±1.5a	7.1 ±1.5a

[a]Mean ± Standard Deviation, n=3; [b]n=50. Values followed by the same letter in the same line are not significantly different at 95% confidence level; d.m.: dry matter; w.m.: wet matter.

There was also a significant increase ($p < 0.05$) in the dietary fibre and mineral contents in the formulations with white and red quinoa in comparison with the control, thus contributing to a suitable intake of fibre and minerals such as Ca, Fe and Zn in the diet (Bath et al., 1992; Stikic et al., 2012).The results of the digital image analysis of the crumb of the products developed are shown in Table 3. There was a significant increase ($p < 0.05$) in the value of the cell area/total area parameter in the crumb of breads that included red or black quinoa in comparison with the bread with white quinoa and the control (Fig. 3). Although significant changes were not seen in the cells/cm2 parameter, a decreasing tendency was observed in the sample with black quinoa. It is worth noting that there was a very significant increase ($p < 0.05$) in the maximum cell area in the crumb of the breads with various varieties of quinoa in comparison with the control bread. These differences may be due to greater α-amylase activity in the quinoa, leading to an increase in the quantity of fermentable sugars produced from the starch (Lorenz and Coulter, 1991; Caussette et al., 1997). Although the maximum cell area increased in the crumb of the breads with quinoa, there was a decrease in the median cell area of those breads, most probably due to the formation of large gas cells which compressed the other gas cells, reducing the median cell area. With regard to texture, the parameters analysed are shown in Table 3. A significant increase ($p < 0.05$) was observed in the firmness parameter of the breads with white or black quinoa in comparison with the control, basically due to the reduction in the percentage of gluten.

The incorporation of quinoa in the bread formulations also led to significant increases ($p < 0.05$) in the gumminess and chewiness parameters, whereas there was a significant decrease ($p < 0.05$) in cohesiveness with respect to the control sample.

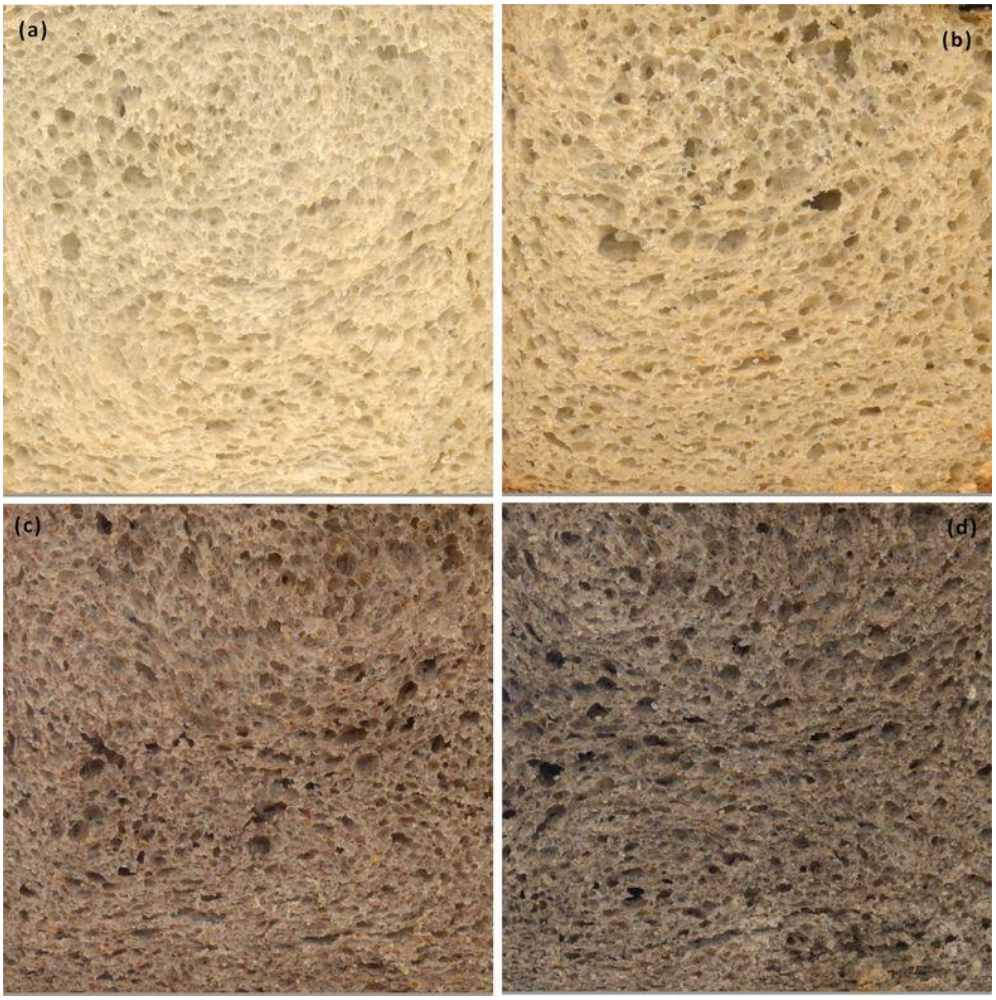

Fig. 3. Effect of the inclusion of quinoa on crumb structure. Bread formulations: a wheat bread; b white quinoa bread; c red quinoa bread; and d black quinoa bread

In general, during storage, there were significant changes in all the texture parameters of the products developed (data not shown). However, a very marked increase was observed in the firmness values of the products formulated with quinoa in comparison with the control sample during 2 days of storage (Fig. 4b). This crumb hardening can be explained partly by the phenomenon of amylopectin retrogradation (Fig. 4a). Retrogradation is a complex phenomenon that depends on many factors, such as the size and structure of the starch granules, and it involves phenomena such as the formation of bonds with proteins and/or the presence of lipids with surfactant properties that can cause differences in the migration of water molecules between gluten and starch during storage (Gray and Bemiller, 2003). Accordingly, the significant increase ($p < 0.05$) in the crumb firmness during storage of the products with quinoa may be due to a greater loss of moisture generated by irregular dough, with layers of gluten surrounding conglomerates of starch granules (Morita et al., 2001). The preliminary sensory analysis indicated that partial replacement of wheat flour with 25% of whole quinoa flour did not significantly affect the general acceptability of the products developed. However, the breads with quinoa were given slightly better scores than the control sample, with the exception of the bread with red quinoa, which received slightly less acceptance. The acceptance of products made with quinoa might be due, among other things, to the formation of aromatic compounds, such as pyridines, characteristic of quinoa flours, generating flavours accepted by consumers (Hansen and Schieberle, 2005).

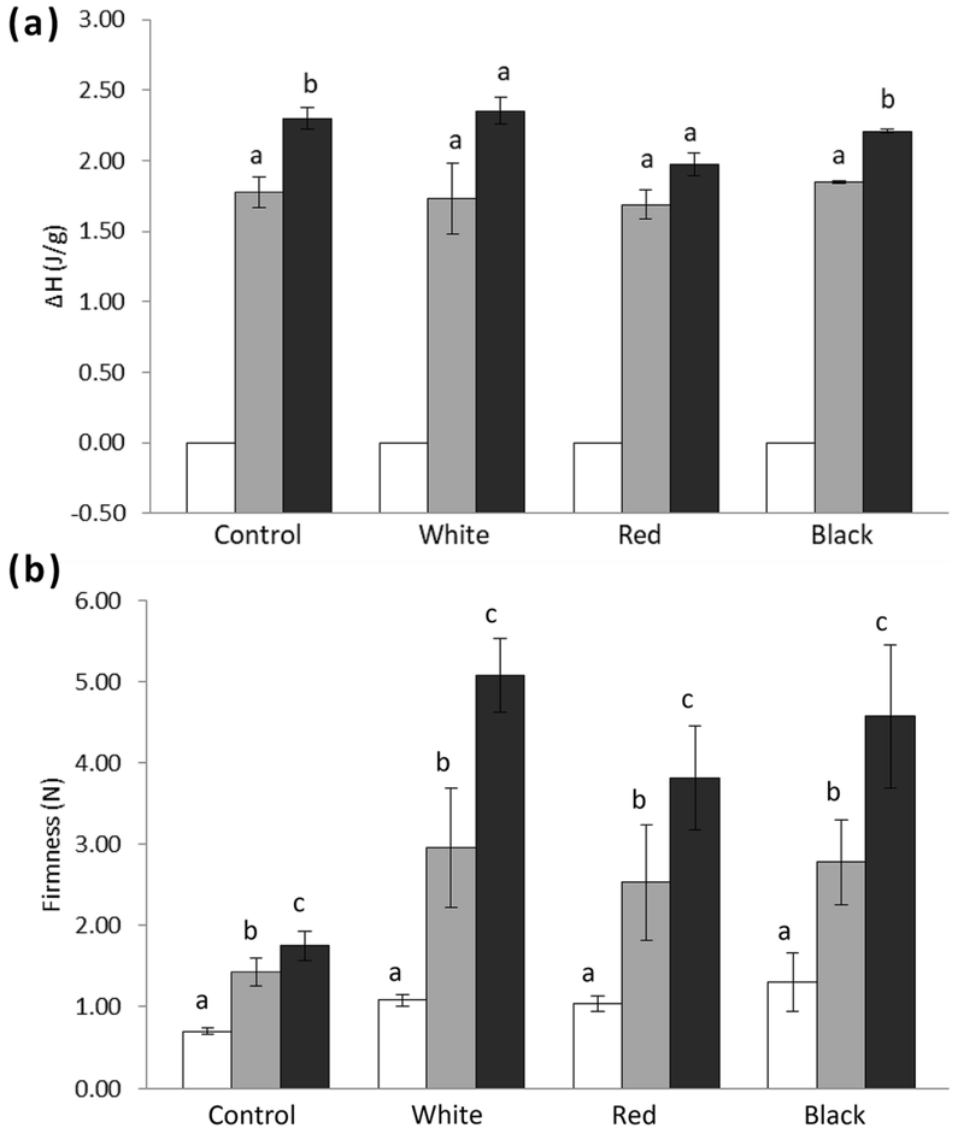

Fig. 4. Firmness and amylopectin retrogradation of control and wheat and quinoa bread samples (n = 3): unfilled square, day 1; grey colored filled square, day 2; black colored filled square, day 3. Mean ± Standard Deviation, n = 3. Values followed by the same letter in the same line are not significantly different at 95% confidence level

CONCLUSIONS

The global proteomic approach offered a general view of the various proteins in the different quinoas and the changes that took place during the breadmaking process, which included hydrolysis and formation of bonds between quinoa proteins and wheat proteins, modifying the protein structure of the doughs formulated. In general, the three varieties of quinoa presented a similar behaviour in terms of pasting properties, thermal characteristics and proximal composition that were different if comparing to wheat flour. The gelatinization thermal transition of starch from red and black quinoa flours appeared in a greater temperature range than white quinoa flour. The replacement of 25% of the wheat flour with whole quinoa flour in making bakery products caused a change in the thermal and pasting properties of the bread doughs, which led to the development of baked products with different physico-chemical and textural characteristics. However, a significant increase ($p < 0.05$) in the nutritional profile together with the overall consumer acceptance of the products developed was conclusive for proposing replacement with quinoa flour as a strategy for nutritional improvement in the manufacture of bread with refined wheat despite the slight decrease in the technological quality of the products developed. Therefore, black quinoa bread presented a higher amount of dietary fibre/ash and a lower amount of starch compared to white and red quinoa breads. These differences produced breads with a lower loaf height and higher crumb firmness, chewiness, and resilience with a similar acceptability by consumers regardless the different formulations.

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CAPÍTULO 2

Development of healthy, nutritional bakery products by incorporation of quinoa

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ABSTRACT

The use of quinoa could be a strategy for the nutritional improvement of bakery products. The inclusion of this pseudocereal, with its suitable balance of carbohydrates, proteins, lipids and minerals, could contribute to attaining the adequate intake values proposed by the FAO (Food and Agriculture Organization) and/or EFSA (European Food Safety Authority) for suitable maintenance and improvement of the population's health. Bakery products made with white, red or black royal quinoa significantly improved the contribution to an adequate intake of polyunsaturated fatty acids (linoleic and linolenic acids) and dietary fibre, which produced an improvement in the soluble/insoluble fibre ratio. There was also an increase in the contribution to the average requirement of Fe and Zn, although the increase in the phytate/mineral ratio would make absorption of them more difficult. Inclusion of flour obtained from the three quinoas studied slightly improved the protein quality of the products that were prepared and positively affected the reduction in their glycaemic index.

INTRODUCTION

Quinoa is a native pseudocereal of Latin America that now has great consumer acceptance in Europe and throughout the world. Because of its suitable balance of carbohydrates, proteins, lipids and minerals and its bioactive compound content, it has been proposed that it should be included as a strategy to improve the nutritional quality of bakery products made with refined flours (Haros and Schoenlechner, 2017; Ballester-Sánchez et al., 2019a). Not only would the incorporation of quinoa flour in formulations increase the protein content but it could also improve the biological value of the proteins in these formulations, since quinoa proteins contribute essential amino acids that are limiting in wheat flours (such as lysine and threonine), and they are more digestible (Vega-Galvez et al., 2010). It could also lead to an increase in the unsaturated fatty acid content and an improvement in the omega 3/omega 6 fatty acid relationship. The main unsaturated fatty acids in quinoa are linoleic and α-linolenic acids, a precursor of long-chain polyunsaturated fatty acids (PUFAs), which are essential fatty acids (Repo-Carrasco et al., 2003; Haros and Schoenlechner, 2017). Moreover, its high fibre and mineral contents could help to attain the daily requirements of these substances and of calcium, iron and zinc in the diet (Haros and Schoenlechner, 2017). However, mineral bioavailability does not depend only on the concentration of the mineral in question in the food (such as Ca, Fe or Zn); there are compounds such as phytates that form complexes with di- and trivalent minerals and prevent their absorption (Lopez et al, 2001). Because of the high proportion of dietary fibre in wholemeal flours made from quinoa

and other grains, their inclusion in bread formulations could have a beneficial effect by improving gastrointestinal transit and reducing levels of cholesterol and as a source of prebiotics, among other functions of dietary fibre (ADA, 2008). On the other hand, there are studies that propose strategies to reduce the glycaemic response in bakery products by the use of whole grains and by incorporating the external parts of the grain (Sanz-Penella et al., 2014). Diets with a high glycaemic index (GI) are associated with the development of metabolic dysfunction and predisposition to type 2 diabetes, as well as with problems of overweight/obesity (Schwingshackl et al., 2015).

The dietary reference intakes (DRIs) proposed by the FAO/WHO (Food and Agriculture Organization/World Health Organization) for the world population, also known as dietary reference values (DRVs) proposed by EFSA (European Food Safety Authority) for the European population, are a set of nutrient reference values that indicate the quantity of a nutrient that must be consumed regularly to maintain the health of a healthy person (or population). The main aim of the first reference values, proposed in the early 1940s, was to prevent nutritional deficiencies in the population (Carbajal, 2003). However, nutrient reference intake values also focus on preventing illness and on promoting health (Aranceta, 2004). There may be differences in the reference values proposed by the two organisations because they are based on the average intakes of the population in question, taking their food behaviour into account. These reference values have become a fundamental tool for evaluating the nutrients provided by a food when it is ingested.

The aim of this study was to make a detailed analysis of the chemical

composition of the raw materials and the products developed, including the nutritional profile of products in which wheat flour was replaced with whole white, red or black quinoa flour. The contribution made to the DRIs/DRVs of nutrients such as linoleic acid, linolenic acid, calcium, iron, zinc and dietary fibre by the ingestion of a bakery product with quinoa was investigated and the impact on the glycaemic index was estimated.

MATERIALS AND METHODS

Materials

White, red and black quinoa seeds (organic "*quinoa real*", royal quinoa), marketed by ANAPQUI (La Paz, Bolivia), were purchased from Ekologiloak (Bizkaia, Spain). The three types of quinoa seeds were ground separately to obtain the corresponding flour by using a commercial blender three times for 30 s at room temperature (Aromatic, Taurus, Oliana, Spain) and were stored at 14 °C. Dehydrated yeast (*Saccharomyces cerevisiae*, Maizena, Spain) was used as a starter and commercial wheat flour from a local supermarket (Carrefour, Spain) was used for the breadmaking process.

Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), dehydrated yeast (1.0 g/100 g flour), sodium chloride (1.6 g/100 g flour) and distilled water (70.8 g/100 g flour). Whole quinoa flour was incorporated in the bread dough formula at a proportion of 25 g/100 g flour. The breadmaking procedure was

performed as described in a previous paper (Ballester-Sánchez et al., 2019b). Measurements were carried out in triplicate.

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Proximate chemical composition

Proximate analysis of moisture, dietary fibre, starch and phytic acid (*myo*-inositol 1,2,3,4,5,6- hexakisphosphate) of the raw materials and breads was performed according to approved methods 925.09, 991.43, 996.11 and 986.11, respectively (AOAC 1996). Protein determination was carried out by the Dumas combustion method (N conversion factor 5.7) according to ISO/TS 16634-2 (2016). Lipid and ash contents were determined according to Official Methods 30-10 and 08-03, respectively (AACC, 2000). Measurements were carried out in triplicate.

Amino acid analysis

Samples (1 g) were hydrolysed with 4 mL of 6 N HCl. The solutions were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Amino acids were determined in the acid hydrolysis, after derivatisation with diethyl ethoxymethylenemalonate, by high-performance liquid chromatography (HPLC) Model 600E multi-system with a 484 UV–Vis detector (Waters, Milford, MA, USA) with a 300 mm × 3.9 mm reversed-phase column (Novapack C18, 4m; Waters) at 18◦C); acetonitrile in binary gradient, the detection at 280 nm, with D-L aminobutyric as standard, Sigma Chermical Co. St. Louis, MO, USA) according to the method of Alaiz et al. (1992). The amino acid composition was expressed as grams of amino acid per 100 g of protein. Measurements

were carried out in triplicate.

Essential amino acid index

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

$$EAAI = 10^{log\ EAA} \quad (1)$$

where

$$EAA = 0.1[\log\left(\frac{a_1}{a_{1s}}x100\right) + \log\left(\frac{a_2}{a_{2s}}x100\right) + \cdots \log\left(\frac{a_n}{a_{ns}}x100\right)] \quad (2)$$

a_1, …, a_n are the amino acid contents in the sample, and a_{1s}, …, a_{ns} are the essential amino acid requirements in the protein standard (FAO, 2007).

Fatty acid composition

The samples were transesterified to convert fatty acid methyl esters (FAMEs), following the methodology previously described by Garces and Mancha (1993). The fatty acid composition and quantification were determined using an Agilent Technologies chromatograph (Santa Clara, United States) with a capillary column (100 m × 0.25 mm i.d. (internal diameter) × 0.25 µm film thickness) and a flame ionisation detector according to IUPAC (International Union of Pure and Applied Chemistry) Method 2.302 (IUPAC, 1992). Measurements were carried out in triplicate.

Mineral composition

The total Ca, Fe and Zn concentrations in samples were determined using a flame absorption spectrometer at the Analysis of Soils, Plants and Water Service at the Institute of Agricultural Sciences, Madrid, Spain. Each sample (0.5 g) was placed in a Teflon perfluoroalkoxy vessel and digested by means of 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) in 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 5% HCl. Measurements were carried out in triplicate.

In vitro digestion and Glycaemic Index (GI)

In vitro starch digestion and glycaemic index estimation were performed according to the modified method reported by 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) ($HI = AUC_{sample}/AUC_{wheat\ bread} \times 100$). The glycaemic index (GI) was calculated with the equation $GI = 0.549 \times HI + 39.71$ used by Laparra et al. (2018) for the inclusion of Latin American crops in bread. The measurements were carried out 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, taking into account the total carbohydrates of each sample (Wolter et al., 2014).

Statistical analysis

One-way ANOVA and Fisher's least significant differences (LSD) were applied to establish significant differences between samples. All statistical analyses were carried out with the Statgraphics Plus 16.1.03 software (Bitstream, Cambridge, MN, USA), and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Raw material and bread chemical composition

The protein contents of the raw materials did not reveal significant differences between the quinoa flours or with the control flour (Table 1). The control flour had a slightly lower protein content in comparison with other coloured quinoa varieties reported in the literature (Diaz-Valencia et al., 2018). These differences could be due mainly to the use of a lower protein conversion factor (N × 5.7) than the one generally used in the literature (N × 6.25). The quinoa nitrogen-to-protein conversion factor used in the literature varies from 5.70 to 6.25 (Haros and Schoenlechner, 2017). In this regard, the European Union proposes setting a single value, but so far, the value has not been agreed (EU, 2017).

In the bread formulations, replacing 25% of the wheat flour with whole quinoa flour produced a significant increase in the protein content in breads made with red and black quinoa in comparison with the control sample (Table 1). The lipid contents in the quinoa flours were significantly higher than those of

the wheat flour, basically because the germ had been removed from the wheat flour (Table 2). However, it must be pointed out that the lipid contents of quinoa grains are higher than those of whole wheat flour mainly because of the greater proportion of the germ in relation to the other anatomical parts of the quinoa grain (Haros and Schoenlechner, 2017). In the comparison of varieties, the lipid contents were significantly higher in the white and red quinoas than in the black variety (Table 2).

The results are in agreement with the values found in the literature, which range between 4.1 and 9.7 g/100 g, indicating great variability between quinoa varieties (Haros and Schoenlechner, 2017). The predominance of unsaturated fatty acids in quinoa seeds is well known (Haros and Schoenlechner, 2017), and therefore, the greater lipid content in bakery products made with quinoa could help to improve the saturated/unsaturated fatty acids ratio in Western diets (He and Corke, 2003).

The ash analysis did not reveal significant differences ($p < 0.05$) between the various quinoa flours or between the products made with them (Table 3). The same tendency was observed with the total dietary fibre (TDF) contents of the quinoa flours, which were significantly higher than those of the wheat flour (Table 4). Although the comparison in this study was made with refined wheat flour, it has been reported in the literature that quinoa grain generally has a higher total dietary fibre content than wheat grain (Haros and Schoenlechner, 2017). Even though there were no significant differences between white and red quinoa with regard to the dietary fibre content (SDF, soluble dietary fibre; IDF, insoluble dietary fibre; and TDF), an increasing tendency was observed in

the quinoa flours: white<red<black (Table 4). Diaz-Valencia et al. (Diaz-Valencia et al., 2018) reported similar behaviour with regard to the total fibre content of coloured quinoas from Peru. The dietary fibre content of the bakery products showed a content that was related to the contents of the various raw materials, with black quinoa bread having the highest total dietary fibre content (Table 4). Soluble/insoluble relationships close to 1:2 have demonstrated a more effective physiological action (Jaime et al., 2002). The bakery products that showed a ratio close to the recommended value were the ones made with red and white quinoa (Table 4). The improvement in the ratio in the bakery products could have a hypocholesterolaemic effect, attributable to the higher SDF content, as reported by Konishi et al. (2000) in their study of the effect of quinoa seed pericarp on mice. It has also been reported that SDF reduces gastric emptying, the glucose absorption rate and postprandial insulin, and therefore, an increase in the content of this fibre in bakery products could help to improve the control of glycaemia in blood (Salas-Salvadó et al., 2007). With regard to the intake of total dietary fibre, both the FAO and EFSA propose an adequate intake (AI) of 25 grams per day for adults (FAO, 2010; EFSA, 2017).

Table 1. Amino acid composition of raw materials and breads.

Amino acid g/100 g prot	Target FAO[#]	Flours				Breads			
		Control	White Quinoa	Red Quinoa	Black Quinoa	Control	White Quinoa	Red Quinoa	Black Quinoa
Proteins, % d.m. [a]		13.3±0.1abc	13.0±0.7abc	12.8±0.7ab	13.5±0.2abc	12.5±0.3a	13.2±2.5abc	14.7±0.2c	14.4±0.2bc
Asx		4.5±0.1a	10.5±0.2c	10.44±0.04c	10.6±0.1c	4.4±0.3a	6.0±0.2b	6.0±0.1b	5.8±0.1b
Glx		36.9±1.9c	16.7±0.4a	16.6±0.2a	16.51±0.06a	37.5±1.1c	31.9±1.2b	32.3±0.7b	31.9±0.8b
Ser		5.3±0.3a	5.2±0.1a	5.17±0.05a	5.22±0.04a	5.3±0.5a	5.4±0.1a	5.41±0.07a	5.3±0.1a
Gly		3.8±0.2a	5.8±0.1c	5.56±0.04c	5.80±0.07c	3.8±0.4a	4.3±0.1b	4.4±0.1b	4.3±0.1b
Arg		3.6±0.2a	9.1±0.3c	9.1±0.2c	8.7±0.1c	3.4±0.4a	4.4±0.1b	4.62±0.07b	4.3±0.1b
Ala		3.0±0.1a	5.0±0.1c	4.95±0.01c	5.0±0.1c	3.2±0.3a	3.7±0.1b	3.8±0.1b	3.6±0.1b
Pro		14.5±2.2e	6.66±0.06a	8.3±0.3ab	8.37±0.03abc	10.5±0.4cd	10.2±0.6bcd	8.9±0.8bcd	10.8±0.3d
Essential amino acids (EAA)									
His	1.5	3.00±0.07c	3.81±0.05d	3.80±0.01d	3.91±0.08d	2.3±0.2a	2.61±0.08b	2.58±0.04b	2.66±0.05b
Val	3.9	3.9±0.1a	4.9±0.1c	4.85±0.04c	5.0±0.1c	4.1±0.2ab	4.2±0.2b	4.3±0.1b	4.2±0.1ab
Met	1.6	0.6±0.3a	0.6±0.4a	0.7±0.2a	0.71±0.07a	0.9±0.1a	0.7±0.1a	0.87±0.01a	0.7±0.1a
Cys	0.6	1.7±0.1d	1.0±0.1ab	1.06±0.05ab	1.0±0.2a	1.4±0.3cd	1.23±0.04abc	1.37±0.08c	1.3±0.1bc
Ile	3.0	3.5±0.1a	4.2±0.1c	4.23±0.08c	4.19±0.06c	3.7±0.2ab	3.7±0.1ab	3.77±0.07b	3.7±0.1ab
Leu	5.9	6.8±0.3a	7.2±0.1a	7.27±0.07a	7.24±0.09a	6.8±0.7a	7.0±0.3a	7.1±0.1a	6.9±0.1a
Phe	3.8*	4.7±0.2ab	4.3±0.1a	4.34±0.05a	4.28±0.03a	4.6±0.5ab	4.6±0.1ab	4.7±0.1b	4.6±0.1ab
Tyr		2.7±0.4bcd	3.0±0.1d	2.9±0.1d	2.9±0.1cd	2.49±0.06abc	2.1±0.1a	2.4±0.1ab	2.2±0.2a
Lys	4.5	1.8±0.1a	6.2±0.1c	6.05±0.06c	6.21±0.07c	2.0±0.2a	2.8±0.2b	2.76±0.01b	2.7±0.1b
Thr	2.3	2.8±0.1a	4.31±0.08c	4.19±0.06c	4.29±0.03c	2.9±0.3a	3.3±0.1b	3.31±0.05b	3.24±0.09b
EAAI		2.66	2.84	2.85	2.86	2.68	2.69	2.73	2.69

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. aDry matter. #Amino acid pattern suggested by FAO for adults (g/100 g protein). *Suggested composition for aromatic amino acids Phe+Tyr (FAO, 2008). EAAI: essential amino acid index.

Table 2. Fatty acid composition of raw materials and breads and adequate intake contributions

Organic Acid		**Units**[a]	**Flours**			
			Control	**White Quinoa**	**Red Quinoa**	**Black Quinoa**
Lipids		g/100 g	1.2±0.1a	5.4±0.6c	5.3±0.2c	4.5±0.4b
Palmitic acid	C16:0	mg/g	0.33±0.03a	0.56±0.07c	0.57±0.03c	0.45±0.05b
Stearic acid	C18:0	mg/g	0.02±0.01a	0.05±0.01c	0.05±0.01c	0.04±0.01b
Oleic acid	C18:1n9c	mg/g	0.14±0.01a	1.6±0.2c	1.7±0.1c	1.2±0.1b
Linoleic acid	C18:2n6c	mg/g	0.68±0.05a	2.7±0.3b	2.6±0.1b	2.3±0.2b
Linolenic acid	C18:3n3	mg/g	0.03±0.01a	0.41±0.05b	0.37±0.02b	0.36±0.04b
Organic Acid or Reference		**Units**[a]	**Breads**			
			Control	**White Quinoa**	**Red Quinoa**	**Black Quinoa**
Lipids		g/100 g	1.09±0.09a	2.2±0.1bc	2.3±0.2c	2.02±0.07b
Palmitic acid	C16:0	mg/g	0.29±0.03a	0.38±0.02b	0.40±0.03b	0.36±0.01b
Stearic acid	C18:0	mg/g	0.02±0.01a	0.03±0.01b	0.04±0.01b	0.03±0.01b
Oleic acid	C18:1n9c	mg/g	0.14±0.01a	0.54±0.03c	0.61±0.06d	0.46±0.01b
Linoleic acid	C18:2n6c	mg/g	0.60±0.05a	1.11±0.06b	1.18±0.10b	1.06±0.04b
□-Linolenic acid	C18:3n3	mg/g	0.09±0.04a	0.12±0.01b	0.12±0.02b	0.11±0.01b
LA/ALA	C18:2n6c/ C18:3n3	g/g	6.6/1	9.2/1	9.8/1	9.6/1
% of contribution of AI E% for LA	FAO	2.5 E%	8	14	15	14
	EFSA	4.0 E%	5	9	9	9
% of contribution of AI E% for ALA	FAO/EFSA	0.5 E%	6	8	8	7

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. aDry matter. AI (adequate intake) contribution (%) for a daily average intake of 100 g of bread. AI E% (percentage of energy intake) for LA (linoleic acid) and ALA (α-linolenic acid) for adult ≥18, (FAO, 2007; EFSA, 2017), E= (Kcal protein + Kcal lipid + Kcal carbohydrates) in 100 g of bread.

Table 3. Mineral (Fe, Ca, Zn) content, phytate level ratio in raw materials and breads, phytate/mineral molar ratio and average requirement contributions.

Parameter		Units[a]	Flours			
			Control	White Quinoa	Red Quinoa	Black Quinoa
Ash		g/100 g	0.41±0.19a	2.37±0.02b	2.32±0.04b	2.5±0.03b
Ca		mg/100 g	20.3±1.6a	30.4±1.4c	22.9±1.2b	33.0±0.8d
Fe		mg/100 g	0.57±0.07a	2.5±0.3b	2.21±0.05b	2.24±0.07b
Zn		mg/100 g	0.65±0.11a	1.8±0.1b	1.97±0.09c	1.87±0.07bc
InsP_6		mg/100 g	2.9±0.4a	15.7±1.5b	15.2±1.0b	16.9±2.5b
			Breads			
			Control	White Quinoa	Red Quinoa	Black Quinoa
Ash		mg/100 g	1.04±0.08a	1.50±0.01b	1.51±0.06b	1.61±0.01b
Ca		mg/100 g	20.5±1.1a	21.7±1.9ab	22.0±1.0ab	23.5±0.2b
Fe		mg/100 g	0.69±0.08a	1.2±0.1c	1.18±0.03bc	1.08±0.01b
Zn		mg/100 g	0.60±0.05a	1.1±0.1b	1.09±0.05b	1.08±0.03b
InsP_6		mg/100 g	1.3±0.1a	3.6±0.3b	3.71±0.05b	4.0±0.3b
InsP_6/Ca<0.24		mol/mol	0.06	0.16	0.17	0.17
InsP_6/Fe<1.0		mol/mol	1.83	2.86	3.14	3.70
InsP_6/Zn<15.0		mol/mol	2.1	3.19	3.40	3.70
AR contribution		mg/day				
Ca	FAO	1000	3	3	3	3
	EFSA	750	2	2	2	2
Fe	FAO	14	5	9	8	8
	EFSA	11/16	6/4	11/8	11/7	10/7
Zn	FAO_{High}	4.2/3	14/20	27/37	26/36	26/36
	$FAO_{Moderatete}$	7.0/4.9	9/12	16/23	16/22	15/22
	FAO_{Low}	14.0/9.8	4/6	8/11	8/11	8/11
	$EFSA_{300}$	9.4/7.5	6/8	12/15	12/15	11/14
	$EFSA_{600}$	11.7/9.3	5/6	10/12	9/12	9/12
	$EFSA_{900}$	14/11	4/5	8/10	8/10	8/10
	$EFSA_{1200}$	16.3/12.7	4/5	7/9	7/9	7/8

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. aDry matter AR (average requirement) contribution (%) for a daily average intake of 100 g of bread. AR in mg per day for males/females ≥18. EFSA: European Food Safety Authority. FAO: Food and Agriculture Organization.

Mean consumption of 100 g of bread made with quinoa flour helped to attain 34%–43% of the daily adequate intake of fibre in adults, and black quinoa bread was the one that produced the highest percentage contribution, increasing the contribution by 19% in comparison with the control sample (Table 4).

Table 4. Dietary fibre content in raw materials and breads and contribution to adequate intake.

Parameter	Units[a]	Flours			
		Control	**White Quinoa**	**Red Quinoa**	**Black Quinoa**
IDF	g/100 g	3.9 ± 0.7a	11.26 ± 0.01b	13.9 ± 0.7bc	17.4 ± 2.3c
SDF	g/100 g	1.11 ± 0.01a	3.37 ± 0.01c	3.9 ± 0.7c	2.25 ± 0.01ab
TDF	g/100 g	5.0 ± 0.7a	14.63 ± 0.02b	17.8 ± 1.5b	19.7 ± 2.3b
		Breads			
		Control	**White Quinoa**	**Red Quinoa**	**Black Quinoa**
IDF	g/100 g	4.8±0.7a	6.38±0.01b	6.9±0.7b	9.1±0.7c
SDF	g/100 g	1.07±0.01a	2.13±0.01bc	2.7±0.7c	1.6±0.7ab
TDF	g/100 g	5.9±0.7a	8.51±0.01b	9.6±1.5bc	10.66±0.01bc
SDF:IDF$_{1:2}$	g/g	1:4.5	1:3.0	1:2.6	1:5.7
AI contribution	**%**	24	34	38	43

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]Dry matter. 1:2 ratio of soluble/insoluble fibre (Jaime et al., 2002) AI (adequate intake) contribution (%) for a daily average intake of 100 g of bread. AI in g per day for dietary fibre in adult ≥18 is 25 (EFSA, 2017).

The starch values of the quinoa flours were significantly lower than that of the wheat flour (Ballester-Sánchez et al., 2019b). However, whole flours have a lower percentage of starch than refined flours, and the quinoa grains used in this study had even lower starch contents than the levels reported for flours of whole cereals such as wheat, barley and corn (Haros and Schoenlechner, 2017). The starch content of the white variety was significantly higher than that of the red and black varieties (Ballester-Sánchez et al., 2019b). Similar results were found in the literature for

white quinoa grown in Holland or in Peru (De Bruin, 1964; Diaz-Valencia, 2018). With regard to the starch contents of the bakery products, no significant differences were observed, but there was a similar tendency to the one seen in the analysis of the raw materials, with the starch content in the breads made with quinoa decreasing to a level of 25% (Table 5). This reduction could lead to a decrease in the glycaemic load of products made with quinoa flour, as described below (Wolter et al., 2014).

Table 5. Effect of quinoa flour addition on glycaemic index and glycaemic load.

Parameter	Units	Breads			
		Control	White Quinoa	Red Quinoa	Black Quinoa
Starch[a]	%	66.2±1.3b	61.8±1.7a	62.6±1.1a	60.0±2.6a
AUC		5362±172c	4578±128a	4572±28a	4917±141b
TSH_{90}[b]	%	82±9a	73±5a	71±4a	71±4a
GI[b]	%	95±1c	86±1a	86.5±0.2a	90±1b
GL[b]	%	28.0±0.5d	20.1±0.3b	23.31±0.08c	19.4±0.3a
HC[c]	SH/min	96±15a	97±7a	95±11a	76±6a
Slope-LB[c]	SH/min	0.13±0.05a	0.35±0.03b	0.36±0.09b	0.19±0.03a

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. AUC: area under the curve of starch digestion, TSH_{90}: total starch hydrolysed at 90 min, GI: glycaemic index. GL: glycaemic load, w.b.: wet basis, HC: hydrolysis coefficient, SH: starch hydrolysed, [a]Dry basis, [b]Wet basis, [c]Slope and coefficient of hydrolysis calculated for each sample using Lineweaver–Burk's transformation of the TSH accumulation curves.

Amino acid composition

The amino acid contents of the raw materials and the breads are shown in Table 1. The predominant amino acid in the quinoa flours was glutamic acid, and it was significantly lower than in the wheat flour. Similar results have been found in cultivars in various regions (Haros and Schoenlechner, 2017;

Motta et al., 2019). The sulfur- containing amino acids (methionine and cysteine) had the lowest levels in the flours analysed, as also reported by other researchers (Motta et al., 2019). In general, the essential amino acid contents in quinoa were higher than those reported in most whole cereal grains, such as wheat, barley, rice and/or corn (Vega-Galvez et al., 2010). Consequently, one would expect that the incorporation of 25% of these flours in bread formulations would produce a significant increase ($p < 0.05$) in the essential amino acid contents. In fact, the concentrations of histidine, threonine and lysine increased significantly in comparison with the control sample.

However, although the incorporation of 25% of quinoa flour produced a general improvement in the amino acid profile of the bakery products developed, the improvement did not reach the value suggested by the FAO (2008) for lysine (4.5 g/100 g of protein). The improvement in the nutritional quality of the protein provided by the quinoa raw materials and the bakery products prepared with quinoa was evaluated by calculating the EAAI (Table 1). The EAAI values of the quinoa flours were slightly higher than that of the wheat flour, indicating a protein of greater nutritional quality (Table 1). However, the incorporation of 25% of quinoa flour in the breads produced almost no change in the EAAI values in comparison with the control sample. Apparently, there were also no losses during baking, taking the lysine values of the raw materials as a reference for the theoretical calculation (data not reported). An increase in the percentage of quinoa flour (over 50%) could attain the lysine values proposed by the FAO (2008).

Fatty acid composition

The analysis of the fatty acid profile of the raw materials showed higher levels in the quinoa flours than in the control flour (Table 2). A noteworthy result was the significantly lower concentrations of palmitic, stearic and oleic acids in the black quinoa flour in comparison with the other quinoas, mainly due to the lower lipid contents of the flour of this variety. However, there were no significant differences in the essential fatty acid contents in the various varieties of quinoa; linoleic acid was the main fatty acid (over 50%), followed by oleic acid (over 20%), as reported in the literature (Haros and Schoenlechner, 2017). The higher concentrations of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in the quinoa flours in comparison with the wheat flour produced a significant change ($p < 0.05$) in the lipid profile of the bakery products developed. Accordingly, intake of these products could help to reduce the risk of suffering certain diseases. It has been reported that replacing saturated fatty acids (SFAs) with polyunsaturated fatty acids (PUFAs) and/or monounsaturated fatty acids (MUFAs) in intake helps to reduce the LDL cholesterol concentration and the total cholesterol/HDL cholesterol ratio, and therefore the risk of suffering heart disease (FAO, 2008). Consequently, adequate intakes of 2.5 E% (percentage of energy intake) per day (FAO, 2008) or 4 E% per day (EFSA, 2017) of the energy intake have been proposed for linoleic acid (LA), and 0.5 E% per day for linolenic acid (ALA) (FAO, 2008; EFSA, 2017). The lipid profile analysis showed a significant increase in LA and ALA in the breads with quinoa, which generated an increase in the contribution to AIs

(Table 2).

Consumption of 100 g of bread with quinoa would contribute up to 15 E% (according to the FAO) or up to 9 E% (according to EFSA) of LA and up to 8 E% of ALA (FAO, 2008; EFSA, 2017). The saturated/unsaturated acid ratio is an indicator for nutritional and functional analysis (He and Corke, 2003). In the present study, the saturated/unsaturated fatty acids ratio of the products made with quinoa was higher than that of the control bread, mainly because of their high linoleic and oleic acid contents, which could help to reduce the incidence of cardiovascular diseases (Field, 2003). Omega 6 (n-6) and omega 3 (n-3) fatty acids are essential for humans and can only be biosynthesised from their ALA and LA precursors (Haros and Schoenlechner, 2017). There is no scientific rationale for recommending a specific n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lie within the recommendations established or previously reported (FAO, 2010; EFSA, 2017). However, in order to facilitate labelling, adequate intake values of 10 g of LA/day and 2 g of ALA/day have been proposed for adults, from which it is possible to establish a ratio of 5:1 (EFSA, 2009). The current n-6/n-3 ratio in Western diets has been estimated as lying in the range of 14:1–20:1 (Field, 2003). Accordingly, intake of the products developed in the present study could help to improve the imbalance in Western diets and thus help to achieve the recommendations of the international organisations.

Mineral and phytate composition

There were no significant differences in the Fe contents of the quinoa flour, whereas the Ca content was significantly higher in the black quinoa, followed by the white and red quinoas (Table 3). The red quinoa had the highest Zn content, followed by the black and white quinoas. Studies on other pseudocereals found higher Ca and Mg contents in coloured genotypes (Mustafa et al., 2011). However, Diaz- Valencia et al. (2018) did not find a colour effect in their study with various varieties of quinoa. The variability of the mineral contents in the grains can be explained by the agroecological conditions in which they are grown, especially the soil (Vega-Galvez et al., 2010). The values reported in the present work are of the same order as those reported for other quinoas and, in general, for other whole grains (Haros and Schoenlechner, 2017). It is known that minerals in wheat and other cereals are located mostly in the outer parts of the grain (Delcour et al., 2010). Accordingly, as was to be expected, the quinoa flours had significantly higher mineral contents than the control, which caused the same tendency in the breads made with those flours. The analysis of the bakery products with quinoa showed a significant increase in mineral contents with the exception of Ca, which was only significant in the product made with black quinoa ($p < 0.05$). The increase in the Fe and Zn contents in the products made with quinoa could have a significant impact on the consumer, helping to attain the DRIs/DRVs proposed by the FAO/EFSA, respectively (FAO, 2001; EFSA, 2017). The contribution of minerals to the diet made by the bakery products is shown in Table 3. Ingestion of 100 g of bread

made with quinoa did not improve the contribution to the average requirement (AR) for Ca, but it increased the contribution to the AR of Fe by 4%–5% (AR_{FAO}: 14 mg/day; AR_{EFSA}: 11/16 mg/day) in comparison with the white bread. With regard to Zn, various studies have demonstrated the negative effect of phytic acid on the bioavailability of this mineral and other di- and trivalent minerals (Haros and Schoenlechner, 2017). Phytates are negatively charged at physiological pH and can therefore form insoluble complexes with cations in the digestive tract, thus reducing their bioavailability (Lopez et al., 2001). Because of this inhibitory effect, particularly for Zn, the FAO and EFSA have both proposed various ARs for the consumption of phytates in the diet. The FAO (2001) considers three levels of bioavailability of zinc, depending on the phytate content in the diet (high, moderate and low bioavailability), whereas EFSA (2017) contemplates four levels of phytate intake per day (300, 600, 900 and 1200 mg per day). The incorporation of 25% of whole quinoa flour in bakery products generated an increase of ~13%/17% (FAO) and ~6%/7% (EFSA) in the contribution to the AR of Zn for males/females, respectively, in comparison with the control sample in diets with high bioavailability (phytate/zinc < 5). In diets with high consumption of phytates or low bioavailability (phytate/zinc ≥15) the contribution increased by only ~4%/5% (FAO) and ~3%/4% (EFSA) for males/females, respectively.

The significantly higher ($p < 0.05$) phytic acid content of the quinoa flours with respect to the wheat flour caused an increase in the phytic acid content in the breads made with quinoa (Table 3). The reduction in the phytate

content in the products made in comparison with the raw materials was basically due to the activity of phytases, which are activated during the kneading and fermentation stages and the first stages of baking, causing hydrolysis of the phytates to myo-inositol with a lesser degree of phosphorylation (Sanz-Penella et al, 2009). Phytate/mineral molar ratios are a useful tool for predicting the inhibitory effect on the bioavailability of minerals in humans (Ma et al., 2005). Phytate/Ca ratios greater than 0.24 in a food indicate that after ingestion the bioavailability of that mineral could be compromised. In the case of Fe, the bioavailability is compromised if the phytate/Fe ratio is greater than 1. Similarly, absorption of Zn is drastically reduced when the phytate/Zn ratio is greater than 15 (Ma et al., 2005). The breads made with 25% of quinoa flour had phytate/Fe ratios greater than 1 (2.9–3.70), which would negatively affect absorption of this mineral. However, the phytate/Ca ratios in the products with quinoa were less than 0.24, and the phytate/Zn ratios were less than 15 in the formulations with inclusion of quinoa flour, thus improving the bioavailability of these two minerals, mainly because of the greater contribution of these minerals, despite the higher concentration of phytates in the quinoa flour (Table 3).

Glycaemic index

The analyses performed for the glycaemic index estimation are shown in Table 5. After 90 min of digestion of the wheat bread it showed 82% of hydrolysed starch. The TSH90 (total starch hydrolysed at 90 min) was reduced by 9%–11% in the breads with quinoa. The wheat bread showed the

significantly ($p < 0.05$) highest glycaemic index (GI) percentage in comparison with the breads made with whole quinoa flour (Table 5). Furthermore, significantly higher values were observed in the GI of the breads made with black quinoa compared with those made with white and red quinoa. In the literature, a reduction of ~5% was reported in the GI of bread made with 100% of quinoa in comparison with the reference control bread (Wolter et al., 2014). With regard to the predicted glycaemic load (pGL), significant differences were observed between all the bread samples analysed (Table 5), with the bread made with black quinoa being the sample that showed the smallest pGL. The Lineweaver– Burk plot, widely accepted and established for calculating the kinetic parameters of starch hydrolysis, was used to transform cumulative curves into linear curves (Sanz-Penella et al., 2014). With this method it is possible to calculate the reciprocal values of (% of starch hydrolysis) and time. The inclusion of 25% of quinoa did not produce significant changes in the hydrolysis coefficients (Table 5). However, the values of the slope of the curve in the Lineweaver–Burk plot showed a smaller slope, indicating faster hydrolysis of starch, in the wheat bread in comparison with the breads with quinoa. This may have been because the fibre and other compounds present in quinoa, such as polyphenols, affected the glucose uptake kinetics, as reported by other researchers (Sanz-Penella et al., 2014; Li et al., 2017).

Moreover, *in vivo* studies have indicated that breads formulated with different sources of dietary fibre or mixtures of them in baked products have a hypoglycaemic effect in humans owing to the reduction in the rate of

absorption of carbohydrates from the diet because of the formation of a viscous gel in the small intestine (Rokka et al., 2013). In this context it must be emphasised that the glycaemic response of foods depends on the texture and size of the particles, but also on the type of starch, the degree of its gelatinisation, the type of association/interaction with other components of the food, and the type of processing of the food. Therefore, the differences found in the products with quinoa were due not only to an effect of dilution of starch as a result of the inclusion of a whole flour but also to the different properties of quinoa starch, among other factors.

CONCLUSIONS

Replacement of 25% of wheat flour with white, red or black quinoa flour produced a general improvement in the nutritional profile of the bakery products developed in this study in terms of an improvement in the contribution to adequate intake of fibre, general increase in protein content with a slight improvement in the amino acid profile, especially in lysine, and an increase in lipid content with an improvement in the saturated/unsaturated fatty acids ratio due to the higher content of linoleic acid in the quinoa flours, helping to attain adequate intake of linoleic and linolenic acids. The mineral content of the quinoa flours produced an improvement in the contribution to the average requirements of Fe and Zn made by the breads with addition of quinoa, although an increase in the phytate/mineral ratio might compromise absorption of these minerals. The breads with quinoa flour also produced

a reduction in the glycaemic index and the predicted glycaemic load, with a tendency for the starch hydrolysis rate to decrease.

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CAPÍTULO 3

Effect of incorporating white, red or black quinoa flours on free and bound polyphenol content, antioxidant activity and colour of bread

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ABSTRACT

Interest in quinoa as a functional food ingredient is currently emerging. The flours from white, red and black quinoa seeds were analysed in terms of total polyphenol content and antioxidant activity.

They were incorporated at 25% on flour basis into the bread dough formula to evaluate their potential to improve the functional properties of wheat breads. The contribution of extractable polyphenols (soluble forms) and the largely unexplored hydrolysable polyphenols (bound forms that can be found in the residues of the former) were taken into account to reflect a realistic health-promoting potential of breads. The red and black quinoa varieties stood out compared to wheat flour, with about double the polyphenol content and up to 4.7-fold increments in antioxidant activity when considering the sum of extractable and hydrolysable polyphenols. The red and black flours were equally effective in intensifying the antioxidant properties of bread despite the baking process (between 2- and 3-fold). They produced significant changes in the parameters that describe crust and crumb colour (L*, a*, b*). A clear darkening was observed compared to the control bread, an appealing attribute for lovers of unconventional and natural products. According to our results, the flours from the coloured quinoa seeds could be considered interesting antioxidant sources and be applied as natural ingredients in bread-making; new, promising and valuable unconventional products for consumers and producers could be developed.

INTRODUCTION

Eating products with antioxidant properties has been popularised due to the belief that some dietary patterns can be useful for preventing certain pathological conditions with a positive effect on people's quality of life (Langhans, 2018). The food industry has reacted to this market opportunity and has shown interest in developing new products with improved antioxidant properties for years. The use of antioxidants of plant-based ingredients has become important in most food innovations as a way to obtain current consumers' confidence, who feel especially attracted by everything that is organic and natural.

Ancient grains are perceived by consumers as being more healthy and natural compared to common cereals. They have aroused much interest as a source of ingredients to develop new functional foods (Boukid et al., 2018). Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal from South America, has become very popular and well appreciated, which is not surprising given its remarkable nutrition composition and other interesting attributes (Repo-Carrasco et al., 2003). Quinoa is also recognised as an excellent source of polyphenols. Health benefits associated with its intake have been described, especially for lowering the risk of oxidative stress-related diseases (Pasko et al., 2010; Simnadis et al., 2015).

Bread is regularly and widely consumed, and is considered traditionally important for human nutrition. Research shows this food matrix as an appropriate vehicle to introduce bioactive compounds in diets deficient in antioxidants, and the resulting breads are very much in demand (Dziki et al.,

2014). Quinoa is a relatively new ingredient in bread, and its impact on polyphenol and antioxidant activity contents has barely been addressed (Álvarez-Jubete et al., 2010; Brend et al., 2012; Chlopicka et al., 2012). Addition of quinoa flour generally increases total polyphenol content and antioxidant activity in the resulting breads.

The present work assesses in-depth the potential interest of incorporating quinoa flour into bread and the changes that occur during the baking process in terms of colour and antioxidant properties related to total polyphenol content, specifically: 1) three types of quinoa (white, red and black Organic quinoa Real©) were used to identify whether a specific variety was more appropriate as an antioxidant source; 2) the contribution of extractable (soluble forms) and hydrolysable polyphenols (bound forms that can be found in the residues of the former) to the total polyphenol content and antioxidant activity was contemplated to reflect a more realistic health-promoting potential of breads. The aforementioned studies (Álvarez-Jubete et al., 2010; Brend et al., 2012; Chlopicka et al., 2012) ignore the hydrolysable polyphenol content of quinoa breads despite the presence of this fraction being appreciable in plant food and, therefore, relevant in dietary intake (Pérez-Jimenez et al., 2013). These compounds seem to exert some biological activity in the colon by contributing to health properties with soluble polyphenols (Pérez-Jimenez et al., 2013); 3) the association between colour and phenolic content in seeds and breads was also analysed.

MATERIALS AND METHODS

Materials

White, red and black *quinoa seeds* (Organic quinoa Real©), commercially available from ANAPQUI (La Paz, Bolivia), were purchased from Ekologikoak (Bizkaia, Spain). Quinoa seeds were ground separately to obtain the corresponding flour in a commercial coffee blender (Aromatic, Taurus, Oliana, Spain). Wheat flour was purchased from a local market and dehydrated yeast (*Saccharomyces cerevisiae*, Maizena, Spain) was used as a starter for the breadmaking process.

Bread-making procedure

Control bread and three types of quinoa breads containing flour of each quinoa variety (white, red and black) were produced. Wheat flour was replaced by quinoa flour at 25 g/100 g of flour. This quinoa concentration was stablished as optimal to bread performance and acceptance by consumers in a previous work (Iglesias-Puig et al., 2015). The control bread dough formula consisted of wheat flour (450 g), dehydrated yeast (2.5 g/100 g flour basis), sodium chloride (1.6 g/100 g flour basis) and distilled water (up to optimum absorption, 500 Brabender Units, 58.0 g/100 g, flour basis, according to (Iglesias-Puig et al., 2015). Wheat flour was replaced with 25% whole quinoa flour on flour basis to the bread dough formula (water absorption, 58.7 g/100 g flour basis). Breads (in duplicated) were elaborated in a breadmaker (BM 3989, Severin, Germany) following the manufacturer indications. The pre-established baking program 1 and the strongest toasting

level were used. Process variables consisted in the following steps: 1st kneading phase and rising phase for 9 min and 20 min respectively; 2nd kneading phase and rising phase for 14 min and 20 min respectively; short stirring for 30 sec; 3rd rising phase for 4 min and 30 sec; last rising phase for 45 min and lastly baking along 60 min at 170 ºC. The obtained breads were dried at 40°C for 3 h by forced-air convection oven drying (Binder, Germany) and ground to a fine powder in a domestic mincer (Moulinex, France).

Colour assessment

Colour was measured by a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan). Colour differences were recorded as CIELab, L* (lightness), a* (redness to greenness) and b* (yellowness to blueness) values. $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Each sample was measured 4 times at different points due to heterogeneity from the quinoa.

Extraction of extractable and hydrolysable phenolic compounds

The extractable polyphenols fractions (EPF) and the hydrolysable polyphenols fractions (HPF) were obtained following the method of Saura-Calixto et al. (2007). The extractable polyphenols fractions (EPF) were obtained from 0.25 g of the flours (wheat and quinoa) and the ground breads in two consecutive 1-hour incubation steps at 24ºC (10 ml acidic methanol/water (50:50, v/v; pH 2) and 10 ml acetone/water (70:30, v/v)). The hydrolysable polyphenols fractions (HPF) were obtained by acidic hydrolysis done with the resulting residues using 20 mL of methanol and 2

mL of concentrated sulphuric acid (85ºC for 20 h). Samples were then centrifuged (5000 g for 10 min), washed two times with distilled water and finally taken to a final volume of 5 ml. EPF and HPF were stored at -20ºC.

Polyphenol content

Polyphenol content (PC) was determined in both the EPF and HPF by the method of Folin-Ciocalteu (Singleton & Rossi, 1965). Total polyphenol content (TPC) was determined as the sum of PC in both the EPF and HPF. For PC determination, absorbance was measured at 724 nm in a microplate spectrophotometer reader (Power Wave HT, BioTek Instruments, Winooski, VT) and compared with a standard curve of Gallic acid (1, 0.70, 0.5, 0.25, 0.125, 0.0625 mM). Results were expressed as mg of Gallic Acid Equivalents (GAE) g-1 sample dry basis (DB). Determinations were performed per triplicate in each extract.

Determination of antioxidant capacity

The antioxidant capacity (AC) was determined in both the EPF and HPF by two spectrophotometric assays: DPPH (α-diphenyl-β-picrylhydrazyl free radical scavenging method) according to Brand-Williams et al. (1995) and FRAP (ferric reducing antioxidant power assay) according to Benzie and Strain (1996). Total antioxidant activity (TAC) was determined as the sum of AC in both the EPF and HPF. DPPH assay: 50 µL of diluted sample were mixed with a 250 µL of DPPH methanolic solution (60 µM) and incubated for 30 minutes at room temperature. Absorbance was read at 517 nm.

FRAP assay: 260 µL of the FRAP reagent were mixed with 40 µL of the diluted sample and incubated for 30 minutes at 37ºC. Absorbance was read at 593 nm. Determinations were made in triplicate in each extract with a microplate spectrophotometer reader (Power Wave HT, BioTek Instruments, Winooski, VT, USA) and the results were expressed as µmol of Trolox equivalents (TE)/g sample dry (d.m.).

Betalains content

Betalains were quantified in the EPF of the quinoa flours with a microplate spectrophotometer reader (Power Wave HT, BioTek Instruments, Winooski, VT, USA) at two wavelengths (480 and 536 nm). Betalain concentration was evaluated as described by Escribano et al., (2017). Determinations were made in triplicate in each extract and the results were expressed as mg / 100 g d.m.

Statistical analysis

ANOVA, followed by the Fisher's Least Significant Differences (LSD) test, was used to compare PC, TPC, AC, TAC of polyphenol extracts as well as the colour values of flours, breads or seeds. Pearson's correlation analysis was used to show the relationship between various parameters. The significance level was set in both cases at $p < 0.01$ and calculations were made using the IBM SPSS Statistics software, v22 for Windows. The pairwise comparisons between the means of the estimated and calculated values before and after

baking, respectively, were performed by a Student's *t*-test (Microsoft Excel 2010). *p* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Polyphenol content and antioxidant activity in the EPF and HPF of flours

PC and AC were determined in the EPF and HPF of flours. TPC (PC in EPF + PC in HPF) and TAC (AC in EPF + AC in HPF) were considered for complete contribution estimation (Table 1). The PC of the EPF in the black quinoa was significantly higher than in the other varieties ($p < 0.01$) (around 1.3-fold). Regarding the PC of the HPF, the red and black quinoas were indistinguishable ($p > 0.01$) and stood out compared to the colouredless variety ($p < 0.01$). The AC determinations revealed that the white quinoa presented significantly lower FRAP and DPPH ($p < 0.01$) values in both the EPF and HPF fractions compared to the coloured varieties, which followed the same pattern as in the polyphenols content. DPPH and FRAP values in the EPF of the black variety were significantly higher than in the red one ($p < 0.01$). Values in the HPF were not consistent; both dark varieties were indistinguishable by DPPH ($p > 0.01$) while FRAP values in the black quinoa flour were significantly lower compared to those of the red variety ($p < 0.01$). The determination of PC and AC of the quinoa grains has been reported (references can be found in review articles on the subject (Tang & Tsao, 2017)). The shown values varied considerably, due mainly to

differences in the employed extraction procedure and analytical protocols used. To compare the polyphenol values that corresponded to the EPF, those works that used the same extractive protocol as ours (methanol/water followed by acetone/water) were chosen. Our PC values were slightly higher, i.e. 5.03-6.60 mg/g *vs.* 1.23-3.41 mg/g (Abderrahim et al., 2015), 1.44-2.1 mg/g (Brend et al., 2012), 3.75 mg/g (Pasko et al., 2009) and ~ 4 mg/g (Pellegrini et al., 2018).

Table 1. Phenolic content and antioxidant activity of flours

	Wheat	Quinoa		
		White	Red	Black
Phenolic content (mg GAE/g d.m.)				
EPF	6.53±0.01b	5.03±0.46a	5.13 ± 0.22a	6.60±0.46b
HPF	7.37±0.35a	12.64±2.21b	23.07±0.34c	24.10±1.84c
Total (EPF +HPF)	13.90±0.36a	17.67±2.26a	28.20±0.41b	30.69±1.89b
Antioxidant capacity (µmol TE/g d.m.)				
DPPH				
EPF	1.75±0.29a	3.37±0.15b	8.28±0.39c	9.30±0.00d
HPF	7.05±1.76a	12.12±4.88b	23.46 ± 1.18c	21.18±0.91c
Total (EPF +HPF)	8.80±1.76a	15.50±4.88a	31.74±1.24b	30.49±0.91b
FRAP				
EPF	1.27±0.03a	2.70±0.45b	10.73±0.13c	13.30±0.02d
HPF	10.60±0.11a	8.25±1.14a	44.94±5.31b	39.64±0.83c
Total (EPF +HPF)	11.87±0.12a	10.95±1.22a	55.67±5.31b	52.93±0.83b

Codes: d.m. (dry matter), EPF (extractable polyphenols fraction), HPF (hydrolysable polyphenols fraction), FRAP (ferric reducing antioxidant power); DPPH (α, α-diphenyl-β-picrylhydrazyl free radical scavenging method); GAE (gallic equivalents); TE (trolox equivalents). Data expressed as mean ± standard deviation. Means within lines followed by the same letter are not significantly different according to LSD post-hoc test at 99% confidence level

Regarding AC, the DPPH and FRAP values for the white quinoa (3.37 and 2.7 mg/g, respectively) agreed with those described by Pellegrini et al. (2018) and Brend et al. (2012), who reported lower values for the coloured varieties (between ~ 1.6- and 3-fold lower).

The PC and AC values that we obtained in the HPF were also compared with those reported in the literature, although data published on the HPF in quinoa are scarce (Abderrahim et al., 2015; Tang et al., 2016). The obtained PC values were much higher than those described in these works, with up to 7-fold increments between the white quinoa varieties, and about 4-fold ones between the coloured ones. Regarding AC, our DPPH and FRAP values were also higher than other reported ones (Tang et al., 2016), around 2- to 9-fold higher. The fact that these authors used much shorter hydrolysis times compared to our assay conditions could be the reason for the less efficient release documented.

When considering the final contribution of both the EPF and HPF to TPC and TAC (EPF plus HPF), both coloured varieties presented the same behaviour and stood out from the white quinoa. This clear trend between higher phenolic content and antioxidant activity values and the darker varieties has been previously described (Brend et al., 2012; Tang et al., 2016). The PC values associated with HPF were much higher in the flours of the three varieties than the values in the EPF (they dobled the white quinoa, and were 4.3- and 3.6-fold more for the red quinoa and the black quinoa, respectively), which constituted between 70% and 82% of the TPC and TAC (EPF + HPF). This remarkable contribution of HPF is consistent with that

described in the literature for others foods and raw materials, and suggests the importance of not underestimating this polyphenol fraction (Pérez-Jiménez et al., 2013).

As our main objective was to include quinoa in bakery products, the TPC and TAC of the three flours were compared to those of wheat. When considering the EPF + HPF sum, the red and black quinoa flours stood out for their significantly higher TPC and TAC values than the wheat flour (around twice and up to 4.7-fold increments, respectively), whereas the latter was indistinguishable from the white quinoa. The PC values in the EPF were significantly higher in the wheat flour compared to the white and red quinoa flours ($p < 0.01$), and equalled to the black variety ($p > 0.01$). The AC analysis in these extracts showed that the wheat flour presented clearly lower AC than the quinoa flours ($p < 0.01$). These contradictory results suggest an overestimation of the value of polyphenols in the wheat flour EPF extract through the interaction of compounds other than phenols with the Folin-Ciocalteu reagent, as mentioned elsewhere (Everette et al., 2010).

Colour analysis of flours and seeds. Relationship with phenolic contents

The colour parameters of flour and seeds were also determined, and differences between both quinoa form presentations were observed (Table 2). The colour of the quinoa flours was a combination of yellowness (b*), redness (a*) and lightness (L*), except for the white quinoa flour that

showed a negative a* parameter related with a greenish tint. L* and a* statistically differed among all the quinoa flours and, as expected, white quinoa flour L* and red quinoa flour a* had the highest values; b* was the equivalent in the white and red flours and was higher than in the black one. In terms of total colour differences (ΔE*) in relation to wheat flour, the quinoa flours presented differences of 3.8 for the black quinoa, and of 15.5 and 19.9 for red and black ones, respectively.

Table 2. Colour parameters of flours and seeds

	Wheat	QUINOA		
		White	Red	Black
FLOURS				
L*	89.9 ± 1.8d	85.6 ± 1.8c	73.8 ± 0.0b	69.7 ± 0.8a
a*	-1.7 ± 0.1a	-1.5 ± 0.2a	2.1 ± 0.0c	0.9 ± 0.1b
b*	10.1 ± 0.3a	13.1 ± 0.8c	12.6 ± 0.0c	11.4 ± 0.2b
ΔE*	-	3.8 ± 0.2a	15.5 ± 1.8b	19.9 ± 2.6b
100-L*	10.1± 1.8d	14.4± 1.8c	26.2± 0.0b	30.3± 0.8a
SEEDS				
L*	n.d.	76.2 ± 1.4c	38.2 ± 1.3b	31.8 ± 0.4a
a*	n.d.	0.2 ± 0.3a	13.0 ± 0.4c	6.5 ± 0.6b
b*	n.d.	19.8 ± 0.4b	18.0 ± 0.9b	8.1 ± 0.5a

$\Delta E^* = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$ (calculated with respect to wheat flour). n.d. (not determined, the wheat grains from which the commercial flour used was obtained were not available). Data expressed as mean ± standard deviation. Means within lines followed by the same letter are not significantly according to LSD post-hoc test at 99% confidence level

The colour parameters were affected by milling, which was expected given the pigment accumulation in the grain outer layer and the processing effect on colour lightening. Lightness (L*) was generally higher in flours compared to seeds, and redness (a*) was lower. The black quinoa seeds obtain the

lowest L* and b* values, as occurred for the corresponding flour, compared to the other quinoa varieties.

An association between colour parameters and phenolic content has been described in different fruits, grains and plants. In the present work, the TPC in the quinoa samples negatively and significantly correlated with the L* values of seeds (r = -0.970, *p = 0.001*). We now know that the different seed colourations are not really due to the polyphenolic compounds of the anthocyanins type as described by Pasko et al. (2009), but to the presence of pigments called betalains with antioxidant activity properties (Abderrahim et al., 2015; Escribano et al., 2017). Abdherrahim et al. (2015) proposed that the regulatory mechanisms involved in the biosynthesis of betalains and polyphenols respond to the same environmental conditions and, therefore, a simultaneous increase in both compounds takes place. This could explain why good correlations between both compounds were observed. Moreover, pigments are extracted in the same aqueous/organic solvents as extractable polyphenols (Abderrahim et al., 2015). We detected the presence of betalains in our quinoa EPFs (3.43, 6.15 and 7.19 mg/100 g in the white, red and black quinoa, respectively). This result agrees with the fact that the correlation found between PC and AC was lower in EPF (FRAP: r = 0.610, *p = 0.07*; DPPH; r = 0.559, *p = 0.16*) than in the HPF (FRAP: r = 0.934, *p < 0.0001*; DPPH; r = 0.884, *p < 0.0001*). It is indicative that betalains contribute, together with polyphenolic compounds, to the antioxidant activity of the EPF.

Effect of quinoa incorporation on breads

Phenolic contents and colour

The PCs of quinoa and the control breads were determined (Table 3). All the quinoa breads presented an equivalent PC in the EPF, around 2-fold higher than the control bread ($p < 0.01$). However regarding PC in the HPF, only the black quinoa bread displayed statistically higher values. No significant differences were observed for the TPC values (EPF + HPF) compared to the control bread ($p > 0.01$), but an increasing trend in content was observed as the used quinoa was darker.

Table 3. Phenolic content (mg GAE/g d.m.) of breads

	BREADS			
	Control	**White quinoa**	**Red quinoa**	**Black quinoa**
EPF	1.74±0.80a	3.35±0.25b	3.89±0.01b	3.60±0.11b
HPF	17.19±0.23a	17.34±0.05a	20.23±2.99a	19.82±0.05b
Total (EPF + HPF)	18.93±0.24a	20.70±0.26a	24.12±2.99a	23.43±0.12a

Codes: d.m. (dry matter), EPF (extractable polyphenols fractions), HPF (hydrolysable polyphenols fractions), GAE (gallic equivalents). Data expressed as mean ± standard deviation. Means within lines followed by the same letter are not significantly different according to LSD post-hoc test at 99% confidence level

The effect of baking on phenolic content was evaluated in the quiona breads by comparing the values in flour mixtures (estimated by taking the percentage of each flour used to make doughs and the corresponding phenolic values in Table 1) with the value determined in breads (Table 3). As Figure 1 shows, a drop between 1.5- and 1.8-fold in the PC in EPF was detected in the quinoa breads ($p < 0.05$). Contrarily, the PC result in the HPF was striking as a statistically significant increase took place after the thermal

process in the white (2-fold) and black quinoa (1.7-fold) breads (*p = 0.0040* and *0.0038,* respectively). The same behaviour and an increase of the same order were noted for the red quinoa bread, but not significantly (*p = 0.0519*). When combining both the EPF and HPF, PC became statistically higher in the white and black quinoa breads after baking. Greater thermal sensitivity of soluble polyphenols than hydrolysable ones has been described in breads containing barley flour (Holtekjolen, as cited in Dziki et al., 2014). Dziki et al. (2014) reviewed the changes in PC and AC during the bread-making process.

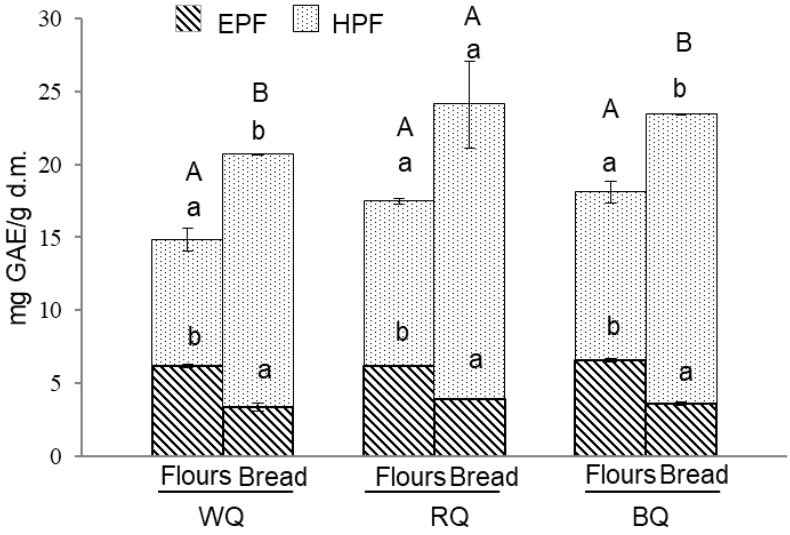

Fig. 1. Effect of baking on the polyphenol content of the 25% quinoa breads. Low-case letters refer to the comparisons of extractable (EPF) and hydrolysable polyphenol fractions (HPF); upper-case letters refer to the comparisons of the EPF + HPF sum. Comparisons were always made between flour mixtures (75% wheat + 25% quinoa) and the bread values within each quinoa type. The bars followed by the same letter are not significantly different according to the Student's *t*-test at the 95% confidence level. WQ: white quinoa, RQ: red quinoa, BQ: black quinoa

Destruction of compounds has sometimes been reported, but unaffected or new compounds seemed to form on other occasions. Reduction has been

associated with either the heat instability of compounds or the formation of complexes with bread proteins and carbohydrates that make them less extractable. The formation of Maillard reaction products has been widely used to explain the increase in the concentration of phenolics, as well as the breackage of covalently bound phenolic compounds. Even an overestimation of the analytical methodology has been suggested (see Dziki et al. (2014) and references therein). Thus the occurrence of different effects of baking on the TPC being influenced by the type of ingredient used during production cannot be ruled out (Gelinas and McKinnon as cited in Dziki et al., 2014).

The inclusion of 25% of quinoa flours influenced the final colour of breads (Table 4). In terms of total colour differences (ΔE*), the three quinoa breads were significantly more coloured than the control bread for both crust and crumbs. Except for crumbs of the white quinoa, the ΔE* values were higher than 5 units and, therefore, indicates that differences can be visually perceptible by consumers. The L* (lightness) values lowered for crumbs when darker varieties were incorporated. The opposite behaviour for parameter a* (redness) occurred, which increased in the following sequence: white quinoa > black quinoa > red quinoa. The differences for yellowing (b*) for crumbs were less evident, but a decreasing trend was observed in parallel to darkening. In summary, the breads containing the darker flours were more reddish- and less yellowish-coloured than those with the white quinoa and the control bread.

Table 4. Colour parameters of breads

	BREADS			
	Control	**White quinoa**	**Red quinoa**	**Black quinoa**
CRUST				
L*	59.5 ± 2.5b	49.2 ± 2.7a	47.1 ± 4.2a	45.3 ± 3.5a
a*	7.2 ± 1.1a	11.3 ± 0.3b	10.6 ± 1.4b	9.4 ± 1.0ab
b*	30.6 ± 1.7c	28.2 ± 2.2bc	24.9 ± 0.5ab	23.9 ± 0.8a
ΔE*	-	9.5 ± 2.9a	13.0 ± 3.1a	15.2 ± 3.2a
100-L*	40.5± 2.5b	50.8± 2.7a	52.9± 4.2a	54.7± 3.5a
CRUMB				
L*	69.1 ± 2.2b	64.2 ± 2.2b	50.8 ± 2.0a	46.3 ± 1.0a
a*	-1.6 ± 0.1a	-1.3 ± 0.1a	4.6 ± 0.4c	2.9 ± 0.2b
b*	14.9 ± 1.3ab	17.5 ± 2.1b	15.5 ± 0.3ab	12.2 ± 0.2a
ΔE*	-	4.2 ± 3.3a	17.3 ± 3.3b	22.8 ± 2.1b

$\Delta E^* = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$ (calculated with respect to control bread). Data expressed as mean ± standard deviation. Means within lines followed by the same letter are not significantly different according to LSD post-hoc test at 99% confidence level

The colour parameters found in breads could be attributed to the colour caracteristic of each quinoa variety (see Table 2), but also to the formation of Maillard products (Gelinas and McKinnon as cited in Dziki et al., 2014). Parameter 100-L* has been proposed as a marker to estimate the formation of hydroxymethylfurfural (intermediate products of the Maillard reaction) (Ramírez-Jiménez et al., 2000). The 100-L* values for quinoa bread crusts (Table 4) were significantly higher compared to the values in the corresponding flours (Table 2) ($p < 0.01$). This was indicative of the presence of hydroxymethylfurfural and therefore, Maillard reaction products could be partly responsible for the increment in the TPC observed after baking (Fig. 1).

Contribution to antioxidant properties

In order to evaluate whether the remarkable AC detected in the quinoa flours remained after baking, this parameter was determined in both the EPF and HPF of the resulting breads, and was compared to that of the control (Fig. 2).

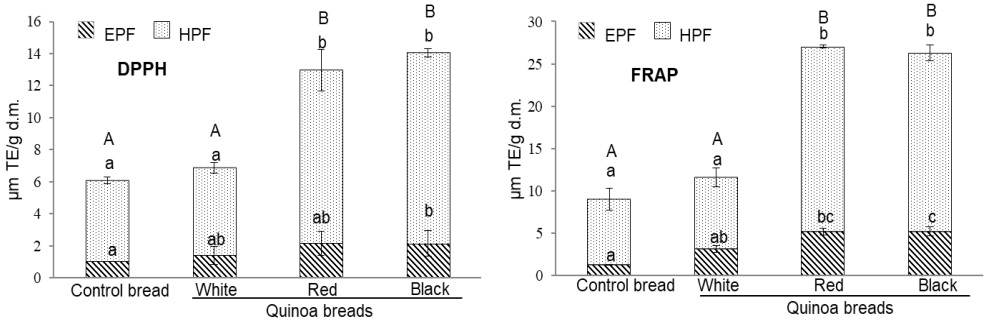

Fig. 2. Antioxidant activity of the extractable (EPF) and hydrolysable (HPF) polyphenol fractions from the control and the 25% quinoa breads. Low-case letters refer to the comparisons of both the EPF and HPF; upper-case letters refer to the comparisons of the EPF + HPF sum. The bars followed by the same letter are not significantly different according to the LSD *post hoc* test at the 99% confidence level

The sum of the AC values in both the EPF and HPF (TAC) was significantly higher ($p < 0.01$) in the red and black quinoa breads than in the control (DPPH: around 2-fold, FRAP: around 3-fold). These results showed that these two flours varieties were equally effective in intensifying the antioxidant properties of wheat bread, unlike the white quinoa bread, which was indistinguishable from the control ($p > 0.01$). In all cases, the contribution of the HPF to the sum was clearly larger than that of the EPF.

Published data about the antioxidant effect that derives from adding quinoa flour to bread formulations are scarce. Specifically, greater antioxidant activity, compared with the corresponding controls, has been described in gluten-free bread with 50% quinoa (Álvarez-Jubete et al., 2010) and 100% quinoa (Álvarez-Jubete et al., 2010; Brend et al., 2012). Chlopicka et al. (2012) characterised the AC of the EPF from wheat bread with 15% and 30% quinoa, and they obtained DPPH and FRAP values within the range of 1.17 µmol/g and 73.75 mg Trolox/100 g (i.e. 2.95 µmol/g), respectively, which are of the same order as our results (DPPH: 1.41-2.17 µmol/g and FRAP: 3.13-5.20 µmol/g). The differences they found in relation to the control bread were not as clear as those that we obtained. By respecting the HPF, we found no data about this for the quinoa breads and, as far as we know, this is the first time that a complete determination in polyphenols is provided for such a food product type. This is interesting if we take into account that both the EPF and HPF are ingested when bread is eaten.

The effect of baking conditions was evaluated by comparing AC in the flour mixtures, estimated by using the DPPH and FRAP values in Table 1, with the experimental values in the corresponding breads (Fig. 2). Baking did not lead to any major losses in AC in the quinoa breads (Table 5). In fact we observed no modifications in the ability to quench DPPH radicals, while occasionally significant increments were noted in the FRAP values, especially for the EPF. This latter feature contradicts the post-baking losses in the PC of these fractions (see Fig. 1).

Table 5. Effect of baking on the antioxidant activity of the 25% quinoa breads

	DPPH (µmol TE/g d.m.)		**FRAP** (µmol TE/g d.m.)	
	Flours[1]	Breads	Flours[1]	Breads
Extractable polyphenol fraction (EPF)				
WQ	2.16±0.18a	1.41±0.55a	1.63±0.09a	3.13±0.41b
RQ	3.38±0.12a	2.17±0.75a	3.64±0.05a	5.15±0.39b
BQ	3.64±0.22a	2.15±0.80a	4.28±0.03a	5.20±0.55a
Hydrolyzable polyphenol fraction (HPF)				
WQ	8.32±2.54a	5.47±0.32a	10.01±0.20a	8.47±1.12a
RQ	11.15±1.03a	10.78±1.29a	19.19±1.41a	21.90±0.14a
BQ	10.58±1.09a	11.89±0.25a	17.86±0.12a	21.08±0.93b
Total (EPF + HPF)				
WQ	10.47±2.50a	6.87±0.64a	11.64±0.22a	11.60±1.19a
RQ	14.53±1.02a	12.95±1.48a	22.82±1.41a	27.06±0.41a
BQ	14.22±1.11a	14.04±0.83a	22.14±0.12a	26.29±1.08a

[1]Estimated by taking into account the percentage of each flour used to make doughs (75% Wheat + 25% Quinoa) and the corresponding antioxidant activity values in Table 1 "online resource") Codes: d.m. (dry matter), TE (Trolox equivalents), WQ (white quinoa), RQ (red quinoa), BQ (black quinoa). Data expressed as mean ± standard deviation. Letters in non-italic and italic refers to the comparisons of DPPH and FRAP determinations, respectively, between flour mixtures and breads. Means within lines followed by the same letter are not significantly different according to Student's *t*-test at the 95% confidence level

The formation of the Maillard reaction products during baking is known. As they are antioxidative agents (see Dziki et al. (2014) and references therein), they likely contribute to increased antioxidant activity in breads. Brend et al. (2012) have described an increase in FRAP levels in 100% red quinoa breads and referred to the same cause.

CONCLUSIONS

The study of both the TPC and TAC of three differently coloured quinoa seeds, by considering soluble and bound fractions, indicated that this pseudocereal could be a good natural source to improve the antioxidant activity of wheat bread. The antioxidant potential was different depending on the quinoa variety used, and was strongly related with seed colour. The red and black varieties stood out and were equally effective in intensifying the antioxidant properties of wheat bread. Future research works will be needed to asses the *in vivo* antioxidant effect. Bread colour was affected by the colour characteristic of each quinoa variety because clear darkening was observed compared to the control bread. The bakery industry could take advantage of this attribute to attract lovers of unconventional products and consumers in general as darkness in products is associated with the un-refined products currently recommended as part of healthy lifestyle habits.

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PARTE 2. Adaptación de métodos de molienda húmeda de maíz y molienda seca de trigo para abordar el fraccionamiento de granos de quinoa.

CAPÍTULO 4

Quinoa wet-milling: effect of steeping conditions on starch recovery and quality

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ABSTRACT

Cereal starches play an important role in the food and non-food industries because of their low cost, availability, and ability to impart a wide range of techno-functional properties. The main objective of this research was to isolate starch, germ, protein, and fiber components from quinoa by a wet-milling procedure. The effect of steeping time and temperature on starch recovery and its quality was investigated. The quinoa steeping conditions, such as time (1, 5, and 9 hours) and temperature (30, 40, and 50 °C), in SO_2 solution with lactic acid were investigated using a 3^2 factorial design in order to optimize the starch separation and its quality. The effect of steeping conditions on starch was evaluated in terms of whiteness, protein, lipid, amylase, and damaged starch contents, as well as thermal and pasting properties. Results showed how the different steeping times and temperatures affected the fraction yields and starch recovery and its quality. Optimization of the wet-milling process used in this study produced the highest starch recovery level and best starch quality after 6.5 hours of steeping at 30 °C. Experimental values were close to the predicted ones, with an error below 2% for all attributes tested.

INTRODUCTION

The primary sources of carbohydrates for the global population are cereals and pseudocereals. Pseudocereals are essentially starch crops; however, they may contain significant quantities of protein and oil, and these constituents frequently determine their suitability for a specific end use. In particular, quinoa is a pseudocereal native to South America, mainly from Peru, Bolivia, Argentina, Colombia, Ecuador, and Chile, but in recent decades other countries such as the United States, Canada, Italy, France, Spain, England, and Sweden have also become producers (Bazile and Baudron, 2015). Structurally, quinoa is composed of three main parts: the perisperm, the embryo or germ, and the pericarp or seed hull (Reguera and Haros, 2017). The perisperm is the primary starch storage portion, the germ is the lipid storage portion, and finally the hull, also called bran, consists mainly of cellulose and hemicellulose. The physicochemical and functional properties of the main components of quinoa, starch, fiber, and protein, are widely described in the literature (Koizol, 1992; Schoenlechner et al., 2010; Kurek, et al., 2018). The objective of milling is to obtain intermediate products that can be used subsequently in the manufacture of other products. Normally, milling schemes are classified as dry- or wet-milling. In dry-milling the aim is to separate the anatomical part of the grain to produce mainly flour, whereas the purpose of wet-milling is to separate the chemical components of the grain, such as starch, proteins, fiber, and lipids, to obtain the purest possible fraction of each component (Haros and Wronkowska, 2017). The

main cereal used in wet-milling is corn (maize). In conventional wet-milling, corn is steeped in an aqueous solution containing sulfur dioxide (0.1–0.3%), an antimicrobial reducing agent, which solubilizes and disperses the proteinaceous matrix that envelops and binds the starch granules (Eckhoff and Tso, 1991; Calzetta-Resio et al., 2006). Modification of the structural characteristics, and the physicochemical and functional properties of starch owing to steeping and milling conditions has been reported in corn (Perez et al., 2001; 2003), wheat (Lorenz and Kulp, 1978), and rice (Lee et al., 2004). Wet-milling is a more complex process than dry-milling, and it is a source of a great variety of products. Although starch is the main product of wet-milling, there are other subproducts that are used for technological and food purposes, such as the fiber-rich and protein-rich fractions. The wet-milling of quinoa has not been widely studied yet, especially the optimum parameters and the steeping conditions such as time, temperature, pH, and stirring, among others. The steeping temperature is usually between 28 and 55 °C, because it must be below gelatinization temperature. The steeping time is conditioned by the type of grain, its morphology, and its size (Haros and Wronkowska, 2017). Changes in these parameters are important in starch isolation and its properties, determining its use (Haros and Wronkowska, 2017). Wright et al. (2002) used steeping with sodium hydroxide for 12 h at room temperature to isolate starch from varieties of sweet and bitter quinoa. Steffolani et al. (2013) and Jan et al. (2017a) isolated starch from the flour of several varieties of quinoa by steeping with NaOH.

The main objective of this research was to develop and optimize a quinoa wet-milling procedure for isolating the fractions of starch, proteins, and fiber at laboratory scale. The effect of steeping time and temperature on starch recovery and its quality was also investigated.

MATERIALS AND METHODS

Raw materials

Commercial Bolivian seeds of quinoa (*Chenopodium quinoa),* Organic red Quinoa Real© were purchased from ANAPQUI (La Paz, Bolivia).

Wet-milling procedure

Previous studies, in which different steeping solution temperatures, times, and pHs were evaluated, were used for reference purposes (Zheng et al., 1998; Calzetta-Resio et al., 2009; Wronkowska and Haros, 2014). Quinoa seeds (50 g) were steeped in 500 mL of sufficient sodium bisulfite to give a sulfur dioxide concentration of 0.25% at pH 5.0, adjusted with lactic acid. The wet-milling tests were performed according to a 3^2 factorial design (Table 1), and each experiment was conducted in duplicate. Two steeping variables – steeping time (1, 5, and 9 hours) and steeping temperature (30, 40, and 50 °C) – were assayed in a laboratory fermenter (Biostat Bplus, Sartorius, Spain) with constant control of temperature, pH at 5.0 adjusted with lactic acid, and stirring at 300 rpm. The steeped quinoa was separated into different fractions in two stages: a) the seeds were milled using a plate

mill (Corona, Lambers & Cia, Colombia) to separate the germ fraction by flotation in water. After separation, the germ fraction was washed with ultrapure water (1 L) to remove the residual starch content (Figure 1); b) the degerminated seed slurry obtained after the first milling was scattered/homogenized with a disperser (PT 10/35 GT, Polytron, Lucerne, Switzerland). The homogenization was performed with 200 mL of water at 15,000 rpm for 1 minute three times.

Table 1. Factorial design for sampling

Run	Name	Steeping Conditions		Coded Value	
		time, h	Temperature, °C	time, x_1	Temperature, x_2
1	WM1-30	1	30	-1	-1
2	WM1-40	1	40	-1	0
3	WM1-50	1	50	-1	1
4	WM5-30	5	30	0	-1
5	WM5-40	5	40	0	0
6	WM5-50	5	50	0	1
7	WM9-30	9	30	1	-1
8	WM-9-40	9	40	1	0
9	WM9-50	9	50	1	1

Then the homogenate was screened through two sieves (300 and 53 µm), where the hulls and protein fractions were retained, respectively. The fractions were washed with ultrapure water and the resulting suspension was centrifuged at 12,000 rpm for 15 min at 4 °C to obtain the starch fraction. After centrifugation it was possible to separate the pure starch from the tailings, the last were at the top of the pellet and were removed manually with a spatula (Wronkowska and Haros, 2014). The fractions were

dried in a forced-air oven at 40 °C overnight, and aliquots of the steepwater (SW) and washing water (WW) were dried at 70 °C in a forced-air oven to determine the soluble and suspended solids (total solids).

All fractions were stored in sealed plastic containers until their analysis in a chamber at 14 °C.

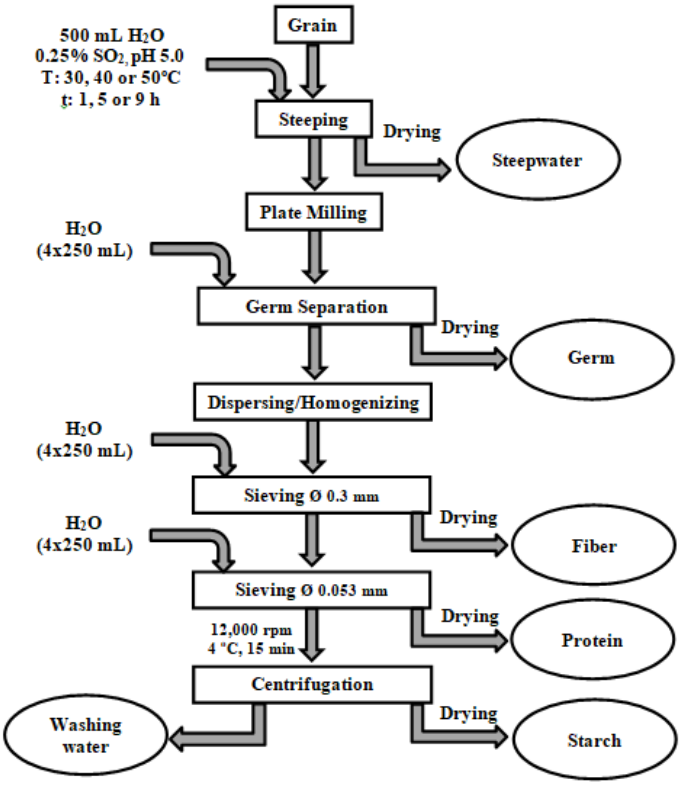

Fig. 1. Laboratory Quinoa wet-milling.

The yield of each fraction, expressed as a percentage, was calculated as the ratio of the totally dried isolated fraction to the initial amount of dried quinoa, as:

$$Yield\ (\%) = \frac{dry\ weight\ of\ separated\ fraction}{initial\ dry\ weight\ of\ grain} x100 \quad (1)$$

The starch recovery was calculated as the ratio of the dry weight of the isolated starch to the dry weight of starch in grain:

$$Starch\ recovery(\%) = \frac{dry\ weight\ of\ isolated\ starch}{dry\ weight\ of\ starch\ in\ grain} x100 \quad (2)$$

Physicochemical starch characterization

Moisture content was determined following the official assay procedure (Method 925.09, AOAC, 1996). Starch content was measured by the total starch assay procedure (AOAC, 1996). The protein and lipid contents were determined by the Dumas combustion method (Nx5.7) according to ISO/TS 16634-2 (2016) and the Soxhlet technique (Method 30-20, AACC 1995) with petroleum ether under reflux conditions, respectively.

The amylose and amylopectin contents were determined using a commercial assay kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) based on the Concanavalin-A method developed by Yun and Matheson (1990). The instrumental color of the starchy fraction was measured with a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan). The parameters determined were L* (lightness), a* (redness to greenness), and b* (yellowness to blueness), and the whiteness index (WI) was

calculated by the following equation: $WI=100–((100–L)^2+a^2+b^2)^{0.5}$. All the analyses were done in triplicate.

Determination of quinoa starch thermal properties

A differential scanning calorimeter (DSC-7, PerkinElmer, USA) was used to measure the thermal properties of the raw materials and starch fractions, and the amylopectin retrogradation. The DSC was calibrated with indium (enthalpy of fusion 28.4 J/g, melting point 156.4 °C). Samples were weighed into DSC pans (LVC 0319-0218, PerkinElmer), and ultrapure water was added to obtain a water:flour ratio of 3:1 in order to ensure complete gelatinization. After sealing, the pans were left for a few hours to equilibrate the humidity, and then they were scanned at a rate of 10 °C/min from 20 to 130 °C. Subsequently, the pans were stored at 4 °C for 2 days, and then heated again in the calorimeter from 20 to 130 °C at 10 °C/min to analyze amylopectin retrogradation. An empty pan (air) was used as a reference, and three replicates of each sample were analyzed. Thermal transitions of starch were defined in terms of onset temperature (T_o), peak (T_p), conclusion temperature (T_c), and enthalpy of gelatinization and amylopectin retrogradation (ΔH_G and ΔH_R, respectively), expressed in J/g of starch (Haros et al., 2004).

Pasting properties of quinoa starch

The pasting properties of the starch fractions were measured using a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriewood, Australia) according

to AACC Method 76-21 (1995). Distilled water (25 mL) was added to 3.0 or 3.5 g of sample placed in the aluminum RVA canister. The suspensions were stirred thoroughly at 160 rpm. The temperature was first maintained at 50 °C for 1 min and then raised to 95 °C at a rate of 12 °C/min, held at 95 °C for 2.5 min, cooled to 50 °C at the same rate, and finally held at 50 °C for 2 min. Pasting parameters evaluated included: pasting temperature (P_{temp}), peak viscosity (PV), peak time (P_{time}), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV-HPV), and setback (CPV-HPV). The experiments were conducted in triplicate.

Factorial design and statistical analysis

In order to study the effect of steeping conditions on starch recovery and quality, a factorial design was used. The independent factors studied were time (1, 5, and 9 hours) and temperature (30, 40, and 50 °C) at three levels. The run conditions of a 3^2 factorial design are shown in Table 1 in terms of experimental conditions and coded values. The design makes it possible to approximate the experimental data (Y_{obs}) with a response surface model expressed in coded values:

$$Y_{obs} = a_0 + a_1 x_1 + a_2 x_2 + a_{12} x_1 x_2 + a_{11} x_1^2 + a_{22} x_2^2 + \varepsilon \qquad (3)$$

In Equation 3, x_1 is the design factor steeping time, and x_2 is the steeping temperature. The coefficients a_1 and a_2 are the main effects of x_1 and x_2, respectively. 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_{12}) represent the interactions between factors. The difference between the experimental data (Y_{obs}) and the model (Y_{calc}) gives the residual (ε). For each response the RS-Q was calculated, which is the fraction of variation of the response explained by the model.

Statistical analysis

The multivariate analyses (stepwise regressions, multiple way analysis of variance, and correlation matrix) of the yields of quinoa fractions after wet-milling, and the physicochemical, thermal, and pasting properties of the starch fractions were performed using the Statgraphics® software package (Statistical Graphics Corporation, Virginia, Washington, DC, USA).

RESULTS AND DISCUSSION

Effect of steeping condition on yields

The composition of raw material used in this investigation was: moisture, 10.68±0.02; starch, 67±3; protein, 12.8±1.0; lipid, 7.3±0.6, and ash contents, 2.32±0.04 g/100 g in dry matter (d.m.).

The fraction yields obtained by quinoa wet-milling were: 7.1–13.3% of germ, 9.1–14.6% of fiber, 0.9–2.6% of protein, 54.9–58.5% of starch, 5.2–17.9% of total solids in steepwater, and 4.1–10.8% in washing water, expressed in dry matter. The starch yields in the current investigation were slightly higher than those reported by Wright et al. (2002), and much higher than those obtained by Jan et al. (2017a) using different steeping conditions and wet-

milling procedure. Wright et al. (2002) performed the steeping step in 0.30% NaOH at room temperature for 12 h. In the case of Jan et al. (2017b), quinoa seeds were steeped in alkali solution (0.20, 0.25, and 0.30% NaOH) at ~4 °C for 24 h.

The analytical data obtained from the factorial design for the yields of quinoa fractions obtained by wet-milling were fitted to multiple regression equations using three levels of two independent factors (Table 2) in order to estimate the dependence of yields (Eq. 3). The results obtained showed that the steeping time factor significantly affected the yields of the quinoa fractions (p<0.01).

Table 2. Factorial design coefficients of yield of by-products of quinoa wet-milling

Coefficient	Constant	Linear		Quadratic		Interaction	R-SQ
	a_o	a_1	a_2	a_{11}	a_{22}	a_{12}	
Germ	11.866	1.763**	1**				0.83
Fiber	9.444	-1.682**		1.479**		0.82**	0.88
Protein	1.118	-0.497**		0.472*			0.79
Starch	57.636		-0.789*	-1.689**		-1.055*	0.72
SW	13.787	4.565**	2.302**				0.94
WW	6.220	-2.152**					0.70

0.05 (*) and 0.01 (**) indicate statistically significant at the 95 and 99% confidence levels, respectively. SW: leached solids in steepwater, % in dry matter. WW: solids in washing water, % in dry matter. a_1 and a_2 are the coefficients of the main single effects of x_1 and x_2, respectively (x_1 is the design factor steeping time, x_2 is the steeping temperature). 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.

In general, when the steeping time increased, the germ yield and SW solids increased significantly (by 19–46% and 76–93%, respectively), whereas the

other yields decreased significantly (fiber, protein, starch, and WW fractions). The total solids (SW) leached in the steepwater increased significantly with steeping time at the expense of degradation of grain components during this step (Perez et al., 2003).

The steeping temperature individually promoted the largest increase in the germ yield (from 7.1-11.4 % (at 30ºC) to 10.5-12.8 % (at 50ºC)) and in the SW solid fraction (from 5.2-13.0 % (at 30ºC) to 8.1-17.9 % (at 50ºC)).

As a global tendency, the starch yields and recoveries decreased with the increase in steeping temperature, as indicated by the a_2 coefficient (Tables 2 and 3, respectively). In contrast, the steeping time, as a single independent variable, did not show any significant effect on the starch yields/recoveries. However, it was reported that corn starch yields increased as the steeping time increased (Perez et al., 2001). These discrepancies could be due to the ability of sulfur dioxide/lactic acid in the steepwater to disperse the protein matrix that envelopes the starch granules. This fact is less significant in quinoa than in corn, which the grain is harder and the starch is strongly linked protein matrix (Dailey, 2002; Perez et al., 2003; Wronkowska and Haros, 2014). In addition, there was a significant effect derived from the interaction between steeping time and temperature (a_{12}, Tables 2 and 3). On the other hand, as a result of the factorial design, the starch yield/recovery presented a maximum value in the domain studied, as represented by the negative quadratic coefficient of steeping time a_{11} (Tables 2 and 3, respectively).

The fiber yields also presented a significant interaction coefficient between the two factors studied (Table 2). The quadratic coefficient of the steeping time factor (a_{11}) was also significant for the fiber and protein yields, in both cases indicating a minimum value of these fractions within the domain studied (Table 2). On the other hand, the quadratic coefficient of the steeping temperature factor (a_{22}) was non-significant for any of the by-products.

It is observed that even though the increase of steeping time causes more solids to leach into the steep water, such an increase was particularly pronounced at 50ºC of steeping. It was reported that when lactic acid was present in the steepwater an increment of the proteolytic activity resulting from the action of that chemical (Perez et al., 2001). One of the main contribution to the increase of leached solids could be due to increased solubility of protein by the action of lactic acid, so the protein fraction decreased significantly with the steeping time (a_1: -0.497). On the other hand, when the steeping time increased the germ fraction augmented and the fiber fraction decreased probably due to the better separation of embryo and higher soluble fiber lost, respectively.

Starch recovery and physicochemical characterization

Results of starch recovery and physicochemical characterization in terms of factorial design coefficients are shown in Table 3. In general, as the steeping temperature increased the starch recoveries decreased, whereas the steeping time showing a maximum in this parameter (Table 3). However, it is

important to take into account that there was also a significant interaction between the two factors, as mentioned earlier. The absolute values of the starch recoveries were within the range 81.9±0.1–87.2±0.7% in dry matter. There are only a few investigations on quinoa wet-milling and the recoveries were not reported. Nevertheless, the results of the current investigation could be compared with previous data for cereals and/or other pseudocereals. The starch recovery/efficiency in corn was around 85.1% d.m. (Perez et al., 2003), in amaranth 67.7% d.m. (Calzetta-Resio et al., 2009), in buckwheat 64.6% d.m. (Wronkowska and Haros, 2014), and in rice 69.6% (Loubes et al., 2016), at the same order of magnitude as the current investigation.

With regard to the protein and damaged starch contents in the starch fraction, neither steeping time nor steeping temperature affected them significantly (Table 3). The results varied in the range 1.56±0.02–1.9±0.8% protein d.m. and 5.5±0.1–7.5±0.2% damaged starch d.m.

It was also observed that the amylose content of the starch decreased significantly only with the linear factor steeping time. This could be due to the higher hydrolysis of amylose during this step, as evidenced by the significantly higher amount of total solids in steepwater after 9 hours of steeping (from 5.2–8.1 to 13.0–17.9% d.m., for 1 and 9 h, respectively). On the other hand, the starch whiteness index quality parameter was significantly affected by the linear steeping temperature factor (Table 3), which decreased slightly with the increase in temperature (from 91 to 89% for 30 °C and 50 °C, respectively). The starch physicochemical properties of

quinoa obtained under the various steeping conditions by wet-milling were similar to the results reported by Steffolani et al. (2013), and slightly higher than those reported by Jan et al. (2017a, 2017b). In the current study the non-detection of lipids owing to efficient separation of the germ during the wet-milling procedure was a valuable result from the point of view of starch quality and in comparison with other investigations (Wright et al., 2002; Steffolani et al., 2013; Jan et al., 2017a).

Effect of steeping conditions on thermal properties

The DSC analysis of quinoa starch revealed how the steeping time and steeping temperature affected the thermal properties. Factorial design showed that the initial and peak temperatures of both gelatinization and retrogradation were significantly affected by both steeping factors. In general, they were higher when the steeping time and temperature increased (T_{oG}: from 51.2±0.1 °C to 55.4±0.5 °C; T_{pG}: from 58.6±0.1 °C to 61.1±0.1 °C; T_{oR}: from 35.6±0.2 °C to 40.0±0.5 °C; T_{pR}: 45.5±0.1 °C to 47.8±0.1 °C), whereas the conclusion temperatures were not affected significantly (T_{cG}: 68.2±0.3–70.1±0.2 and 55.6±0.5–56.8±0.5 °C). In addition, the factors showed a significant interaction in T_o and T_p of gelatinization, as well as a significant effect on the quadratic terms, which indicated the presence of a maximum in T_o (steeping time factor, α_{11}) and a minimum in T_o and T_p (steeping temperature factor, α_{22}) in the response surface, respectively.

Table 3. Factorial design coefficients of physicochemical, thermal, and pasting properties of quinoa starch isolated by wet-milling

Coefficient	Constant	Linear		Quadratic		Interaction	R-SQ
	a_o	a_1	a_2	a_{11}	a_{22}	a_{12}	
Physicochemical characteristics							
Recovery, % d.m.	85.898		-1.176*	-2.514**		-1.574**	0.72
Protein, % d.m.	1.674						0.65
Damage, % d.m.	4.481						0.36
Whiteness Index	91.788		-0.703*				0.74
Amylose, % d.m.	22.937	-2.232**					0.66
Thermal Properties							
Gelatinization							
T_o, °C	51.428	0.558**	1.573**	-0.74*	1.67**	0.957**	0.94
T_p, °C	58.656		0.632**		1.594**	0.796**	0.85
T_c, °C	68.719	-0.513**					0.71
ΔH_G, J/g of starch	13.132						0.45
Retrogradation							
T_o, °C	38.161	1.411**	0.694*				0.79
T_p, °C	47.078	0.822**	0.432*				0.81
T_c, °C	55.749						0.21
ΔH_R, J/g of starch	1.179						0.32
Pasting							
P_{temp}, °C	66.396		0.949*	-1.428*			0.64
P_{time}, min	6.915						0.29
PV, cP	3333.39	-230.25**					0.67
HPV, cP	2669.5	-296.0**					0.68
CPV, cP	3845.83	-181.67*					0.60
Breakdown, cP	664.11						0.55
Setback, cP	1170.61	105.0*		-139.17*			0.64

0.05 (*) and 0.01 (**) indicate statistically significant at the 95 and 99% confidence levels, respectively. DSC, Differential Scanning Calorimetry; T_o, onset temperature; T_p, peak temperature, T_c, conclusion temperature; ΔH_G, enthalpy of gelatinization, J/g in d.m.; ΔH_R enthalpy of retrogradation, J/g in d.m. RVA: Rapid Visco Analyser; P_{temp}, Pasting temperature; P_{time}, Peak time; PV, Peak viscosity; HPV, hot paste viscosity; CPV, final or cool paste viscosity; Breakdown: PV-HPV; Setback, CPV – HPV; cP, centipoise; d.m., dry matter. a_1 and a_2 are the coefficients of the main single effects of x_1 and x_2, respectively (x_1 is the design factor steeping time, x_2 is the steeping temperature). 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.

The increase in T_o and T_p, with a narrower gelatinization temperature range, suggests a partial annealing effect. This behavior was also observed in corn starch (Perez et al., 2001, 2003), in wheat starch (Lorenz and Kulp, 1978), and in rice starch (Lee et al., 2004) with an increase in steeping time. Annealing is defined as the heating of starch in excess water at subgelatinization temperatures, which are the conditions during steeping (Perez et al., 2001; Falade and Ayetigbo, 2015).

It may provoke partial melting of some crystals and realignment of starch chains in the amorphous regions, giving rise to more ordered crystals with higher melting points (Perez et al., 2001).

The steeping conditions did not significantly affect the enthalpy of gelatinization, as was also observed previously in corn wet-milling and rice wet-milling by other researchers (Perez et al., 2001; Lee et al., 2004). It was reported that annealing has no effect on ΔH_G (Knutson, 1990), but it was also stated that the more crystalline structure are before annealing, the less they can be enhanced by the annealing process (Alvani et al., 2012).

In the current investigation, the enthalpies of gelatinization and retrogradation were in the range of 12.4±0.3–13.5±0.1 J/g of starch for ΔH_G and 1.2±0.2–1.7±0.2 J/g of starch for ΔH_R, respectively. The results of enthalpy of gelatinization are in agreement with those reported by Wright et al. (2002) for starches isolated from several varieties of quinoa. On the other hand, Steffolani et al. (2013) reported slightly higher values in quinoa and kañiwa starches.

Results for enthalpy of retrogradation were higher, by ~26%, than those reported by Steffolani et al. (2013) after 14 days of storage at 4 °C. Srichuwong et al. (2017) reported retrogradation temperatures in accordance with our results (36.2–61.7 °C) after 6 days of storage at 4 °C. However, the enthalpy of retrogradation was higher (4.2 J/g starch) than in this study, probably owing to the longer storage time in their case.

Influence of steeping conditions on quinoa starch pasting properties

The values of the pasting property parameters were: from 3898 to 3064 cP for PV, from 3430 to 2231 cP for HPV, from 4340 to 3557 cP for CPV, from 7.00 to 6.72 min for P_{time}, and from 68.44 to 64.50 °C for P_{temp}. These results are in agreement with those reported by Wu et al. (2017) for starch from several varieties of quinoa. Steffolani et al. (2013) observed similar values, with the exception of P_{time} (5.17–4.97 min) and P_{temp} (62.7 °C), which were slightly lower than our results. In general, variation of steeping temperature did not modify the pasting properties, and no significant differences were found in the RVA parameters of quinoa starch with the exception of the pasting temperature (P_{temp}). This parameter increased slightly with the increase in temperature and presented a maximum (Table 3). It was observed that the linear effect of steeping time significantly affected the coefficients of the PV, HPV, and CPV parameters, causing a decrease in them when the time increased. In contrast, setback increased significantly as the steeping time increased. However, all these tendencies seemed to be

significant for the first 5 hours of steeping, whereas longer steeping times did not seem to modify the viscosity profile (Figure 3).

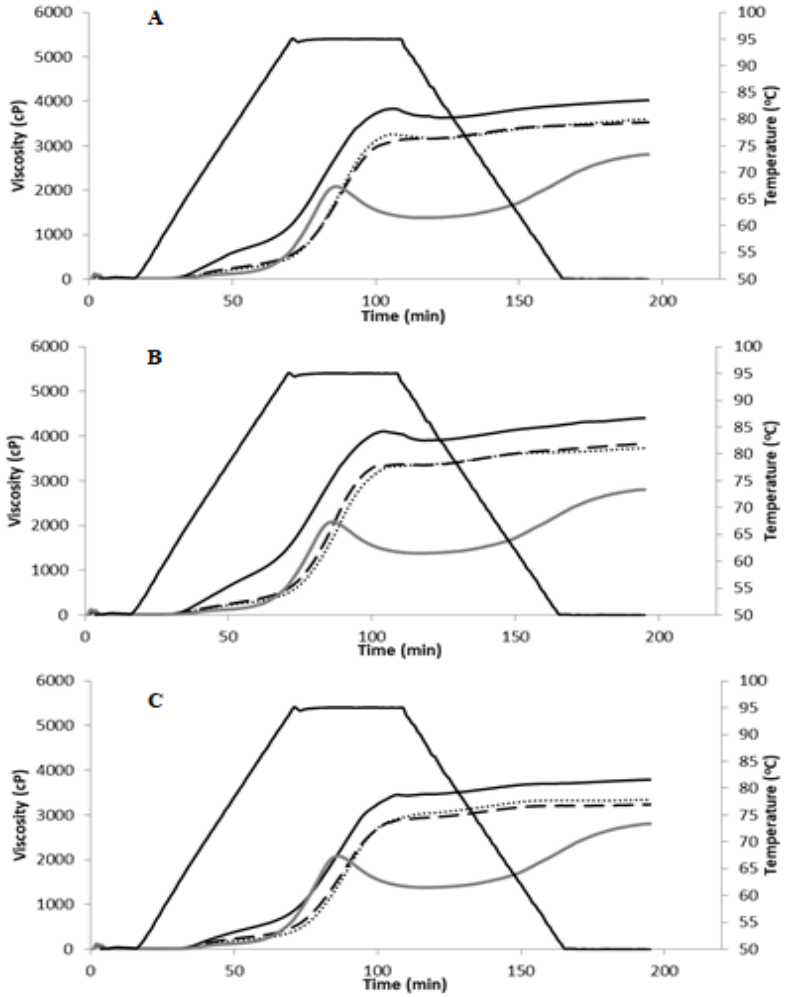

Figure 3. Effect of steeping conditions on pasting profile of starch obtained by wet-milling. **A.** 30 °C; **B.** 40 °C; **C.** 50 °C;
▬ 1 h; ▬ ▬ 5 h; …… 9 h; ▬ quinoa whole flour.

The PV decreased by about 11–14% between 1 hour of steeping and 9 hours of steeping. This parameter indicates the starch water-binding capacity and gives an indication of the viscous load.

A drop in its value is usually brought about by partial hydrolysis (Haros et al., 2004; 2006). The steeping chemicals, such as SO_2 and lactic acid, could hydrolyze the starch and reduce the pasting viscosities because they diffuse into the starch granules during their hydration and swelling (Shandera and Jackson, 1996; Haros et al., 2006). After reaching PV the swollen starch granules are easily broken and disintegrated by stirring, so the viscosity decreases to a minimum, the hot paste viscosity (HPV). During the hold period at 95 °C and mechanical shear stress, the starch granules disrupt and amylase molecules leach out into the solution (Haros et al., 2006). The HPV dropped significantly during the first hour of steeping at all the temperatures studied. After the cycle of heating and cooling a reassociation between amylose molecules occurs. If the concentration is sufficient they form a gel and the viscosity increases up to a final viscosity (CPV) which involves the retrogradation phenomenon. In the current investigation CPV also dropped significantly in the starch fraction after steeping, probably owing to the partial loss of amylose. As described above, the amylose content of starch decreased significantly with the steeping time factor (Table 3), from 23.1±0.8–26.5±0.4% to 19.2±0.2–24.0±0.2% for 1 hour and 9 hours of steeping, respectively, which could explain the changes observed in pasting properties (Figure 3).

The values of the pasting property parameters were: from 3898 to 3064 cP for PV, from 3430 to 2231 cP for HPV, from 4340 to 3557 cP for CPV, from 7.00 to 6.72 min for P_{time}, and from 68.44 to 64.50 °C for P_{temp}. These results are in agreement with those reported by Wu et al. (2017) for starch from several varieties of quinoa. Steffolani et al. (2013) observed similar values, with the exception of P_{time} (5.17–4.97 min) and P_{temp} (62.7 °C), which were slightly lower than our results.

Optimization of steeping conditions

The calculation of the optimum experimental conditions to be used was performed for the recovery parameter, taking into account the starch physicochemical properties of quinoa in terms of protein content, damaged starch, and whiteness index. The effects of time (x_1) and temperature (x_2) on starch recovery were satisfactorily simulated by Equation (3). The maximum expected starch recovery (85.7%) occurred at 6.5 h of steeping time at 30 °C. Expected responses for steeping time and temperature factors in comparison with values reported by other authors are shown in Table 4. In general, the expected values were higher than those reported in other studies. This expected response was corroborated experimentally. The expected responses were tested, and the results were: 86.7±1.6% (d.m.) for starch recovery, 62.3±0.8% for starch yield, 1.74±0.05% of protein, and 91.8±0.08% of whiteness. The differences between the experimental and expected responses presented a deviation of only 2%.

Table 4. Expected yield, recovery, and physicochemical composition of quinoa starch fractions, and comparison with other investigations

Parameter of Starch Fraction	Units	Current Investigation[a]	Jan et al., 2017a,b	Steffolani et al., 2013	Wright et al., 2002
Yield	%.	61.89	48.52	NR	53.3
Recovery	%	85.68	NR	NR	NR
Protein	%	1.78	0.95	1.09	0.46
Lipids	%	ND	0.40	1.94	NR
Whiteness Index	%	91.45	NR	NR	NR
Amylose	%	23.9	12.1	17.4	20.6
Damaged Starch	%	4.65	NR	NR	NR

[a]expressed in dry matter. ND: Not detected; NR: Not reported. Steeping conditions: Jan et al. (2017a, 2017b): 0.25% NaOH, 24 h; Steffolani et al. (2013): 0.25% NaOH, 12 h; Wright et al. (2002): 0.30% NaOH, 12 h.

CONCLUSIONS

The quinoa wet-milling process proved to be a potential procedure for obtaining various valuable components of quinoa grains. The factorial design showed that the variables steeping time and temperature affect the parameters significantly, increasing or decreasing their values, depending on the parameter analyzed. The wet-milling process developed in this study achieved a high level of starch recovery from quinoa. Maximum response values were obtained when the steeping time was set at 6.5 hours and the steeping temperature at 30 °C.

It is still necessary to study quinoa starch more deeply, because its propitious properties may have application potential in areas such as novel food additives, fat replacement, pharmaceuticals, cosmetics, papermaking, and textiles. Finally, the economic cost of steeping operating conditions and

the starch quality/recovery obtained by using a wet-milling procedure should be evaluated in order to find the most suitable conditions at industrial level.

Acknowledgments

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CAPÍTULO 5

Isolation of red quinoa fibre by wet- and dry-milling and aplication as a potential functional bakery ingredient

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ABSTRACT

Quinoa is recognised internationally for its nutritional and health properties. It has interesting attributes, such as being an excellent source of fibre and polyphenols and being gluten-free, and therefore this grain is used as a replacement for cereals. The main aim of this work was to study the effect of two milling methods, wet and dry, to obtain a dietary fibre-rich fraction from red Quinoa Real, and to determine its potential as a functional ingredient in bakery products. Wet milling produced a higher yield (10.1%) and recovery (58.2%) of the fibre fraction and higher purity (72%) than the values obtained by dry milling (9.1, 52.5 and 59%, respectively). With regard to functional properties, dry milling produced fibre with higher total antioxidant activity than that obtained by wet milling (FRAP: 1.1 times more). The fibre-rich fractions obtained by the two processes did not differ considerably in terms of colour, but the process affected their granulometry, which was lowest in the fibre obtained by wet milling, and the dispersity was greatest. Moreover, the bread products made with a 5% incorporation of either of the two fibres presented enrichment in terms of nutrients, dietary fibre and antioxidant capacity in comparison with the control sample. The inclusion of fibre isolated by dry milling produced bread products of higher technological quality with regard to specific loaf volume and less crumb firmness.

INTRODUCTION

Pseudocereals are a source of starch, proteins, lipids, dietary fibre, vitamins and minerals (Haros and Schoenlechner, 2017). As they also contain bioactive compounds such as polyphenols, pseudocereals have attracted increasing attention because of their potential for the development of functional foods (Wang and Zhu, 2016; Haros and Schoenlechner, 2017; Ballester-Sanchez et al., 2019a). Research in recent years has concentrated on the development of functional foods with an impact on health in regard to the prevention of metabolic disorders such as obesity and hypertension (Haros and Schoenlechner, 2017). Dietary fibre is of particular importance because of its involvement in the maintenance of a good state of health. However, its use in the food industry still presents a challenge because of its various physico-chemical and techno-functional characteristics, and its inclusion in the making of foods could affect their final quality positively or negatively as a result of changes in colour, texture, taste and water holding capacity, among other things (Lazaridou et al., 2007).

Among pseudocereals, quinoa is a raw material with technological, sensory, nutritional and functional potential for use as a source of food ingredients in products with or without gluten, such as bread and other bakery products, snacks and breakfast cereals, infant foods, fermented beverages, pasta and traditional, exotic or haute cuisine foods (Haros and Schoenlechner, 2017; Ballester-Sánchez et al., 2019ab). Various methods for obtaining dietary fibre from diverse sources have been proposed, and the isolation conditions tend to be the parameters that most affect the chemical composition and techno-

functional properties (Tejada-Ortigoza et al., 2017). The fibre in cereals/pseudocereals can be obtained by a process of milling followed by fractioning to obtain intermediate products (primary processing) that can then be used to make end products (secondary processing). The types of cereal milling are normally classified as dry milling or wet milling. In dry milling the aim is the individual separation of the anatomical parts of the grain, after conditioning with small quantities of water, in order to obtain, mainly, refined flour, bran and germ (Haros and Schoenlechner, 2017). However, as its name indicates, wet milling is performed with considerable quantities of water and the aim is to separate the chemical components of the grain – starch, proteins, fibres and lipids/oil – in order to obtain fractions of those components with the greatest purity possible (Haros and Schoenlechner, 2017). Traditionally, in the countries where quinoa originates, stone mills or rollers have been used to produce whole quinoa flour. Owing to the small size of quinoa grains (1.0–2.6 mm), and the morphological and structural differences between quinoa and cereals, the fractioning and/or separation systems typically used for wheat, corn or rice are not very efficient. Various methods have been employed for laboratory-scale fractioning of quinoa. However, there is still a need for intense investigation of the application of dry and wet milling processes in order to fraction quinoa with a high degree of recovery and purity (Haros and Schoenlechner, 2017).

There have been reports of the health benefits associated with the consumption of quinoa to reduce the risk of suffering diseases connected

with oxidative stress such as cancer and diabetes and cardiovascular and neurodegenerative disorders (Simnadis et al., 2005; Pasko et al., 2010). This beneficial effect is mainly attributable to polyphenols, compounds with antioxidant properties, among others (Langhans, 2018), the presence of which in quinoa has been demonstrated (Tang et al., 2015 and 2016). The data concerning polyphenols that are available in the literature for foods generally correspond to polyphenols obtained in organic/aqueous extracts (extractable or soluble polyphenols), but they do not take account of the significant quantities of polyphenols that remain in the residues (hydrolysable polyphenols) (Pérez-Jiménez et al., 2013). These latter compounds are associated with dietary fibre, and therefore it is especially important to determine their concentrations in fibre fractions. In this regard, the availability of a fibre-rich fraction with antioxidant properties would offer many advantages for the health.

The main aim of this work was to obtain a fibre-rich fraction from red Quinoa Real (royal quinoa) by dry milling and wet milling. The yields of the two methods were determined and the physico-chemical and nutritional properties of the resulting fibre fractions were compared. The polyphenol concentration and antioxidant activity of the extractable and hydrolysable fractions were also determined, providing a complete estimation of the contribution of each of the fibre fractions. The potential of the fibres obtained as a breadmaking ingredient was also studied by including them at a proportion of 5% in bread formulations, and the results were compared

with control samples in terms of technological, nutritional and functional quality.

MATERIALS AND METHODS

Materials

Commercial Spanish wheat flour and red quinoa seeds (organic Quinoa Real), marketed by ANAPQUI (La Paz, Bolivia), were purchased from local suppliers (La Meta, S.A., and Ekologiloak, Spain). Compressed yeast (Saccharomyces cerevisiae, Levital, Spain) was used as a fermentation starter.

Dietary fibre isolation procedure

In this work two methods were used for isolating dietary fibre: wet milling (WM) and dry milling (DM). The yield of each fraction obtained by milling was calculated as:

$$Yield\ (\%) = \frac{dry\ weight\ of\ separated\ fraction}{initial\ dry\ weight\ of\ grain} \times 100$$

The dietary fibre recovery was calculated as:

$$Fibre\ recovery(\%) = \frac{dry\ weight\ of\ isolated\ fibre}{dry\ weight\ of\ fiber\ in\ grain} \times 100$$

Wet milling procedure

Quinoa seeds (300 g) were steeped in 3 L of sufficient sodium bisulfite to give a sulfur dioxide concentration of 0.25% at pH 5.0, adjusted with lactic

acid. The wet milling process was carried out following the methodology proposed by Ballester-Sánchez et al. (2019c) with slight modifications (Fig. 1). Previously, the experimental variables of the steeping conditions (time/temperature) were optimised for isolation of the dietary fibre fraction. The calculation of the optimum experimental conditions to be used was performed for the yield parameter, taking into account the physico-chemical properties of the dietary fibre fraction of quinoa in terms of starch, protein, lipid and ash contents. The fibre fractions were stored in vacuum-sealed polyethylene bags until their analysis. The assays were carried out in triplicate.

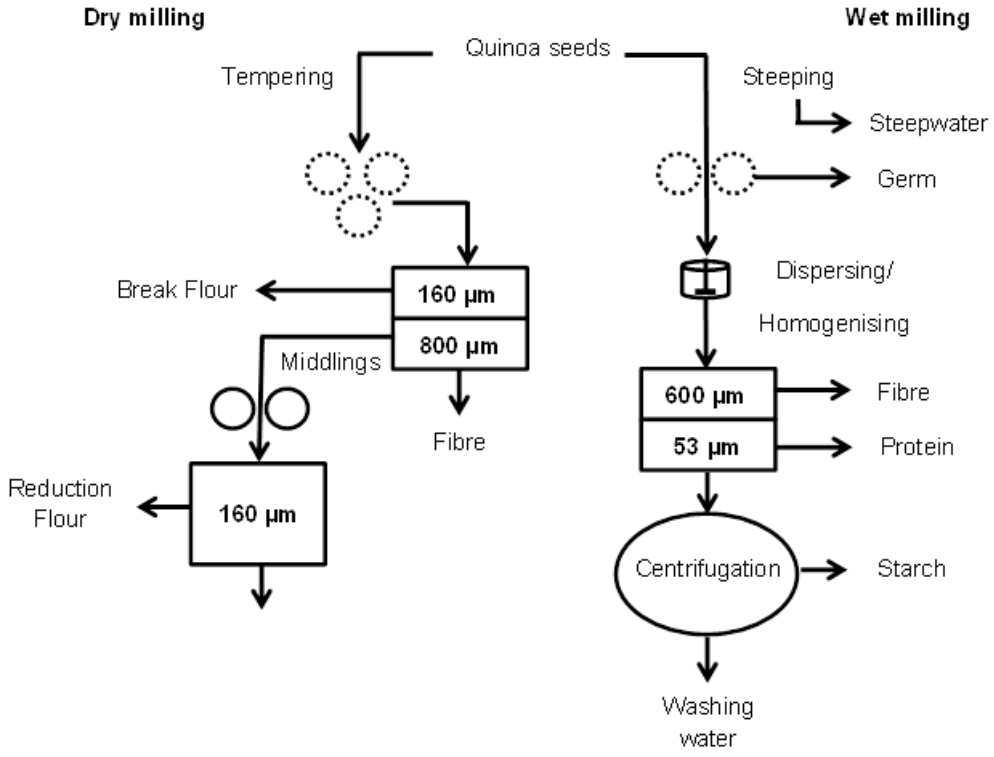

Fig. 1. Flow chart of dry and wet milling of quinoa.

Dry milling procedure

Before milling, the quinoa seeds were tempered according to Method 26-95 (AACC, 2000) to reach the desired moisture of 16% w/w. Quinoa seeds (500 g) were ground using a CD1 laboratory mill (Chopin Technologies, Villenueve La Garenne, France) following the International Approved Method 26-70.01 (AACC, 2000) (Fig. 1). Tempered quinoa was first passed through a set of break rolls. Bran, middlings and break flour were produced and separated using a rotary sieve, and these portions were collected separately. Then the bran stream was run once more to optimize the flour extraction of the mill. The bran and break flours obtained were not processed further (fibre fraction and shorts, respectively). The middlings were processed further through a set of reduction rolls and split into shorts and reduction flour. The fibre fractions were stored in vacuum-sealed polyethylene bags until their analysis. The milling procedure was carried out in triplicate.

Chemical proximal composition

Moisture, dietary fibre and starch in seeds, dietary fibre fractions and breads were determined according to Approved Methods 925.09, 991.43, 996.11 and 986.11, respectively (AOAC, 1996).. Protein determination was carried out by the Dumas combustion method (N conversion factor 5.7) according to ISO/TS 16634-2 (2016). Lipid and ash contents were determined according to official methods ((Method AACC 30-25.01, 2000 and Method ICC 104/1, 1990). All the analyses were done in triplicate.

Colour analysis

The colour of the seeds, dietary fibre fractions and breads was measured by a digital colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Japan). Colour differences were expressed in accordance with the CIELab colour space. The parameters measured were L* (lightness), a* (redness to greenness) and b* (yellowness to blueness). Each sample was measured 3 times to minimize the heterogeneity in the seed/fibre colours. ΔE* was calculated to estimate the difference in colour between the samples and the control.

Fibre particle size distribution

The particle size distribution of the dietary fibre fractions was determined by laser diffraction analysis (Malvern Instruments Ltd., Malvern, England), using a testing instrument equipped with a sample dispersion unit for dry samples. Measurements were run in triplicate at room temperature. Size distribution was quantified as relative volume of particles in size bands presented as size distribution curves (Malverin MasterSizer Micro software v. 5.60). Particle size distribution was described by the following parameters: largest particle size (D90), median diameter (D50 – the size at which 50% of particles, by volume, are smaller and 50% are larger), smallest particle size (D10), and mean particle diameter (D[4.3]). The D-values (D10, D50 and D90) are the intercepts for 10%, 50% and 90% of the cumulative mass, respectively.

The dispersity was calculated using the following equation:

$$Đ = \frac{D90 - D10}{D50}$$

Extraction of extractable and hydrolysable phenolic compounds

Polyphenol extractions were performed as was done previously (Ballester-Sánchez et al., 2019b), following the method of Saura-Calixto et al. (2007) with some modifications. The extractable polyphenol fractions (EPF) were obtained from 1 g of sample (grain flours and dietary fibres) in two consecutive 1-hour incubation steps (10 mL acidic methanol/water 50:50, v/v; pH 2 and 10 mL acetone/water 70:30, v/v). The hydrolysable polyphenol fractions (HPF) were obtained by acidic hydrolysis performed with the resulting residues using 20 mL of methanol and 2 mL of concentrated sulphuric acid (85 °C for 20 h). Residues were eliminated by filtering through glass Buchner funnels and the pH was adjusted to 5.5 as described by Hartzfeld et al. (2002). Samples were stored at –20 °C until use.

Polyphenol content

The Folin–Ciocalteu method was used to determine polyphenol content (PC) in both EPH and HPF (Singleton and Rossi, 1965). Determinations were conducted in three independent extracts with a spectrophotometric microplate reader (Spectrostar Nano, BMG Labtech, Ortenberg, Germany),

and results were expressed as mg of gallic acid equivalents (GAE)/g sample dry matter (d.m.).

Antioxidant capacity

Antioxidant capacity (AC) was determined as was described previously (Ballester-Sánchez et al., 2019b), using two different methodologies adapted to microplate format, capacity to reduce α-diphenyl-β-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power assay (FRAP) according to Brand-Williams et al. (1995) and Benzie and Strain (1996), respectively. Determinations were conducted in three independent extracts with a spectrophotometric microplate reader (Spectrostar Nano, BMG Labtech, Ortenberg, Germany), and results were expressed as µmol of Trolox equivalents (TE/g sample dry matter (d.m.).

Breadmaking procedure

The control bread formula consisted of wheat flour (300 g), compressed yeast (3 g/100 g), sodium chloride (1.6 g/100 g) and distilled water (up to optimum absorption, 500 Brabender Units, 64.1 g/100 g flour basis, AACC Method 54-21, 1995). The dietary fibre fraction isolated by wet and dry milling was incorporated in the control bread dough formula at a proportion of 5 g/100 g on flour basis. In the positive control bread formula part of the wheat flour was replaced with whole quinoa flour, 26 g/100 g on flour basis, to achieve a similar proportion of fibre as in the 5% fibre formulations. The ingredients were mixed for 7 min, and the doughs were kneaded and divided

into pieces weighing 100 g. The doughs were fermented in a chamber at 85% of relative humidity (Termaks, Bergen, Norway) for 75 min at 28 °C. After the fermentation step, the dough was baked in an electric oven (Binder, Germany) at 180 °C for 29 min. Finally, the bread was cooled at room temperature for 60 min for subsequent analysis. Each formula was prepared in triplicate.

Technological parameters

The technological parameters analysed were the weight (g) and specific volume (mL/g) of the loaf, the shape ratio (cm/cm) and the crumb firmness, using a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) with a 35 mm flat-end aluminium compression disc (Gámbaro et al., 2004). The experiments were conducted in triplicate.

Statistical analysis

One-way and multi-way ANOVA and Fisher's least significant differences (LSD) were applied to establish significant differences between samples using the Statgraphics Plus 16.1.03 software. The pairwise comparisons to estimate the effect of baking on the AC of the breads were performed by a Student's t test (Microsoft Excel, 2010). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Yields and recoveries of fractions obtained by wet and dry milling

The operating conditions for steeping the quinoa grain prior to the wet milling step were based on obtaining the maximum yield of the fibre fraction with the greatest possible purity (Ballester-Sánchez et al., 2019c). The conditions were 1.25 h of grain steeping in a solution of SO_2 at 30 °C, which theoretically should provide a yield of 11.0% according to the theoretical design. The fractions obtained by wet milling of quinoa are shown in Figure 2A. The yields were: 7.1% of germ, 2.6% of protein, 62.2% of starch and 10.1% of fibre (WM-fibre), expressed in dry matter (Table 1). These yields are related to those reported by Ballester-Sánchez et al. (2019c) in a process optimised for the recovery of quinoa starch by wet milling.

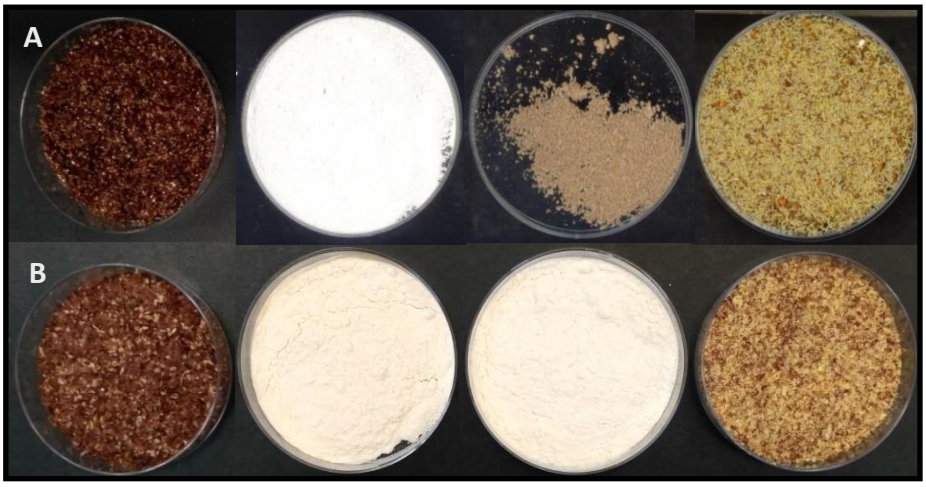

Fig. 2. Fractions obtained by wet and dry milling of quinoa.

The few works that have been published on wet milling of quinoa concentrate their attention on the isolation of the individual fractions for subsequent analysis, without reporting values for the yield and/or recovery of the fractions (Haros and Schoenlechner, 2017).

However, yields of fibre fractions obtained from pseudocereals by wet milling have been reported. In the present study the yields obtained were slightly lower than those observed by Zheng et al. (1998) in the fibre-rich fraction obtained by wet milling of buckwheat, but their purity was considerably lower. Similarly, Calzetta Resio et al. (2009) reported fibre-rich yields from amaranth ranging between 21.6 and 35.5%, but with a high protein content.

The fractions obtained by dry milling of quinoa are shown in Figure 2B. The yields were 9.1% of bran (DM-fibre), 25.5% of break flour, 27.3% of reduction flour and 37.5% of shorts, expressed in dry matter. There are various methods for dry milling of quinoa grain on laboratory scale, and the use of roller mills (Brabender Quadra) or cutting and grinding mills (Wiley) and various kinds and sizes of sieves has been described in the literature (Haros and Schoenlechner, 2017). However, there are no reports describing fractioning of quinoa using the CD1 mill (Chopin, France). This mill, designed for dry milling of soft wheat/bread wheat, has been used for fractioning of barley, with slightly higher reported yields of break flour and bran fractions and lower yields of shorts and reduction flour fractions in comparison with those obtained in the present study (Flores et al., 2005). However, the starch concentration in the bran fraction reported by those authors was almost

double the concentration observed in the present study, indicating lower purity.

The recovery obtained by wet milling was 58.2%, significantly higher than the 52.5% recovery obtained by dry milling (Table 1). As the purpose of wet milling is to isolate the components of the grain, and in order to do so the grain is steeped in special conditions, it is to be expected that the yield and purity of the fibre fraction would be higher than those obtained by dry milling.

Physico-chemical characteristics of quinoa fibre isolated by milling

Chemical Composition

The composition of the quinoa that yielded the fractions isolated by wet and dry milling is shown in Table 1. No significant differences in the soluble fibre content were observed in the fibre obtained by the two methods (Table 1). However, the quantity of insoluble fibre was significantly ($p < 0.05$) greater in the WM-fibre than in the DM-fibre. This difference affected the total fibre content, which was higher ($p < 0.05$) in the WM-fibre than in the DM-fibre. These differences were basically due to the fact that in the dry milling the insoluble fibre was redistributed between the shorts and flour fractions (Fig. 2B), as reported in the case of dry milling of barley using the same kind of mill (Flores et al., 2005). Little research has been done on the isolation of fibre from quinoa. The methods described in the literature generally use complete milling of the grain and then treatment with enzymes and/or

ultrasound in aqueous medium or else fractioning by sieving (Kurek et al., 2018; Opazo-Navarrete et al., 2019). Kurek et al. (2018) reported a fibre fraction purity after enzyme treatment similar to the purity obtained in the present study, and a lower value when ultrasound was used. Similarly, Opazo-Navarrete et al. (2019) reported less purity in the fibre fraction after fractioning by sieving. In this regard, the dry milling method developed for soft wheat/bread wheat and adapted to quinoa in the present study is more efficient for performing the fractioning of this pseudocereal.

On the other hand, in the WM-fibre there was a significantly smaller lipid content than in the DM-fibre ($p < 0.05$). As the lipids are mainly concentrated in the germ (embryo), the presence of 3.7% lipids in d.m. in WM-fibre compared with 6.0% d.m. in the DM-fibre is indicative of better separation of the germ in wet milling (Table 1). Moreover, in wet milling the germ is separated whole, whereas in dry milling it suffers splitting, which could contaminate the other fractions (Figures 2A and 2B). In wet milling one would expect that washing the WM-fibre fraction with water would help to achieve a higher elimination of starch from that fraction, but that was not significant (Table 1). This may have been due to the short steeping time used in the present study, 1.25 h, compared with the 6.25 h needed for efficient separation of starch from red Quinoa Real (Ballester-Sanchez et al., 2019c).

Color

With regard to the colour of the two fibre fractions, there were significant differences, mainly in the lightness parameter (Table 1).

Table 1. Chemical composition, antioxidant capacity and physical parameters of quinoa and its fibre isolated by wet and dry milling.

Parameter	Units	Whole quinoa flour	WM-fibre fraction	DM-fibre fraction
Dietary fibre				
Yield	g/100 g d.m.	--	10.1±0.3b	9.1±0.1a
Recovery	g/100 g d.m.	--	58.2±1.6b	52.5±0.8a
Soluble	g/100 g d.m.	1.5±0.1a	8.16±0.62b	8.23±0.35b
Insoluble	g/100 g d.m.	15.86±0.06a	60±1c	50±1b
Total	g/100 g d.m.	17.4±0.1a	68.2±1c	59±1b
Main components				
Moisture	g /100 g d.m.	10.60±0.03a	12.0±2.7a	16.9±0.3b
Starch	g /100 g d.m.	64.3±0.7c	27.9±0.2b	22.9±1.7a
Protein	g /100 g d.m.	15.2±0.1a	14.2±0.4a	15.3±1.2a
Lipid	g /100 g d.m.	6.83±0.01c	3.7±0.3a	6.0±0.3b
Ash	g /100 g d.m.	2.21±0.07a	2.71±0.04b	3.13±0.05c
Phenolic content				
EPF	mg GAE/g d.m.	4.93±0.07b	3.84±0.28a	6.63±0.06b
HPF	mg GAE/g d.m.	34±1a	77±5b	83±7b
Total (EPF+HPF)	mg GAE/g d.m.	39±1a	81±5b	89±7b
Antioxidant capacity				
DPPH				
EPF	µmol TE/g d.m.	6.04±0.09c	3.63±0.86a	5.16±0.09b
HPF	µmol TE/g d.m.	63±9a	196±11b	205±6b
Total (EPF+HPF)	µmol TE/g d.m.	69±9a	200±11b	210±5b
FRAP				
EPF	µmol TE/g d.m.	6.9±0.1b	3.6±0.4a	6.9±0.1b
HPF	µmol TE/g d.m.	43±1a	152±4b	165±4c
Total (EPF+HPF)	µmol TE/g d.m.	50±2a	156±5b	172±4c
Colour				
L*	-	38±1a	36.1±0.4a	39±1b
a*	-	14.0±0.4b	9.8±0.5a	9.3±0.4a
b*	-	15.3±0.6b	10.9±0.5a	10.9±0.7a
ΔE	-	-	6.5±0.5a	6.6±0.2a
Particle size				
D[4.3]	µm	-	903±17a	994±11b
D10	µm	-	444±19a	564±21b
D50	µm	-	851±18a	947±11b
D90	µm	-	1455±14a	1503±35a
Đ	µm	-	1.19±0.03b	0.99±0.06a

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. Abbreviations: d.m. dry matter; WM, wet milling; DM, dry milling; EPF, extractable polyphenol fraction; HPF, hydrolysable polyphenol fraction; DPPH, α-diphenyl-β-picrylhydrazyl; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalent; TE, Trolox equivalent; L*, (lightness); a*, (redness to greenness); b*, (yellowness to blueness); $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$; D90, largest particle size; D50, median diameter; D10, smallest particle size; D[4.3], mean particle diameter; Đ, dispersity.

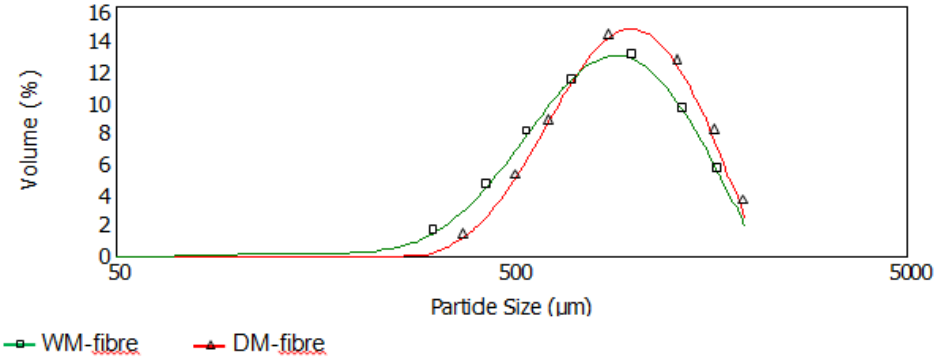

Fig. 3. Particle size distribution of quinoa fibre fraction obtained by wet milling (WM-fibre) and dry milling (DM-fibre).

The reduction in the value of the L* parameter of the WM-fibre may have been due to loss of pigments and/or waxes during steeping, or to changes in those compounds caused by the acid pH of that step, as occurs with other grains (Bayram et al., 2004; Mukherje et al., 2019). On the other hand, the higher contamination of germ in the fibre fraction obtained by dry milling could lead to higher lightness values simply because of the germ's whitish colour (Figure 2). Although the value of L* was significantly higher in the DM-fibre, that was not enough to induce a significant marked colour change (ΔE*) between the two fibre fractions (Figure 2).

Particle size distributions

Particle size is an important parameter because it affects the functionality of the fibre in the human organism (Peerajit et al., 2012), and it also has an effect on the quality of products made with it (Noort et al., 2010; Wang and Zhu, 2016). The two fibres had a clearly unimodal distribution (Fig. 3). The

D50 value of the WM-fibre was significantly lower than that of the DM-fibre, indicating that the quinoa wet milling method developed in the present study produced fibre with a smaller particle size than the fibre obtained with the dry method (Table 1).

During the steeping process the quinoa grains become softer and break up easily, thus leading to higher disintegration of the outer layers in the milling process. The dispersity (Đ), which is generally used to evaluate the particle size distribution of samples, was significantly higher in the WM-fibre than in the DM-fibre (Table 1). This may have been due to the formation of small clusters of fibre particles isolated by wet milling owing to the anisotropic nature of natural fibres and their hydrophilic nature because of the various kinds of forces of attraction between their various structural monomers during the drying process, leading to the formation of small clusters (Dugyala et al., 2013; Sorieul et al., 2016).

Polyphenol content (PC) and Antioxidant Activity (AC)

The WM-fibre showed significantly ($p > 0.05$) lower PC values in the EPF (extractable polyphenol fraction) than the raw material, whereas they were the same in the case of the DM-fibre ($p > 0.05$). This result was to be expected, given that EPF consists of polyphenols that are soluble and can therefore be leached in the steeping water used during the washes applied to obtain it, as reported by Mukherje et al. (2018) and Yu et al. (2018) with regard to steeping of soy flour and wet milling of buckwheat, respectively. In both fibres the HPF (hydrolysable polyphenol fraction) was clearly enriched

in comparison with the quinoa flour (around 2.5-fold). HPF represents the polyphenols bound to cell wall macromolecules and to dietary fibre, so its value increases in the fibre samples (Pérez-Jiménez and Saura-Calixto, 2005). In general, a positive linear correlation between total polyphenol content and antioxidant activity has been reported in extracts of quinoa (Pasko et al., 2009, Tang et al., 2015; Pellegrini et al., 2018). In our case, AC followed the same tendency as PC except with regard to the level of DPPH in the EPF of DM-fibre, for which the values obtained were significantly lower ($p < 0.05$) than those obtained for whole quinoa flour even though the corresponding PC values were equivalent ($p > 0.05$). That could be due to the loss of non-phenolic compounds with antioxidant activity (Dini et al., 2010) and efficient in scavenge the DPPH free radical. Both the whole quinoa flour and the fibres had much higher PC and AC values in HPF than in EPF (PC: 6.9-fold in whole quinoa flour, between 12.5 and 20.1-fold in fibre; AC_{DPPH}: 10.5-fold in whole quinoa flour, between 54.0 and 39.7-fold in fibre; AC_{FRAP}: 6.2-fold in whole quinoa flour, between 42.1 and 23.8- fold in fibres). This notable contribution of HPF agrees with what has been reported in the literature for other foods and ingredients (Pérez-Jiménez et al., 2013) and for whole quinoa flours (Ballester-Sánchez et al., 2019a). HPF is not digested by digestive enzymes and travels directly to the colon, where it performs a beneficial activity, especially on a gastrointestinal level and possibly in other organs and tissues, after fermentation by microbiota (Pérez-Jiménez et al., 2013). The total polyphenol content (TPC: PC in EPF + PC in HPF) and the total antioxidant activity (TAC: AC in EPF + AC in HPF) were calculated to estimate the

complete contribution of the two fibres. The values showed that the two milling processes yielded fibres with a bioactivity that was significantly higher ($p < 0.05$) than that of the whole quinoa grain from which they were obtained (TPC: around 2-fold increase, $TAC_{DPPH/FRAP}$: around 3-fold increments). Furthermore, dry milling was more effective than wet milling with regard to TAC_{FRAP} (1.1 fold increase), although no differences were observed between them with regard to TPC.

Quinoa fibre as a breadmaking ingredient

Various bread formulations were prepared in order to study the behaviour of the quinoa fibre isolated by milling as a breadmaking ingredient. The composition of the wheat flour used in the breadmaking was: moisture, 11.36 ± 0.03; starch, 72 ± 1; protein, 13.74 ± 0.09; lipids, 1.13 ± 0.03; soluble fibre, 1.2 ± 0.5; insoluble fibre, 3.2 ± 0.3; total fibre, 4.4 ± 0.1; and ash, 0.57 ± 0.01 g/100 g in dry matter (d.m.). The physico-chemical parameters of the products that were made are shown in Table 2. Proximal characterisation of the products with quinoa fibres and with whole quinoa flour showed a significantly higher moisture content than that of the wheat bread (Table 2). The fibres contain cellulose and other polysaccharides that retain a quantity of water up to several times higher than their own weight. Therefore, the increase in the amount of fibre was accompanied by an increase in the moisture content of the final product, as reported by other researchers who included fibre and/or bran (Ajmal et al., 2006; Ballester-Sánchez et al., 2019b). There were also significant differences in the total starch content of

the loaves, which presented the same tendency as was found in the analysis of the corresponding raw materials (quinoa bread < DM-fibre bread < WM-fibre bread < wheat bread). The products made with 5% of fibres isolated by wet or dry milling had contents that were significantly lower than those of the bread with whole quinoa flour and higher than the control with regard to protein content, lipids and ash (Table 2). However, no significant differences in any of these parameters were observed between the DM-bread samples and the WM-bread samples. With regard to dietary fibre contents, there were no significant differences in the soluble and insoluble fibre of the formulations made with the two fibres despite the differences found in their composition, nor in the products made with 26% of whole quinoa flour. The total fibre content of the formulations with quinoa fibre in the present study was higher than or similar to the values reported by other researchers who incorporated a high proportion of fractions rich in defatted rice bran or pearled wheat (Ajmal et al., 2006; Blandino et al., 2013).

There were significant differences in the colour of the crust of the bread samples (Table 2). The DM-fibre bread and control samples had significantly higher ($p < 0.05$) L* values than the other samples. Both the WM-fibre bread and the DM-fibre bread had lower values of a* (redness) and of b* (yellowness) despite the inclusion of a coloured ingredient (Table 1). Although the tendency of the colour parameters of the ingredients was observed in the crumb, it seems that the browning reactions during baking and the granulometry of the fibres used eclipsed that tendency in the crust. The changes in the colour parameters of the crust were perceived visually

and were quantified with ΔE*. Less variation was observed in the colour of the DM-fibre bread, followed by the WM-bread and finally by the sample with whole quinoa flour in comparison with the control sample (Table 2). In general, the incorporation of whole quinoa flour and its fibre caused darkening of the crumb (lower L*) and a higher red component (a*), following the tendency of the ingredients, as commented above.

With regard to the technological parameters, no significant differences were observed in loaf weight (Table 2). However, a lower specific volume was observed in the WM-fibre bread (2.7 cm3/g) than in the other bread formulations, followed by the product with whole quinoa flour (3.8 cm3/g). The replacement of flour with gluten-free ingredients and the incorporation of fibre in the bread formulations made the formation of the viscoelastic network more difficult and caused a reduction in the specific loaf volume (Wang and Zhu, 2016; Ballester-Sanchez et al., 2019b). As the dietary fibre content in the products made with the fibre fractions obtained by milling was similar ($p < 0.05$), the reduction in the specific volume might be due exclusively to the smaller particle size of the WM-fibre (Table 2). In previous research, the incorporation of wheat bran with smaller granulometry produced the same tendency in bread products (Noort et al., 2010; Wang and Zhu, 2016). This caused a change in the shape ratio of the central slice in the products formulated with quinoa ingredients, producing slightly flatter loaves, especially with the inclusion of WM-fibre, although without significant differences (Table 2).

Table 2. Chemical composition, antioxidant capacity and technological characteristics of breads with quinoa and quinoa fibre isolated by wet and dry milling

Parameter[a]	Units	Control bread	Quinoa Bread		
			Whole flour	WM-fibre	DM-fibre
Main components					
Moisture	g /100 g d.m.	26±1a	30±2b	31±3b	32±1b
Starch	g /100 g d.m.	71.1±1.0d	59.0±1.4a	68.4±0.4c	65.8±0.8b
Protein	g /100 g d.m.	13.00±0.04a	13.5±0.1c	13.18±0.01b	13.20±0.04b
Lipid	g /100 g d.m.	0.19±0.03a	1.19±0.08c	0.40±0.06b	0.35±0.03b
Ash	g /100 g d.m.	0.026±0.003a	0.038±0.001c	0.029±0.002ab	0.030±0.002b
Dietary fibre					
Soluble	g /100 g d.m.	0.7±0.2a	1.9±0.7b	1.7±0.6ab	1.9±0.7b
Insoluble	g /100 g d.m.	3.6±0.9a	6.5±0.1b	6.3±0.5b	6.0±0.1b
Total	g /100 g d.m.	4.3±1.1a	8.3±0.7b	7.9±1.1b	7.9±0.8b
Phenolic content					
EPF	mg GAE/g d.m.	1.84±0.14a	3.05±0.15c	2.18±0.17b	2.19±0.04b
HPF	mg GAE/g d.m.	12.7±0.1a	18.1±0.6bc	18.9±0.8c	17.7±0.2b
Total (EPF+HPF)	mg GAE/g d.m.	14.49±0.04a	21.15±0.54c	21.06±1.01c	19.84±0.24b
Antioxidant capacity					
DPPH					
EPF	µmol TE/g d.m.	0.33±0.01a	2.06±0.10c	0.87±0.10b	0.85±0.10b
HPF	µmol TE/g d.m.	2.7±0.2a	21.2±1.4b	25.1±1.3c	23.2±1.7bc
Total (EPF+HPF)	µmol TE/g d.m.	3.0±0.2a	23.3±1.5b	26.0±1.3c	24.1±1.7bc
FRAP					
EPF	µmol TE/g d.m.	1.03±0.01a	3.24±0.14c	1.45±0.11b	1.62±0.04b
HPF	µmol TE/g d.m.	3.6±0.2a	11.7±0.3b	12.9±0.9c	11.9±0.1b
Total (EPF+HPF)	µmol TE/g d.m.	4.6±0.2a	15.0±0.3c	14.4±1.0bc	13.5±0.1b
Crust colour					
L*	-	65±1c	52.7±0.6a	57±1b	66±1c
a*	-	6.8±1.4b	9.2±0.5c	6.2±0.3ab	4.6±0.7a
b*	-	30.7±3b	26.2±0.8a	23±1a	24±1a
ΔE	-	-	13.6±0.8b	11±1b	6.8±1a
Crumb colour					
L*	-	64.5±0.4c	47±1a	48±1a	53±2b
a*	-	-1.10±0.08a	4.34±0.3c	5.0±0.2c	3.2±0.2b
b*	-	12.7±0.5b	14.3±0.7c	11.7±0.5ab	11.1±0.1a
ΔE	-	-	18±1b	18±1b	13±2a
Technological					
Loaf weight	g	81.7±0.3a	81±1a	81±1a	82.6±0.1a
Shape ratio	cm/cm	1.57±0.27a	1.65±0.08a	2.19±0.64a	1.65±0.08a
Specific volume	cm^3/g	4.8±0.5b	3.8±0.1ab	3.0±0.6a	4.3±0.6b
Crumb firmness	N	3.6±0.5a	7.7±0.5c	7.5±0.6c	4.9±0.6b

[a]Mean ± standard deviation (n=3), values followed by the same letter in the same line are not significantly different at 95% confidence level. Abbreviations: d.m. dry matter; WM, wet milling; DM, dry milling; EPF, extractable polyphenol fraction; HPF, hydrolysable polyphenol fraction; DPPH, α-diphenyl-β-picrylhydrazyl; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalent; TE, Trolox equivalent; L*, (lightness); a*, (redness to greenness); b*, (yellowness to blueness); $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$

There was a significant increase in the crumb firmness of the quinoa bread and WM-fibre bread formulations that was related to the reduction in the specific volume of those products (Wang and Zhu, 2016; Ballester-Sanchez et al., 2019b).

The effect of the incorporation of fibre on the polyphenol content and antioxidant activity of the bread was evaluated. Table 2 shows that the breads with fibre had significantly higher PC and AC values in both polyphenol fractions than the control without that ingredient ($p < 0.05$). The differences were more marked for AC in HPF, which seems logical because, as commented earlier, both fibres were clearly richer in polyphenols of that kind (Table 1). When bread is consumed, both the EPF fraction and the HPF fraction are ingested, and therefore it is useful to refer to them in terms of TPC (PC EPF + HPF) and TAC (AC EPF + HPF). The TPC and TAC values in the breads with fibre were also significantly higher than the values found in the wheat bread, especially in the case of the activity (around 1.4-fold in TPC, around 8.3-fold and 3.0-fold in TAC_{DPPH} and TAC_{FRAP}, respectively). Neither of the fibre breads stood out particularly, and significant differences between them were only found in the PC and ACFRAP of the HPF fraction (WM-fibre bread > DM-fibre bread). These differences in favour of the WM-fibre bread were only reflected in TPC and only led to a 1.1-fold increase. The PC and AC values of the fibre breads were similar to those of the bread in which quinoa flour was incorporated in the proportion required to give the same quantity of fibre.

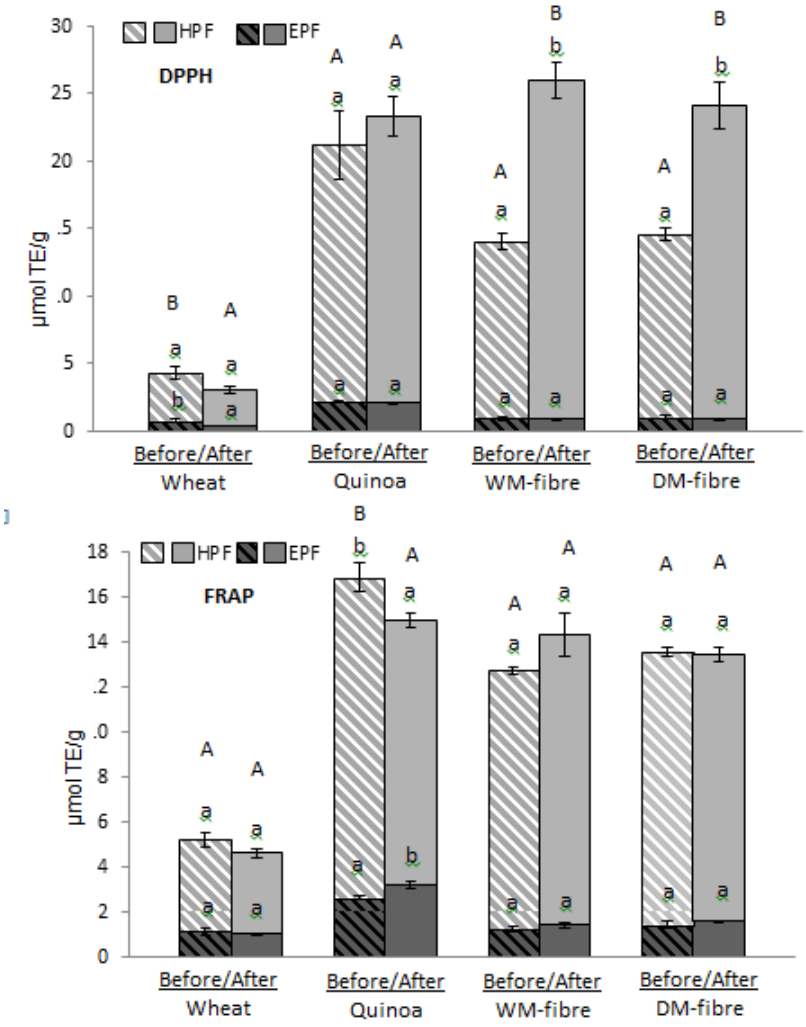

Fig. 4. Effect of baking on the antioxidant capacity of the breads. Within each bread type, comparisons are all made between flours or flour/fibre mixtures "Before" (hatched) and the corresponding bread values "After" (solid) obtained from Table 2. Bars followed by the same letter are not significantly different according to the Student's *t* test at the 95% confidence level. Small letters refer to comparisons of the extractable polyphenol fractions (EPF) and the hydrolysable polyphenol fractions (HPF); capital letters refer to comparisons of the sum of EPF + HPF.

Although the TPC and TAC values of the fibres were noticeably higher than those of the whole quinoa flour (Table 1), it must be borne in mind that the proportion of quinoa flour that was incorporated in the bread was higher than the proportion of the two fibres (26% *vs* 5%).

The effect of baking on AC (Figure 4) was evaluated by comparing the values estimated before cooking (hatched columns in Figure 4) with the values determined in the corresponding breads baking and shown in Table 2 (solid columns in Figure 4).

To calculate the estimated value before baking, the AC values of the wheat flour were obtained (AC_{DPPH} "EPF: 0.8 ± 0.1 µmol TE/g, HPF: 3.5 ± 0.4 µmol TE/g, EPF + HPF: 4.3 ± 0.3 µmol TE/g"; AC_{FRAP} "EPF: 1.2 ± 0.1 µmol TE/g, HPF: 4.1 ± 0.3 µmol TE/g, EPF + HPF: 5.2 ± 0.5 µmol TE/g") and they were used, together with the AC values of the raw materials (Table 1), to calculate the percentage of activity provided by each of the components in the various formulations (control bread: 100% wheat flour, quinoa flour bread: 74% wheat flour + 26% whole quinoa flour, and fibre breads: 95% wheat flour + 5% fibre). No AC losses were detected in the fibre breads although it has been reported that the active antioxidant compounds present in flour could be damaged or degraded as a consequence of heating during baking (Leenhardt et al., 2006; Holtekjølen et al., 2008). Specifically, no alterations were seen in the FRAP values, and the DPPH values increased significantly ($p < 0.05$). This result might be due to the formation of Maillard reaction products during baking, as it has been reported that they contribute to the antioxidant activity in bread (Dziki et al., 2014). The bread with quinoa flour

behaved differently, indicating that the type of ingredient used influences the effect of baking on antioxidant properties, as suggested in a previous study on quinoa bread (Ballester-Sánchez et al., 2019a).

CONCLUSIONS

The results of this study show that quinoa can be a source of dietary fibre with different physico-chemical and functional properties from those that are currently marketed. Wet milling of quinoa produced a higher yield of dietary fibre, with a lower lipid content than in the dietary fibre obtained by dry milling. The fibre-rich fractions obtained by the two processes did not differ considerably in terms of colour, but the process affected their particle size, which was lower in the fraction obtained by wet milling and it had highest dispersity. The DM-fibre showed better bioactive properties with regard to total antioxidant activity, although the process of obtaining it was less favourable with regard to yield and purity in comparison with the WM-fibre. The breads made with the incorporation of 5% of the fibre-rich fractions isolated from quinoa by dry and wet milling were richer in dietary fibre and antioxidant capacity in comparison with the wheat bread. However, the inclusion of fibre isolated by wet milling generated a lower technological quality with regard to specific loaf volume and crumb firmness.

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IV. DISCUSIÓN GENERAL

DISCUSIÓN GENERAL

Esta investigación ha empleado principalmente dos líneas estratégicas para revalorizar el grano de quinoa, por un lado se ha considerado, desde el punto de vista tecnológico y sensorial, la aptitud de la harina integral de quinoa para ser incorporada en productos de panadería consiguiéndose una clara mejora del aporte nutricional y del potencial funcional de los mismos; y por otro lado, la viabilidad de adaptar los procesos tradicionales de fraccionamiento de cereales en el grano de quinoa para la obtención de aislados de los diferentes componentes/compuestos del grano para ser utilizados como nuevos ingredientes en la industria alimentaria y en particular en la industria panadera.

Desarrollo de productos de panadería a base de harina de quinoa

La primera estrategia consistió en estudiar el efecto de la sustitución de 25% de harina por harina integral de quinoa real boliviana blanca, roja y negra sobre las propiedades tecnológicas (reológicas y térmicas) de las mezclas de harinas/masas panarias, la mejora de las propiedades nutricionales y funcionales, así como la calidad y aceptación global del producto (Capítulos 1, 2 y 3).

Como se ha comentado en la Introducción, existe una amplia diversidad genética entre las distintas variedades de semillas de quinoa que hace que la composición sea en general muy variable (Saturni y col., 2010; Miranda y col., 2012ab; Vidueiros y col., 2015). Además, incluso dentro de la misma

variedad, las condiciones agronómicas y geográficas repercuten en la variabilidad composicional (Reguera y col., 2018). En el presente trabajo se incluyeron semillas de quinoa pertenecientes a 3 variedades de quinoa caracterizadas por presentar diferente coloración. Debido a que su composición y propiedades tecnológicas podrían variar, se estudiaron sus harinas para tener un mayor entendimiento de su comportamiento durante el proceso de panificación. En general, las 3 variedades mostraron una composición en consonancia con lo informado en la bibliografía para otras semillas de quinoa similares o pertenecientes a otras variedades y procedencias. Solo se encontraron discrepancias en el contenido en polifenoles y actividad antioxidante debido a la variedad de procolos de extracción existentes y a la falta de métodos analíticos de cuantificación oficiales, haciendo, por tanto, difíciles las comparaciones entre laboratorios (Pérez-Jiménez y Saura-Calixto, 2005). En concreto, las harinas de quinoa blanca, roja y negra destacaron por su contenido en aminoácidos esenciales y en ácido glutámico (D'Amico et al., 2017; Motta et al., 2019); también mostraron valores elevados de ácidos grasos monoinsaturados y poliinsaturados, sobre todo ácido linoleico y oleico (Valencia-Chamarro, 2017; Pereira y col., 2019); de minerales como el Fe, Ca y Zn (Mustafa y col. 2011; Reguera y Haros, 2017; Diaz-Valencia y col., 2018). Además, mostraron elevado contenido en fibra dietética (Repo-Carrasco-Valencia, 2011); así como un destacado contenido en polifenoles totales y actividad antioxidante, con una mayor contribución de la fracción de polifenoles hidrolizables (PH), es decir, de las formas vinculadas a la fibra (Abderrahim y

col., 2015; Tang y col., 2016), con respecto a la de polifenoles extraíbles, formas solubles (Filho y col., 2017). Esta remarcable contribución de la fracción de PH concuerda con los datos de la bibliografía para otras materias primas y sugiere la importancia de esta fracción de polifenoles a pesar de que habitualmente se subestima (Pérez-Jiménez y col., 2013).

Se vieron diferencias entre las variedades de quinoa para algunos de los componentes, en particular, las variedades blanca y roja mostraron valores de lípidos significativamente superiores a los de la variedad negra, y la variedad blanca mostró menor contenido en polifenoles totales que las dos variedades coloreadas. En el caso de la fibra dietética total, aunque las diferencias no fueron significativas, se observó una clara tendencia a valores inferiores en la variedad blanca.

La comparación con la harina de trigo indicó el potencial de las harinas de quinoa para ser utilizadas en la mejora nutricional y funcional de productos de panadería. Las 3 harinas de quinoa mostraron contenidos en los compuestos recién nombrados superiores a los de la harina de trigo salvo en el contenido de almidón. Por lo que respecta al contenido en polifenoles y actividad antioxidante, en términos generales, solo las variedades coloreadas destacaron con respecto al trigo. Esto puede ser debido a la presencia de betalaínas, pigmentos con actividad antioxidante conocida (Abderrahim y col., 2015), cuya presencia se detetectó en las variedades roja y negra. Estos pigmentos podrían estar contribuyendo, junto con los polifenoles, a la mayor actividad antioxidante encontrada en las variedades

coloreadas y explicarían porque el potencial antioxidante se mostró estrechamente relacionado con el color de la semilla.

En cuanto al estudio de las propiedades tecnológicas de las harinas de quinoa del presente trabajo, se estudiaron los cambios en el perfil de proteínas tras el horneado para estimar el efecto de la adición de harina de quinoa blanca, roja y negra en el producto final. Se observaron sobre todo hidrólisis de sus proteínas principales (globulina 11S y albúmina 2S) y polimerizaciones con proteínas del trigo, cambios que se han descrito como responsables del sabor (Martínez-Anaya, 1996; Hansen y Schieberle, 2005). Algunos de los cambios observados fueron debidos a diferencias varietales de las quinoas que indicaron similitudes entre las harinas de quinoa blanca y roja. Las proteínas presentes en la quinoa tienen además un probado comportamiento tecno-funcional como espumantes, emulsionantes y gelificantes (Janssen y col., 2017), por lo que su presencia podría afectar al desarrollo del producto final. Como parte del estudio tecnológico, se analizaron las propiedades reológicas de las harinas. Los valores observados se encuadran dentro de la amplia variabilidad de resultados descritos en la bibliografía para diferentes quinoas (Wu y col., 2014; Li y Zhu, 2017; Alonso-Miravalles y O´Mahony, 2018). La elevada P_{temp} de las harinas de quinoa, así como el reducido valor de la viscosidad de pegado durante el calentamiento (HPV), podrían implicar características de cocción deficientes o un aumento en el volumen de los productos elaborados con estas durante el proceso de panificación, respectivamente (Hoseney, 1984; Lee y col., 2006; Onyango y col., 2010). Por último, las propiedades térmicas de las harinas de quinoa

real del presente estudio, fueron similares a los informados para otras variedades de quinoa blanca y roja oriundas de Perú, Bolivia y China (Li y Zhu, 2017) e inferiores a las variedades procedentes de los Estados Unidos y bolivianas (Wu y col., 2014). Como las propiedades térmicas de una harina dependen tanto de la estructura y tamaño de sus gránulos de almidón, como de otros componentes como las proteínas, lípidos o fibras presentes en estas, las diferencias encontradas en la bibliografía podrían deberse principalmente a los diferentes genotipos empleados (Coulter y Lorenz, 1990; Wang y Zhu, 2016; Li y Zhu, 2017).

La inclusión de las harinas de las variedades blanca, roja y negra de quinoa dio lugar a productos de panadería mejorados nutricionalmente y con más capacidad antioxidante, lo cual puede tener implicaciones favorables en la salud como se discute a continuación. La mejora nutricional que supone la inclusión de harinas integrales de quinoa en el desarrollo de productos está ampliamente estudiada y aceptada, sin embargo, esta mejora no se ha abordado en relación con la contribución a la ingesta promedio de ciertos nutrientes de los productos desarrollados para alcanzar los requerimientos de ingestas propuestos por la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) y por la Agencia Europea de Seguridad Alimentaria (EFSA). Esta relación parece fundamental en la estrategia para el desarrollo de alimentos que puedan tener impacto en la salud del consumidor. En este sentido, la incorporación de un 25 % de harina integral de quinoa supuso una mejora en el contenido de proteínas con mayor valor biológico en el producto final. El aporte de lisina proveniente de las

proteínas de quinoa real aumentó el contenido de esta hasta en un 40% en las proteínas del pan con quinoa respecto al pan de trigo, muy por encima del porcentaje del 26% observado por Stikic y col. (2012) en sus productos con inclusión de un 20% de semillas de quinoa de la variedad Puno cosechada en Perú. Además, no se observaron pérdidas de lisina tras el proceso de panificación. Sin embargo, este incremento en el contenido en lisina no fue suficiente para alcanzar el valor recomendado por la FAO para adultos (4,5 g/100g proteína) (FAO, 2008).

La harina de quinoa provocó una mejora en la relación de fibra soluble:insoluble en los productos panarios, especialmente en aquellos elaborados con las variedades blanca y roja, debido sobre todo al aumento de la fracción soluble. Relaciones de fibra soluble: insoluble cercanas a 1:2 han demostrado una acción fisiológica más efectiva (Jaime y col., 2002). Sería esperable un efecto hipocolesterolémico, así como una reducción del tiempo para producir el vaciamiento gástrico, de la tasa de absorción de glucosa y la insulina postprandial, lo que podría ayudar a mejorar el control de la glucemia en sangre (Konishi y col., 2000; Salas-Salvadó y col., 2007). Además, el aumento del contenido de fibra dietética total hace que el consumo promedio de 100 g de pan elaborado con harina de quinoa pueda contribuir a alcanzar entre el 34 y 43% de la ingesta adecuada de fibra en adultos de 25 gramos diarios recomendada por la FAO y la EFSA (FAO, 2010; EFSA, 2017).

El mayor contenido tanto en ácidos grasos monoinsaturados (MUFA), como de ácidos grasos polinsaturados (PUFA) en las harinas integrales de quinoa

provocó cambios en el perfil lipídico de los productos desarrollados, reduciendo el contenido de ácidos grasos saturados y mejorando la relación insaturados:saturados. Esta misma tendencia fue observada por otros investigadores por la inclusión de harina de quinoa en productos libres de gluten o por la incorporación de semillas de quinoa en productos de panadería (Álvarez-Jubete y col., 2009; Calderelli y col., 2010). El aumento de ácido linoleico (LA) y ácido linolénico (ALA) hace que el consumo de 100 g de pan con quinoa pueda llegar a contribuir hasta un 15% (según la FAO, 2008) o hasta 9% (según la EFSA, 2017) a la ingesta adecuada de LA y hasta en un 8% a la ingesta adecuada de ALA (FAO, 2008; EFSA, 2017). Como consecuencia, la ingesta de los productos desarrollados podría contribuir a disminuir la concentración del colesterol LDL y la relación colesterol total/colesterol HDL, y por ende el riesgo de padecer enfermedades cardiovasculares (FAO, 2008; Field, 2003; Elmadfa and Kornsteiner, 2009). Así mismo, podría ayudar a mejorar el desequilibrio en las dietas occidentales en cuanto a la relación omega-6/omega-3, actualmente estimada en un intervalo de 14: 1–20: 1 (Bhardwaj y col., 2016), a acercarse más a la relación establecida por los valores de ingesta adecudada de LA y ALA de 5:1 (EFSA, 2009).

De forma general, la incorporación de un 25% de harina integral de quinoa, independientemente de la variedad, en los productos de panadería incrementó significativamente el contenido de minerales, sin embargo, la ingesta de 100 g de pan con harina integral de quinoa tan solo mejoraría la contribución al requerimiento promedio de Fe y Zn. Además, diversos

estudios han demostrado el efecto negativo del ácido fítico sobre la biodisponibilidad de cationes di y trivalentes (Fredlund y col., 2006). En este sentido, las ratios molares fitato/mineral son utilizados para predecir el efecto inhibitorio en la biodisponibilidad de minerales en humanos por la presencia de fitatos (Ma y col., 2005). El contenido de fitatos en los productos elaborados se redujo hasta en 4 veces con respecto al contenido de las harinas que les dieron origen, debido a la activación de las fitasas endógenas durante el proceso de panificación (Sanz-Penella y col., 2009). Por lo que, tan solo la ratio fitato/Fe fue superior a 1, alcanzando el valor umbral de inhibición y quedando comprometida la biodisponibilidad de este mineral.

También se estudió si la adición de las harinas de quinoa potenciaba las propiedades funcionales de los productos panarios en cuanto a contenido en polifenoles (CP) y actividad antioxidante (AA). La harina integral de quinoa es un potencial ingrediente en el sector de la industria panadera y el impacto de su adición en los parámetros recién mencionados apenas ha sido abordado (Álvarez-Jubete y col., 2010; Brend y col., 2012; Chlopicka y col., 2012). La principal novedad del presente estudio radica en el hecho de que no existe ningún trabajo hasta la fecha que incluya la caracterización completa de panes de quinoa teniendo en cuenta la aportación de los polifenoles extraíbles (PE) y de los polifenoles hidrolizables (PH), a pesar de que ambas fracciones de polifenoles son ingeridos durante su consumo. Está descrito que cada uno de ellos ejerce su función en el organismo (Acosta-Estrada y col., 2014) por lo que el presente estudio supone una forma más

realista de reflejar el potencial saludable de los productos desarrollados. A pesar de la termolabilidad atribuida a los polifenoles, las propiedades antioxidantes de las harinas de quinoa se mantuvieron inalteradas tras el horneado. No se puede descartar la contribución de productos de la reacción de Maillard (Dziki y col., 2014), formados durante el horneado, a la actividad antioxidante final de los panes y que podrían contrarrestrar posibles pérdidas en la actividad de los polifenoles aportados por las harinas. Además, se consiguió una mejora en los panes con quinoa con respecto al pan control de trigo. En el caso del CP, mostraron valores totales (PE + PH) superiores, si bien las diferencias no fueron significativas. Lo que es realmente relevante es que los panes con harina de las quinoas coloreadas mostraron propiedades antioxidantes significativamente mejoradas con respecto al pan control y por tanto podrían contribuir en la disminución del riesgo de enfermedades relacionadas con el estrés oxidativo y por ende con el desencadenamiento del SM como se ha comentado en la Introducción (Skyler y col., 2017; McCracken y col., 2018).

En los productos de panadería tiene una gran importancia su efecto en el incremento de la glucemia. Los productos elaborados con quinoa mostraron una reducción del índice glucémico y la carga glucémica, determinados *in vitro*, con respecto al pan control; sumado al aumento en el contenido de fibra dietética comentado anteriormente, podría suponer un efecto hipoglucemiante en humanos debido a la reducción en la tasa de absorción de carbohidratos de la dieta por la formación de un gel viscoso en el intestino delgado (EI, 1999; Rokka y col., 2013). En este sentido, diferentes

investigadores han clasificado la quinoa como un alimento con bajo índice glucémico y se ha observado reducción *in vitro* en el índice y la carga glucémica de productos de quinoa con respecto al pan de trigo (Wolter y col., 2014; Xu y col., 2019). Laparra y Haros (2018) detectaron un comportamiento diferente en productos con quinoa blanca en un estudio *in vivo* con animales de experimentación, informando un índice glucémico similar al obtenido en el pan blanco. Sin embargo, en ese trabajo se observó una mayor expresión del receptor de peroxisoma proliferador activado gamma (PPAR gamma), asociado a una mejora en la sensibilidad a la insulina, lo que podría estar indicando un papel preventivo clave en el desarrollo de resistencia a la insulina, diabetes tipo 2, niveles elevados de triglicéridos y niveles bajos de HDL por parte de los panes de quinoa ensayados (Kelly y Scarpulla, 2004; Laparra y Haros, 2018). Semillas de quinoa germinadas mostraron una reducción significativa respecto a las semillas sin germinar en cuanto al índice glucémico, por lo que esta estrategia debería tenerse en cuenta a la hora de formular alimentos con este pseudocereal para colectivos específicos (Lorusso y col., 2017; Lopes y col., 2019).

Desde el punto de vista tecnológico, el efecto de la incorporación de la harina integral de quinoa resultó en una merma ligera, aunque significativa, de la calidad final del producto en cuanto a la altura de la pieza panaria, mayor firmeza de la miga y por ende reducción del tamaño medio de los alveolos, además de un cambio de coloración con respecto al pan control,

sin que afecte de manera considerable la aceptabilidad sensorial del producto. Esta tendencia se ha informado en otras investigaciones básicamente debido a la dilución del gluten y la mayor concentración de fibra en las harinas de quinoa (Park y col., 2005; Wang y col., 2015). En general, la inclusión de harina integral de quinoa en productos panarios muestran una aceptación global generalizada y una elevada intención de compra hasta con inclusiones del 50% (Lorenz y Coulter, 1991; Stikic y col., 2012; Iglesias-Puig y col., 2015; El-Sohaimy y col., 2019), proporcionando una alternativa de mejora nutricional y funcional al tradicional pan blanco, elaborado con harina de trigo refinada.

Fraccionamiento del grano

La segunda estrategia del presente trabajo, pretendió fraccionar las diferentes partes anatómicas y/o componentes del grano de quinoa que pudieran resultar ingredientes con valor añadido en la industria alimentaria. En concreto, se centró la atención en la fracción amilácea por sus múltiples aplicaciones en la industria alimentaria, cosmética y farmacéutica, como se ha comentado en la Introducción; y también en la fracción de fibra, por su clara asociación con la salud y porque su empleo en la industria alimentaria todavía supone un desafío tecnológico y sensorial. Para la obtención del almidón se adaptó la molienda húmeda de maíz al grano de quinoa (Capítulo 4). La molienda húmeda también se adaptó para la obtención de la fracción de fibra y además, esta se comparó con la fracción de fibra (salvado)

obtenida por el proceso tradicional de molienda seca de trigo, cuyo principal producto es la harina refinada (Capítulo 5).

En cuanto a la molienda húmeda de quinoa, los resultados indicaron que las variables tiempo y temperatura de la etapa de maceración afectaron la eficiencia de la separación de los principales componentes del grano y sus rendimientos, tal y como se informó previamente en maíz (Pérez y col., 2001). Las características de la fracción amilácea en cuanto a la pureza, propiedades térmicas y reológicas también fueron afectadas por el proceso de maceración. La optimización del proceso de molienda húmeda utilizado en este estudio produjo la mayor recuperación y calidad de almidón después de 6,5 h de remojo a 30 °C. El rendimiento de almidón del presente trabajo (62%) fue significativamente superior al reportado por Wright y col. (2002) (53%) para la molienda húmeda de quinoa dulce y amarga de Bolivia tras un macerado del grano en una solución NaOH (0,3%), a temperatura ambiente, durante 12 horas y al obtenido por Jan y col. (2017ab) tras una maceración alcalina (NaOH, 0.25%) a temperatura ambiente, durante 24 horas (48%). Los resultados del diseño factorial en cuanto al efecto de las variables operativas de la etapa de maceración mostraron que el tiempo de maceración no afectó de forma significativa al rendimiento del almidón de quinoa, mientras que la variable temperatura si lo hizo, por lo que podemos presuponer que no optimizar la variable temperatura pudo provocar un menor rendimiento en los trabajos anteriormente citados. En lo relativo a las propiedades fisicoquímicas del almidón de quinoa obtenido, cabe destacar

un mayor contenido de proteínas con respecto a otras investigadociones (Wright y col., 2002; Steffolani y col., 2013; Jan y col., 2017ab) que usaron soluciones alcalinas en la etapa de maceración, lo que permite una mejor separación de las proteínas (Haros y Wronkowska, 2017). La separación del almidón de las proteínas por métodos físicos en los procesos de molienda húmeda supone una dificultad debido a la fuerte asociación entre ellos (Haros y Wronkowska, 2017). La presencia de proteínas en almidón destinado a farmacia, producción de jarabes de glucosa o fructosa supone un problema por propiciar reacciones de Maillard durante el proceso de producción o almacenamiento y por ende colorear el producto final (Hernández-Medina y col., 2008). En el caso particular de los pseudocereales, los cuales poseen gránulos de almidón de menor tamaño (1-3 μm) que los de cereales (1-40 μm), la separación del almidón de la proteína, tradicionalmente realizado por medio de hidrociclones, supone un desafío. La filtración en flujo tangencial podría ser una alternativa al proceso de separación basado en la diferencia de densidad, que ha mostrado resultados interesantes y eficientes en la separación del almidón y las proteínas de amaranto (Middlewood y Carson, 2012ab). Por otro lado, a diferencia de otros estudios (Wright y col., 2002; Steffolani y col., 2013; Jan y col., 2017ab), la fracción de almidón obtenida en esta Tesis, mostró estar libre de lípidos, debido fundamentalmente al paso del grano, durante la primera etapa de la molienda, por un molino de discos estriados, lo que permitió la separación del germen íntegro. La molienda húmeda de quinoa optimizada en el presente trabajo supone una alternativa para la obtención

de almidones con características fisicoquímicas y funcionales diferentes a los convencionales, pudiendo ser destinadas a la producción de alimentos infantiles, conservas y bebidas debido a su baja viscosidad (Peralta, 2007). Además, su elevada temperatura de gelatinización podría tener un impacto en la textura y esponjosidad de productos horneados (Ortega, 2008).

Las diferentes metodologías de aislamiento de la fibra generaron fracciones de características variables, y por consiguiente diferencias en sus propiedades tecno-funcionales y posibles usos como ingrediente alimentario. En este sentido, se optimizaron los parámetros tiempo y temperatura de maceración para la máxima recuperación y pureza de la fracción rica en fibra por molienda húmeda y se comparó con el aislamiento de la fracción de salvado de quinoa obtenida por molienda seca. La optimización del proceso de molienda húmeda para el aislamiento de la fracción de fibra produjo la mayor recuperación y pureza tras 1,25 horas de maceración a 30 °C. Existen escasas investigaciones con el fin de aislar la fibra dietética de quinoa por molienda húmeda debido a que los estudios en molienda húmeda de quinoa concentran su atención en el aislamiento de las fracciones individuales para su posterior caracterización química, sin informar el rendimiento y/o la recuperación de las distintas fracciones (Haros y Schoenlechner, 2017). Sí se han informado rendimientos de fracciones de fibra obtenidas de otros pseudocereales por molienda húmeda. En el presente estudio, el rendimiento obtenido (10.1%) fue ligeramente más bajo que los observados por Zheng y col. (1998) para la

fracción rica en fibra obtenida por molienda húmeda de trigo sarraceno, y por Calzetta Resio y col. (2009) para las fracciones de fibra de amaranto, sin embargo, la presencia de otros componentes del grano en estas fracciones fueron indicativos de una menor pureza.

La recuperación de la fracción de fibra obtenida por molienda húmeda fue significativamente superior y de mayor pureza que la obtenida por molienda seca, aun habiéndose observado menor contenido de fibra soluble, debido probablemente a su pérdida durante la etapa de maceración. Además, la menor presencia de lípidos en la fracción de fibra obtenida por molienda húmeda indicó nuevamente una separación e integridad del germen superior en comparación con la molienda seca.

Tras la determinación de la distribución del tamaño de partícula de las fracciones de fibra, la obtenida por molienda húmeda mostró menor tamaño y mayor índice de polidispersidad que la aislada por molienda seca, lo que podría influir en la funcionalidad de la fibra en el organismo humano (Peerajit y col., 2012), así como también en el comportamiento tecnológico de los productos elaborados con ella (Noort y col., 2010; Wang y Zhu, 2016).

El proceso de molienda húmeda provocó una reducción del contenido polifenólico de las formas extraíbles (PE) de la fracción de fibra con respecto al proceso en seco, sin embargo, esta reducción no afectó al contenido fenólico total (PE + PH). La extracción de PE se realiza en solventes orgánicos/acuosos (Pérez-Jiménez y col., 2013), por lo que es muy probable que la maceración del grano de quinoa generara pérdidas por lixiviación similares a las observadas por Mukherje y col. (2018) y Yu y col. (2018)

durante la maceración de soja y trigo sarraceno, respectivamente. El contenido en PH resultó claramente superior en ambas fracciones de fibra en comparación con la harina de quinoa, era esperable dado que este tipo de polifenoles estan en el grano unidos a componentes de la fibra.

Con el objetivo de probar el potencial uso de la fracción de fibra como ingrediente panario, un 5% de cada una de las fracciones de fibras fue introducido en formulaciones panarias. La incorporación de la fibra proveniente de la molienda húmeda provocó una reducción del volumen específico con respecto al pan elaborado con la fibra de la molienda seca, probablemente debido al menor tamaño de partícula de la fibra obtenida por molienda húmeda. Estudios previos observaron una correlación positiva entre la reducción del volumen específico de las piezas panarias y el tamaño de fibra de trigo incorporado como ingrediente, mostrando una mayor reducción cuanto menor era el tamaño de partícula de la fibra (Noort y col., 2010; Sanz-Penella y col., 2012). A pesar de que se observaron algunas diferencias en la composición próximal, contenido en polifenoles y actividad antioxidante entre ambas fibras, este comportamiento no se reflejó en los panes correspondientes ya que estos mostraron valores equivalentes para los citados parámetros. Lo más destacable es que la incorporación de ambas fibras dio lugar a panes con contenido mejorado en nutrientes, fibra dietética, polifenoles y capacidad antioxidante con respecto al control (100% harina trigo). Además, lo productos con fibra no solo no presentaron pérdidas en la actividad antioxidante, sino que esta aumentó debido probablemente a la actividad antioxidante que presentan algunos de los

compuestos generados por la reacción de Maillard durante el horneado (Dziki y col., 2014).

Se valoró la ventaja de adicionar fibra de quinoa, a modo de ingrediente alimentario, como práctica alternativa al uso de la correspondiente harina integral. Para ello, los productos adicionados con fibra se compararon con un pan elaborado con 26% de harina de quinoa (porcentaje estimado para alcanzar una proporción de fibra similar a la de los panes con un 5% de esta). Los productos con fibra no destacaron con respecto a los adicionados con harina integral en cuanto a composición proximal, contenido en polifenoles y actividad antioxidante. En cuanto a los parámetros tecnológicos, los panes con harina integral de quinoa y fibra de molienda húmeda fueron muy similares con valores estadísticamente equivalentes. Sin embargo, los panes adicionados con fibra de molienda seca mostraron una menor merma tecnológica por lo que en este sentido, su uso como ingrediente panario supondría una ventaja con respecto a la inclusión de la harina integral de quinoa.

Los resultados del presente trabajo, proporcionan una referencia de las propiedades fisicoquímicas y funcionales de las fibras aisladas, así como una visión del potencial uso de la fibra de quinoa aislada por molienda húmeda y seca para la industria alimentaria en general y la panadera en particular.

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V. CONCLUSIONES

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

1. En general, el estudio de tres variedades de quinoa, claramente diferenciables por su color (blanco, rojo y negro), mostró un comportamiento similar en términos de propiedades reológicas, características térmicas y composición proximal, con excepción del contenido en lípidos, que fue superior en las variedades blanca y roja. El análisis pormenorizado de las harinas integrales de las tres variedades mostró un mejor perfil aminoacídico de sus proteínas y perfil de ácidos grasos en comparación con el grano de trigo, debido a un mayor contenido de aminoácidos esenciales y de acidos grasos poliinsaturados. Estos datos eran indicativos del potencial de la harina integral de las quinoas estudiadas para la mejora nutricional de productos de panadería.

2. El estudio del contenido fenólico y de la capacidad antioxidante total de las tres semillas de quinoa, considerando las fracciones solubles (extraíbles) e insolubles (hidrolizables), indicó que este pseudocereal podría ser una buena fuente natural de antioxidantes. El potencial antioxidante fue diferente según la variedad de quinoa utilizada y se relacionó fuertemente con el color de la semilla, siendo las variedades roja y negra las que más destacaron probablemente debido a la presencia en ellas de pigmentos tipo betalaínas.

3. El reemplazo del 25% de la harina de trigo con harina de quinoa blanca, roja o negra produjo una mejora general en el perfil nutricional de los productos de panadería desarrollados en términos de aumento del contenido de proteínas con una leve mejora en el perfil de aminoácidos, especialmente en lisina, y un aumento en el contenido de lípidos con una mejora en la relación de ácidos grasos saturados/insaturados. El contenido mineral de las harinas de quinoa podrían ayudar a mejorar la contribución a los requerimientos diarios promedio de Fe y Zn en los productos con quinoa, aunque un aumento en la relación molar fitato/mineral podría comprometer la absorción de estos minerales.

4. Desde el punto de vista de la funcionalidad biológica, los productos elaborados con los tres tipos de harina integral de quinoa mejoraron la contribución a la ingesta adecuada de fibra y la relación de ácidos grasos saturados:insaturados en comparación con el pan de trigo. Los productos elaborados con harina de las variedades coloreadas presentaron una capacidad antioxidante significativamente mayor que el pan control. Además, los productos con harina integral de quinoa podrían reducir el índice glucémico y la carga glucémica, con una tendencia a disminuir la tasa de hidrólisis del almidón tras su ingesta.

5. La sustitución de un 25% de la harina de trigo por harina integral de quinoa en la fabricación de productos de panadería provocó un cambio en las propiedades térmicas y reológicas de las masas de pan resultando

productos horneados con menor altura y una mayor firmeza, masticabilidad y resiliencia de la miga. Sin embargo, un aumento significativo en el perfil nutricional junto con la aceptación general por parte de los consumidores fue concluyente para proponer el reemplazo con harina de quinoa como estrategia para la mejora nutricional en la fabricación de pan a pesar de la leve disminución en la calidad tecnológica de los productos desarrollados.

6. El proceso de molienda húmeda demostró ser un procedimiento viable para el fraccionamiento de quinoa permitiendo la obtención eficiente de las fracciones ricas en almidón y en fibra.

7. Las variables operativas tiempo y temperatura de maceración afectaron significativamente las características fisicoquímicas del almidón aislado, en concreto a la temperatura de gelatinización y retrogradación, así como el pico de viscosidad (PV), la viscosidad mínima durante la cocción (HPV) y la viscosidad final (CPV). El proceso de molienda húmeda desarrollado en este estudio logró una alta recuperación de almidón de quinoa y una separación eficiente de sus componentes que dio lugar a una fracción libre de lípidos. Los valores máximos de respuesta se obtuvieron a las 6,5 horas de maceración a 30 °C.
8. El proceso de molienda en húmedo desarrollado dio lugar a una fracción rica en almidón con propiedades fisicoquímicas y funcionales diferentes a otros almidones y por tanto, con nuevas posibilidades de uso.

9. La comparación de los procesos de molienda húmeda y seca aplicados para la obtención de la fracción de fibra mostró que la molienda húmeda es más favorable en cuanto a los parámetros de rendimiento y pureza.

10. Los procesos de molienda húmeda y seca aplicados a la harina integral de quinoa roja rindieron fracciones de fibra que, por sus características, se pueden considerar fuente de fibra dietética antioxidante. Por tanto, ambas fracciones podrían ejercer una doble funcionalidad biológica con impacto positivo en la salud. Las propiedades antioxidantes fueron más destacadas con respecto a la harina de quinoa en la fracción obtenida por molienda seca.

11. Las características de las fibras obtenidas por ambos procesos de molienda indicaron el potencial de las mismas para su aplicación como ingrediente alimentario. Su inclusión al 5% en productos panarios generó productos más ricos en nutrientes, fibra dietética, contenido en polifenoles y capacidad antioxidante que el pan de trigo. Por tanto, se consiguieron panes claramente mejorados en su composición, aunque de menor calidad tecnológica con respecto al volumen específico de la pieza panaria y la firmeza de la miga.

12. Los productos panarios con ambas fracciones ricas en fibra no presentaron una composición próximal ni propiedades antioxidantes

mejoradas con respecto al pan adicionado con harina integral de quinoa en el porcentaje estimado para alcanzar una proporción de fibra similar. Sin embargo, en los panes elaborados con fibra aislada por molienda en seco, se observó una menor merma tecnológica, por lo que su uso como ingrediente panario, como práctica alternativa al uso de harina integral, podría suponer una ventaja para la industria a la hora de obtener productos de panadería con mayor contenido en fibra antioxidante.

13. Se ha resuelto con éxito el desarrollo de metodologías de aislamiento de componentes individuales del grano de quinoa, lo cual supone un avance importante en su revalorización. Alguno de los componentes obtenidos, en concreto la fracción rica en fibra, se ha aplicado con éxito a productos panarios pero podría aplicarse a otras matrices alimentarias, pobres en fibra y propiedades antioxidantes, para la formulación de nuevos alimentos funcionales. La fracción rica en almidón, por sus características particulares, presenta un gran potencial tanto en la industria alimentaria como en la no alimentaria. Por tanto, la presente investigación abre la puerta a nuevos campos de exploración científica.

VI. ANEXO

La presente tesis doctoral ha dado lugar a 5 publicaciones científicas en revistas del Área de Ciencias de los Alimentos:

Ballester-Sánchez, J., Yalcin, E., Fernández-Espinar, M. T., Haros, C. M. (2019). Rheological and thermal properties of royal quinoa and wheat flour blends for breadmaking. *European Food Research and Technology*, 245, 1571-1582. Factor de impacto (JCR 2018): 2,056.

Ballester-Sánchez, J., Millán-Linares, M.C., Fernández-Espinar, M. T., Haros, C. M. (2019). Development of healthy, nutritious bakery products by incorporation of quinoa. *Foods*, 8(9), 379. Factor de impacto (JCR 2018): 3,011.

Ballester-Sánchez, J., Gil, J. V., Haros, C. M., Fernández-Espinar, M. T. (2019). Effect of Incorporating White, Red or Black Quinoa Flours on Free and Bound Polyphenol Content, Antioxidant Activity and Colour of Bread. *Plant Foods for Human Nutrition*, 74, 185-191. Factor de impacto (JCR 2018): 2,598.

Ballester-Sánchez, J., Gil, J. V., Fernández-Espinar, M. T., Haros, C. M. (2019). Quinoa wet-milling: Effect of steeping conditions on starch recovery and quality. *Food Hydrocolloids*, 89, 837-843. Factor de impacto (JCR 2018): 5,839.

Ballester-Sánchez, J., Fernández-Espinar, M. T., Haros, C. M. (2020). Isolation of red quinoa fibre by wet and dry milling and application as a potential functional bakery ingredient. *Food Hydrocolloids*, 101, 105513. doi.org/10.1016/j.foodhyd.2019.105513. Factor de impacto (JCR 2018): 5,839.

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ORIGINAL PAPER

Rheological and thermal properties of royal quinoa and wheat flour blends for breadmaking

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Abstract
The increasing interest in quinoa in Europe has generated a large number of studies with this seed as a partial substitute for refined wheat flour in bakery products as a strategy to improve their nutritional value. However, the wide genetic diversity of this seed offers very different compositions in different varieties, which would lead to different technological behaviours in the breadmaking process. The aim of this work was to make a comparative study of the protein profile and rheological and thermal properties of three varieties of quinoa widely available commercially in Europe to study their technological potential as breadmaking ingredients with 25% replacement of wheat flour by whole quinoa flour. The results obtained during the analysis offered a view of the proteins present in the various quinoas, and of the processes of hydrolysis and generation of new bonds between wheat and quinoa proteins during the breadmaking process. The changes in the thermal and pasting properties of the bread doughs that included whole quinoa flour led to the development of baked products with different physico-chemical and textural properties, producing an increase on crumb staling. However, replacement of 25% of the wheat flour with whole quinoa flour produced only a slight decrease in the technological quality of the products. A significant increase ($p<0.05$) in dietary fibre, minerals, lipids, and proteins in comparison with a whole wheat product, together with the overall consumer acceptance of the products that were developed, was conclusive for proposing replacement with quinoa flour as a strategy for nutritional improvement in the manufacture of bakery products.

Keywords Quinoa · Bread characteristics · Protein profile · Thermal parameters · Pasting properties

Introduction

Bread is one of the most common foods made with cereals in the world. However, the main cereal used for breadmaking is flour obtained by dry milling of wheat grain, which removes valuable nutrients and bioactive compounds [1]. Whole cereal and pseudocereal flours can be included in bakery products as a strategy to improve their nutritional profile without needing to use whole products completely [2–4]. Among the pseudocereals, quinoa (*Chenopodium quinoa*) is a dicotyledon originally from South America, although, because of its adaptation characteristics and wide genetic diversity, it is now grown in nearly every continent in the world, including Europe [5]. Because its composition is similar to that of cereals, it has a suitable balance of carbohydrates, proteins, lipids, and minerals, and it can be sold without restrictions in Europe in accordance with Regulation (EU) 2015/2283 [6], which means that a large number of varieties are marketed in countries of the European Union, all of which has created increasing interest in society. Moreover, unlike wheat, which contains gluten-forming proteins (gliadins and glutenins), the main proteins in quinoa are albumins and globulins, bound together by disulfide bridges [7]. The most abundant of these proteins is of type 11S, also known as globular chenopodin, with a molecular size of 50–60 kDa [8], followed by those of type 2S albumin, which are polypeptides of a relatively small size, about 9–10 kDa [9, 10]. The predominance of globulins and albumins in quinoa is technologically significant, because they have foaming, emulsifying, and gelling properties, which in some cases are similar to the techno-functional properties of soya or casein proteins [11].

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Article

Development of Healthy, Nutritious Bakery Products by Incorporation of Quinoa

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Abstract: The use of quinoa could be a strategy for the nutritional improvement of bakery products. The inclusion of this pseudocereal, with its suitable balance of carbohydrates, proteins, lipids and minerals, could contribute to attaining the adequate intake values proposed by the FAO (Food and Agriculture Organization) and/or EFSA (European Food Safety Authority) for suitable maintenance and improvement of the population's health. Bakery products made with white, red or black royal quinoa significantly improved the contribution to an adequate intake of polyunsaturated fatty acids (linoleic and linolenic acids) and dietary fibre, which produced an improvement in the soluble/insoluble fibre ratio. There was also an increase in the contribution to the average requirement of Fe and Zn, although the increase in the phytate/mineral ratio would make absorption of them more difficult. Inclusion of flour obtained from the three quinoas studied slightly improved the protein quality of the products that were prepared and positively affected the reduction in their glycaemic index.

Keywords: *Chenopodium quinoa*; bakery products; DRIs/DRVs (Dietary Reference Intakes/Dietary Reference Values) and AI (Adequate Intake); FAO (Food and Agriculture Organization); EFSA (European Food Safety Authority); protein quality; polyunsaturated fatty acids; dietary fibre; mineral availability; glycaemic index estimation

1. Introduction

Quinoa is a native pseudocereal of Latin America that now has great consumer acceptance in Europe and throughout the world. Because of its suitable balance of carbohydrates, proteins, lipids and minerals and its bioactive compound content, it has been proposed that it should be included as a strategy to improve the nutritional quality of bakery products made with refined flours [1,2]. Not only would the incorporation of quinoa flour in formulations increase the protein content but it could also improve the biological value of the proteins in these formulations, since quinoa proteins contribute essential amino acids that are limiting in wheat flours (such as lysine and threonine), and they are more digestible [3]. It could also lead to an increase in the unsaturated fatty acid content and an improvement in the omega 3/omega 6 fatty acid relationship. The main unsaturated fatty acids in quinoa are linoleic and α-linolenic acids, a precursor of long-chain polyunsaturated fatty acids (PUFAs), which are essential fatty acids [1,4]. Moreover, its high fibre and mineral contents could help to attain the daily requirements of these substances and of calcium, iron and zinc in the diet [1]. However, mineral bioavailability does not depend only on the concentration of the mineral in question in the food (such as Ca, Fe or Zn); there are compounds such as phytates that form complexes with di- and trivalent minerals and prevent their absorption [5]. Because of the high proportion of dietary fibre in wholemeal flours made from quinoa and other grains, their inclusion

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Effect of Incorporating White, Red or Black Quinoa Flours on Free and Bound Polyphenol Content, Antioxidant Activity and Colour of Bread

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Abstract
Interest in quinoa as a functional food ingredient is currently emerging. The flours from white, red and black quinoa seeds were analysed in terms of total polyphenol content and antioxidant activity. They were incorporated at 25% on flour basis into the bread dough formula to evaluate their potential to improve the functional properties of wheat breads. The contribution of extractable polyphenols (soluble forms) and the largely unexplored hydrolysable polyphenols (bound forms that can be found in the residues of the former) were taken into account to reflect a realistic health-promoting potential of breads. The red and black quinoa varieties stood out compared to wheat flour, with about double the polyphenol content and up to 4.7-fold increments in antioxidant activity when considering the sum of extractable and hydrolysable polyphenols. The red and black flours were equally effective in intensifying the antioxidant properties of bread despite the baking process (between 2- and 3-fold). They produced significant changes in the parameters that describe crust and crumb colour (L*, a*, b*). A clear darkening was observed compared to the control bread, an appealing attribute for lovers of unconventional and natural products. According to our results, the flours from the coloured quinoa seeds could be considered interesting antioxidant sources and be applied as natural ingredients in bread-making; new, promising and valuable unconventional products for consumers and producers could be developed.

Keywords Quinoa flours (white, red and black) · Bread · Free and bound polyphenols · Total polyphenol content · Antioxidant activity · Colour

Abbreviations

AC	Antioxidant capacity
EPF	Extractable polyphenol fraction
HPF	Hydrolysable polyphenol fraction
PC	Polyphenol content
TAC	Total antioxidant capacity
TPC	Total polyphenol content

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Introduction

Eating products with antioxidant properties has been popularised due to the belief that some dietary patterns can be useful for preventing certain pathological conditions with a positive effect on people's quality of life [1]. The food industry has reacted to this market opportunity and has shown interest in developing new products with improved antioxidant properties for years. The use of antioxidants of plant-based ingredients has become important in most food innovations as a way to obtain current consumers' confidence, who feel especially attracted by everything that is organic and natural.

Ancient grains are perceived by consumers as being more healthy and natural compared to common cereals. They have aroused much interest as a source of ingredients to develop new functional foods [2]. Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal from South America, has become very popular and well appreciated, which is not surprising given its remarkable nutrition composition and other interesting attributes [3]. Quinoa is also recognised as an excellent source of polyphenols. Health benefits associated with its intake have been

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Quinoa wet-milling: Effect of steeping conditions on starch recovery and quality

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ABSTRACT

Cereal starches play an important role in the food and non-food industries because of their low cost, availability, and ability to impart a wide range of techno-functional properties. The main objective of this research was to isolate starch, germ, protein, and fiber components from quinoa by a wet-milling procedure. The effect of steeping time and temperature on starch recovery and its quality was investigated. The quinoa steeping conditions, such as time (1, 5, and 9 h) and temperature (30, 40, and 50 °C), in SO_2 solution with lactic acid were investigated using a 3^2 factorial design in order to optimize the starch separation and its quality. The effect of steeping conditions on starch was evaluated in terms of whiteness, protein, lipid, amylase, and damaged starch contents, as well as thermal and pasting properties. Results showed how the different steeping times and temperatures affected the fraction yields and starch recovery and its quality. Optimization of the wet-milling process used in this study produced the highest starch recovery level and best starch quality after 6.5 h of steeping at 30 °C. Experimental values were close to the predicted ones, with an error below 2% for all attributes tested.

1. Introduction

The primary sources of carbohydrates for the global population are cereals and pseudocereals. Pseudocereals are essentially starch crops; however, they may contain significant quantities of protein and oil, and these constituents frequently determine their suitability for a specific end use. In particular, quinoa is a pseudocereal native to South America, mainly from Peru, Bolivia, Argentina, Colombia, Ecuador, and Chile, but in recent decades other countries such as the United States, Canada, Italy, France, Spain, England, and Sweden have also become producers (Bazile & Baudron, 2015). Structurally, quinoa is composed of three main parts: the perisperm, the embryo or germ, and the pericarp or seed hull (Reguera & Haros, 2017). The perisperm is the primary starch storage portion, the germ is the lipid storage portion, and finally the hull, also called bran, consists mainly of cellulose and hemicellulose. The physicochemical and functional properties of the main components of quinoa, starch, fiber, and protein, are widely described in the literature (Koizol, 1992; Schoenlechner, Wedner, Siebenhandl-Ehn, & Berghofer, 2010; Kurek, Karp, Wyrwisza, & Niu, 2018). The objective of milling is to obtain intermediate products that can be used subsequently in the manufacture of other products. Normally, milling schemes are classified as dry- or wet-milling. In dry-milling the aim is to separate the anatomical part of the grain to produce mainly flour, whereas the purpose of wet-milling is to separate the chemical components of the grain, such as starch, proteins, fiber, and lipids, to obtain the purest possible fraction of each component (Haros & Wronkowska, 2017). The main cereal used in wet-milling is corn (maize). In conventional wet-milling, corn is steeped in an aqueous solution containing sulfur dioxide (0.1–0.3%), an antimicrobial reducing agent, which solubilizes and disperses the proteinaceous matrix that envelops and binds the starch granules (Calzetta-Resio, Tolaba, & Suárez, 2006; Eckhoff & Tso, 1991). Modification of the structural

Abbreviations: ΔH_G, enthalpy of gelatinization; ΔH_R, enthalpy of retrogradation; a^*, redness to greenness; a_1, the main effect if x_1; a_{12}, the mixed coefficient that represents the interactions between factors; a_2, the main effect of x_2; a_{ii}, the square coefficients that indicate if any of the variables has a maximum or minimum in the experimental domain; b^*, yellowness to blueness; CPV, final or cool paste viscosity; DSC, Differential Scanning Calorimetry; g, grams; h, hour; HPV, hot paste viscosity; L^*, lightness; Ptemp, pasting temperature; Ptime, peak time; PV, peak viscosity; rpm, revolutions per minute; RS-Q, adjusted square coefficient of the fitting model; RVA, Rapid Visco Analyser; SW, leached solids in steepwater; T_c, conclusion temperature; T_o, onset temperature; T_p, peak temperature; WI, whiteness index; WQF, whole quinoa flour; WW, solids in washing water; x_1, the design factor steeping time; x_2, the design factor steeping temperature; Y_{calc}, data from the model; Y_{obs}, the experimental data; ε, the difference between the experimental data and the model, the residual

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Isolation of red quinoa fibre by wet and dry milling and application as a potential functional bakery ingredient

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ABSTRACT

Quinoa is recognised internationally for its nutritional and health properties. It has interesting attributes, such as being an excellent source of fibre and polyphenols and being gluten-free, and therefore this grain is used as a replacement for cereals. The main aim of this work was to study the effect of two milling methods, wet and dry, to obtain a dietary fibre-rich fraction from red Quinoa Real, and to determine its potential as a functional ingredient in bakery products. Wet milling produced a higher yield (10.1%) and recovery (58.2%) of the fibre fraction and higher purity (72%) than the values obtained by dry milling (9.1, 52.5 and 59%, respectively). With regard to functional properties, dry milling produced fibre with higher total antioxidant activity than that obtained by wet milling (FRAP: 1.1 times more). The fibre-rich fractions obtained by the two processes did not differ considerably in terms of colour, but the process affected their granulometry, which was lowest in the fibre obtained by wet milling, and the dispersity was greatest. Moreover, the bread products made with a 5% incorporation of either of the two fibres presented enrichment in terms of nutrients, dietary fibre and antioxidant capacity in comparison with the control sample. The inclusion of fibre isolated by dry milling produced bread products of higher technological quality with regard to specific loaf volume and less crumb firmness.

1. Introduction

Pseudocereals are a source of starch, proteins, lipids, dietary fibre, vitamins and minerals (Haros & Schoenlechner, 2017). As they also contain bioactive compounds such as polyphenols, pseudocereals have attracted increasing attention because of their potential for the development of functional foods (Ballester-Sánchez, Gil, Haros, & Fernández-Espinar, 2019; Haros & Schoenlechner, 2017; Wang & Zhu, 2016). Research in recent years has concentrated on the development of functional foods with an impact on health in regard to the prevention of metabolic disorders such as obesity and hypertension (Haros & Schoenlechner, 2017). Dietary fibre is of particular importance because of its involvement in the maintenance of a good state of health. However, its use in the food industry still presents a challenge because of its various physico-chemical and techno-functional characteristics, and its inclusion in the making of foods could affect their final quality positively or negatively as a result of changes in colour, texture, taste and water holding capacity, among other things (Lazaridou & Biliaderis, 2007).

Among pseudocereals, quinoa is a raw material with technological, sensory, nutritional and functional potential for use as a source of food ingredients in products with or without gluten, such as bread and other bakery products, snacks and breakfast cereals, infant foods, fermented beverages, pasta and traditional, exotic or haute cuisine foods (Haros & Schoenlechner, 2017; Ballester-Sánchez, Gil, Haros et al., 2019). Various methods for obtaining dietary fibre from diverse sources have been proposed, and the isolation conditions tend to be the parameters that most affect the chemical composition and techno-functional properties (Tejada-Ortigoza, García-Amezquita, Serna-Saldívar, & Welti-Chanes, 2016). The fibre in cereals/pseudocereals can be obtained by a process of milling followed by fractioning to obtain intermediate products (primary processing) that can then be used to make end products (secondary processing). The types of cereal milling are normally classified as dry milling or wet milling. In dry milling the aim is the individual separation of the anatomical parts of the grain, after conditioning with small quantities of water, in order to obtain, mainly, refined flour, bran and germ (Haros & Schoenlechner, 2017). However, as its name indicates, wet milling is performed with considerable quantities of water and the aim is to separate the chemical components of the grain – starch, proteins, fibres and lipids/oil – in order to obtain fractions of those components with the greatest purity possible (Haros &

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