Tesis Doctoral

Departament Medicina Preventiva i Salut Pública, Ciències de l'Alimentació, Toxicologia i Medicina Legal

Facultat de Farmàcia, Universitat de València

Programa de Doctorado en Ciencias de la Alimentación

USO DE HARINA DE ARROZ INTEGRAL GERMINADA A PARTIR DE VARIEDADES ECUATORIANAS DE GRANO LARGO PARA LA ELABORACIÓN DE PAN LIBRE DE GLUTEN

Tesis Doctoral presentada por: **Fabiola Cornejo Zúñiga**

Dirigida por:
Dra. Cristina Molina Rosell

Valencia, 2015

Dª Cristina Molina Rosell, Profesor de Investigación del Instituto de Agroquímica y Tecnología de Alimentos del Consejo Superior de Investigaciones Científicas.

Hace constar:

Que la memoria titulada "Uso de Harina de Arroz Integral Germinada a Partir de Variedades Ecuatorianas de grano largo para la Elaboración de Pan libre de Gluten" presentada por Dña. Fabiola Cornejo Zúñiga por la Universidad de Valencia, ha sido realizada en el Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) bajo su dirección y que reúne las condiciones necesarias para optar al grado de doctor.

Valencia, Abril de 2015

Dra. Cristina Molina Rosell

AGRADECIMIENTO

Esta investigación ha sido posible gracias a la financiación procedente de la Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación del Ecuador (SENESCYT), el Consejo Superior de Investigaciones Científicas (CSIC), la Escuela Superior Politécnica del Litoral (ESPOL), el Ministerio de Economía y Competitividad (Proyecto AGL2011-23802), la Generalitat Valenciana (Proyecto Grupo de Excelencia Prometeo 2012/064) y Fondos Europeos para el Desarrollo (FEDER).

Deseo expresar mis más sinceros agradecimientos a todas las instituciones y personas que de alguna manera han hecho posible el realizar este trabajo en especial a:

A la Dra. Cristina Molina Rosell, por la confianza y amistad que me brindó, gracias por enseñarme a ser mejor investigadora, espero con el tiempo seguir sus pasos.

A la Dra. Guillermina Font y Dr. Jordi Mañes, que me brindaron su amistad y apoyo en España.

A los directivos y amigos de la ESPOL, en especial a mi amiga, Grace Vásquez, gracias por apoyarme y darme animo en momentos difíciles. También a mi ayudante Janaina, la mejor ayudante que he tenido.

En general a instituciones como LEMAT, CIBE, INIAP por brindarme la oportunidad de realizar mis investigaciones en sus laboratorios.

A mi esposo (Boris) y mis hijos (Doménica, Boris José y Luciana) por su paciencia cuando dedicaba mi tiempo a realizar la tesis. Ustedes fueron mi fuerza para seguir siempre adelante.

A mi madre y hermanos por darme ánimo para continuar con mi sueño.

A Dios por permitirme cumplir otra meta más, porque en esta travesía tuve la oportunidad de aprender y por poner en mi camino a cada una de las personas que ayudaron a que esto fuera posible.

DEDICATORIA

Este trabajo es dedicado a: mi familia: Boris, Doménica, Boris José, Luciana, a mi mamá: Olga y en especial a la memoria de mi papá: Diego José Cornejo Redrován.

RESUMEN

Se estima que el 1% de la población mundial sufre de celiaquía, enfermedad autoinmune con intolerancia permanente al gluten. Generalmente, los productos de panificación libres de gluten son elaborados a base de arroz de granos cortos. En este estudio se exploró el uso de arroz integral germinado de variedad de grano largo en el desarrollo de pan libre de gluten. Inicialmente, se evaluó el comportamiento tecnológico de seis variedades ecuatorianas de arroz de grano largo (INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09 y F50) y su comportamiento como materia prima en la fabricación de pan sin gluten, así como establecer posibles correlaciones entre las características fisicoquímicas (propiedades químicas, de hidratación, térmicas y reológicas) de la harina de arroz con las características (humedad, volumen específico, color y textura) del pan obtenido. Los resultados demostraron que con arroz de grano largo es posible obtener pan sin gluten y sus características fisicoquímicas son comparables con panes sin gluten comerciales. Específicamente se determinó que parámetros como capacidad de ligación de agua (CLA), poder de hinchamiento (PH), volumen de hinchamiento (VH) y entalpia de gelatinización de la harina de arroz definen el comportamiento de los panes libres de gluten.

Asimismo, entre los retos científicos en la elaboración de productos libres de gluten se encuentra el incremento del valor nutricional de los mismos. En este estudio se abordó la utilización de harina de arroz integral germinado como ingrediente funcional en la fabricación de pan sin gluten. Se evaluó el efecto de diferentes etapas de germinación

en las propiedades fisicoquímicas de la harina de arroz germinada (propiedades reológicas y de hidratación) y las del pan de arroz germinado (color, textura y volumen específico). Además, se determinó los componentes nutricionales del pan de arroz germinado como la composición proximal, la digestibilidad *in vitro* de la proteína y almidón, el contenido de ácido fítico, el contenido de componentes bioactivos (GABA, γ-orizanol, compuestos fenólicos) y actividad antioxidante de los panes procedentes de diferentes tiempos de germinación (etapa de remojo, 12, 24 y 48 horas). Se observaron cambios significativos en las propiedades de hidratación y reológicas de la harina de arroz integral en las diferentes etapas de germinación. Se pudo evidenciar la acción enzimática sobre el gránulo de almidón durante la germinación, debido a una disminución de la viscosidad y de la capacidad de absorción de agua (CAA) de la harina. A medida que el tiempo de germinación aumentó, la suavidad de la miga del pan se incrementó. A 48 horas de germinación, a pesar de tener una calidad nutricional superior a las otras etapas, la calidad fisicoquímica del pan no fue apropiada. En general el uso de harina de arroz integral germinada como ingrediente funcional puede ser adecuada para panificación hasta las 24 horas de germinación. Las harinas procedentes de tiempos más prolongados de germinación serían recomendables para combinar con otras harinas y obtener mejoras nutricionales de los panes.

ABSTRACT

Approximately 1% of the world population are celiac patients, autoimmune disease that presents intolerance to gluten. Generally, gluten free bakery products are made from short grain rice. The aim of this study was to determine the breakmaking potential of germinated brown rice from long grain rice variety. Initially, six long-grain rice varieties (INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09 and F50) were evaluated to identify any flour characteristic (hydration and pasting) governing their breadmaking behavior. Results confirmed the suitability of long-grain rice varieties for breadmaking and were comparable to those reported in commercial gluten free breads (GFB). Results suggested that the most important parameters of rice flour when defining breadmaking performance of GFB would be water binding capacity (WBC), swelling power (SP), swelling volume (SV), and enthalpy.

On the other hand, a scientific challenge in GFB is to increase their nutritional value. With this aim, germinated brown rice was used as a functional ingredient to develop GFB. The effect of germination time on physicochemical characteristics of brown rice flour and its effect on gluten free bread qualities were investigated. Also, the proximate composition, phytic acid, *in vitro* protein digestibility and *in vitro* enzymatic hydrolysis of starch, glucose and starch content, as well as the most relevant bioactive compounds (GABA, γ-oryzanol and total phenolic compounds) and antioxidant activity of breads prepared with GBR at different germination conditions was determined. Germination was carried out at 28 °C and 100 % RH for 12, 24 and 48 h; brown rice and

soaked brown rice were also analyzed. Significant changes on hydration and pasting properties of brown rice flour were found during germination. The starch degradation by enzyme activity could be evidenced with the decrease in viscosity and water binding capacity. As germination time increase, a significant softness of the crumb was obtained. However, at 48 h of germination, although it provides GBR bread with nutritionally superior quality the bread quality was inferior. Overall, germinated rice flour showed appropriate functionality for being used as raw ingredient in gluten free breadmaking until 24 hour of germination. Longer germination times could provide nutritionally enriched flour for making flour blends.

ÍNDICE GENERAL

INTRODUCCIÓN

El arroz es uno de los cultivos de mayor producción a nivel mundial, alcanzando en el 2012 una cifra de 738 millones de toneladas de arroz en cáscara (FAOSTAT, 2014). En general, la producción del arroz se localiza mayoritariamente en el continente asiático; siendo China, India e Indonesia los mayores productores (Rosell & Gómez, 2014). Adicionalmente, cerca de 2.5 billones de personas en el mundo dependen del arroz como su principal fuente de alimentación y, más de 100 millones de hogares obtienen la mayor parte de sus ingresos a través de este cultivo.

En el Ecuador, el cultivo del arroz también constituye una de sus principales actividades agrícolas, en el 2010 el país ocupó el puesto 26 en el listado de productores de arroz a nivel mundial (FAOSTAT, 2014). Se estima que el cultivo de arroz da ocupación alrededor de 80 mil familias, de las cuales el 80 por ciento son pequeños productores, con menos de 20 has. Además, existen más de mil plantas pilladoras con capacidades que van desde 1 a 10 Tm/h (INEC, 2010).

En los últimos años, la producción del arroz en el Ecuador se ha incrementado tanto que han existido excedentes del mismo. Generalmente, los excedentes de arroz son adquiridos por el gobierno ecuatoriano y almacenados en silos pertenecientes a la "Unidad Nacional de Almacenamiento" (UNA). Este organismo, junto con el Banco Nacional de Fomento (BNF), se encarga de la recolección, compra y custodia del arroz a ser comercializado en el país. El almacenamiento de los excedentes de arroz permite que el precio del arroz no descienda, debido a que una caída en el precio podría afectar directamente a la economía del agricultor arrocero. Este arroz almacenado es por lo general comercializado internacionalmente, principalmente a países de América Latina. Lamentablemente, la calidad del grano de arroz ecuatoriano es inferior a la de otros países como Estados Unidos, por lo que la comercialización del grano depende del requerimiento de la región y de las relaciones internacionales que existan entre los gobiernos suramericanos, para que puedan ubicarse en el mercado internacional. Adicionalmente, este cultivo ha sido afectado por la incidencia de plagas, por lo que el Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP) ha desarrollado hibridaciones resistentes a estas plagas y con mayor rendimiento por hectárea; pudiendo estos cambios genéticos variar las características fisicoquímicas, funcionales y reológicas del arroz.

Por otro lado, en el Ecuador existe una gran demanda de consumo de pan, provocando que el país importe trigo; debido a que no es productor del mismo. Consecuentemente, el gobierno ecuatoriano estableció como meta en el "Plan Nacional de Buen Vivir 2013-2017" reducir las importaciones de trigo para el año 2017 (SENPLADES, 2013). En efecto, en 2014 el gobierno lanzó un proyecto de sustitución entre el 5 al 10% de harina de trigo por harina de banano, lo que constituiría según el gobierno, un ahorro de 15 millones de dólares en importaciones. Sin embargo, el desarrollo industrial de la harina de banano no ha sido analizado, lo que está provocando problemas en el avance del proyecto. Por ejemplo, entre las dificultades tecnológicas para la industrialización de la harina de banano se encuentran: la forma de pelado y el proceso de secado que requiere, lo que incrementaría los costos de producción. Además, existen estudios en los que se ha remplazado harina de trigo por banano, y a pesar que la sustitución ha mejorado las características nutricionales, las propiedades físicas del pan obtenido han sido perjudicadas (Ho, Aziz, & Azahari, 2013; Zuwariah & Aziah, 2009). En general, desde el ámbito nutricional y económico para el país, se puede utilizar otros cereales, frutas y tubérculos autóctonos como sustitutos del trigo en la elaboración de pan, entre los que podemos destacar al arroz.

El arroz tiene muchas ventajas sensoriales como nutricionales. Entre ellas, posee un sabor neutro, posee bajo contenido de sodio, es fácil de digerir, es hipoalergénico y no posee gluten. La ausencia de gluten en el arroz ha atraído el interés de varios investigadores debido a su adecuación para enfermos de celiaquía, quienes no pueden consumir ésta proteína en sus dietas (Gujral, Haros, & Rosell, 2003; Kadan, Robinson, Thibodeaux, & Pepperman, 2001; McCarthy et al. 2005). La celiaquía es una enfermedad autoinmune que se caracteriza con una inflamación del intestino delgado y daño en su revestimiento produciendo una mala absorción de nutrientes cuando se consume alimentos que contienen gluten. Entre los síntomas que conlleva esta enfermedad se encuentran diarrea, vómito, constipación, fatiga, deficiencia de hierro, hipoplasia del esmalte dental, fractura, artritis, retardo de la pubertad, corta estatura, infertilidad, ansiedad, depresión, entre otros. Por otra parte, existen enfermedades asociadas a la celiaquía, como diabetes mellitus, dermatitis herpetiforme, síndrome de Down y enfermedades neurológicas. La celiaquía es una enfermedad crónica, que afecta a personas de cualquier edad, raza o grupo étnico. Actualmente,

el único tratamiento disponible para los celiacos es una dieta estricta libre de gluten, por el resto de su vida. En la actualidad, se estima que el 1% de la población mundial padece esta enfermedad y sólo del 10 al 15% de esta población ha sido diagnosticado y tratado (Guandalini & Assiri, 2014). Según las estadísticas, cada veinte años se duplica el número de pacientes celiacos (Rubio–Tapia et al., 2009). En general, entre los cereales que se consideran tóxicos para los celíacos se encuentran el trigo, el centeno y la cebada; la avena se considera que puede estar contaminada de trazas de gluten. El arroz, maíz, sorgo y trigo sarraceno son considerados cereales aptos para los celíacos. Lamentablemente, una dieta libre de gluten es muy difícil de mantener, porque en el mercado los productos libre de gluten son escasos y costosos; además de ser deficientes en micronutrientes y fibra (Matos & Rosell, 2012, Capriles & Arêas, 2014).

El gluten es una proteína esencial en la formulación de productos de panificación. En efecto, esta proteína está compuesta por dos fracciones proteicas: la gliadina y la glutenina. La gliadina contribuye a la viscosidad de la masa de pan y la glutenina es responsable de la elasticidad de la masa de pan. Esta masa visco-elástica tiene la habilidad de formar una película bien fina que retiene el gas procedente de la fermentación, permitiendo que la masa se expanda; además contribuye directamente a la formación de una estructura alveolar en la miga, que tras el horneado, confiere palatabilidad y textura suave (Cauvain, 2007). Por lo tanto, la falta de gluten en productos de panificación, representa un reto para los científicos en alimentos, ya que las harinas libres de gluten son incapaces de formar una masa visco-elástica (Arendt, Moore, & Bello, 2008). Con el fin de formar una miga aceptable, las harinas libres de gluten requieren captar mayor contenido de agua que la harina de trigo (Capriles & Arêas, 2014). Para mejorar las características fisicoquímicas del pan sin gluten a base de arroz, se han utilizado compuestos como gomas, hidrocoloides, ingredientes a base de proteínas, enzimas, entre otros (Arendt et al., 2008; Matos. & Rosell., 2014; Rosell, 2009). No obstante, se ha demostrado que la calidad del pan está relacionada con las propiedades fisicoquímicas de la harina de arroz, y estas a su vez dependen de la variedad de arroz (Han, Cho, Kang, & Koh, 2012; Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011; Yu, Ma, Menager, & Sun, 2010) , el tamaño de partícula de la harina, la longitud del grano de arroz (de la Hera, Gomez, & Rosell, 2013), las condiciones de

almacenamiento del arroz (Park, Kim, Park, & Kim, 2012; Tananuwong & Malila, 2011), los tipos de modificaciones realizadas a la harina de arroz (Guha & Ali, 2011), la estructura y composición química (Kim, Song, & Shin, 2010; Zhu et al., 2010), entre otros.

En general, los panes de arroz libres de gluten se fabrican a partir de harinas refinadas provenientes de variedades de arroz de grano corto. Hay estudios que han establecido que la longitud del grano de arroz, el contenido de amilosa, el grado de refinación de la harina y la capacidad absorción de agua son factores determinantes en la calidad de los panes sin gluten (de la Hera, Martinez, & Gómez, 2013; Han et al., 2012; Kadan et al., 2001; Rosell & Gómez, 2006; Torres et al., 1999). El tamaño del grano de arroz está relacionado con el contenido de amilosa. Los granos largos de arroz comúnmente presentan un alto contenido de amilosa, alta temperatura de gelatinización y una mayor tendencia a la retrogradación. Noomhorm et al. (1994) demostraron que una baja temperatura de gelatinización beneficiaba el proceso de panificación. Han et al. (2012) obtuvieron mejores resultados con las variedades que poseían un contenido intermedio de amilosa y bajo índice de absorción de agua. Además, Kadan et al. (2001) analizaron la textura del pan de arroz con variedades de grano largo y grano corto, concluyendo que combinar parte de la variedad de grano largo con un 10% de variedad de grano corto proporcionaba una textura más suave en el pan. En general, algunos estudios han demostrado que variedades de arroz con granos medios y cortos presentan migas más suaves y panes con volúmenes específicos altos (de la Hera et al., 2013; Rosell & Gómez, 2006). Cabe recalcar, las comparaciones entre grano largo y corto de estas investigaciones, no incluían un análisis de la variedad del grano. Las variedades de arroz ecuatoriano son consideradas arroz de grano largo y extra largo, con longitudes superiores a 7 mm. Consecuentemente según los estudios mencionados, las variedades ecuatorianas no serían apropiadas para la elaboración de pan sin gluten. Pero como se ha mencionado anteriormente, no existen estudios previos que identifiquen el efecto de la variedad de arroz de grano largo.

Por otro lado, un inconveniente de los productos dirigidos a la población celiaca es que sólo se enfocan hacia la eliminación de alergenos, en este caso el gluten. En el caso de los productos de panificación se utilizan mezclas de polímeros que pudieran originar productos con características sensoriales similares a los que contienen gluten. A fin de incrementar el valor nutricional de los panes sin gluten, algunos estudios han utilizado harina de pseudo-cereales (Alvarez, Auty, Arendt, & Gallagher, 2009; Miñarro, et al., 2012), harina de soya (Marco & Rosell, 2008), prebióticos (Korus, Grzelak, Achremowicz, & Sabat, 2006; Sabanis, Lebesi, & Tzia, 2009), almidones resistentes (Korus, Witczak, Ziobro, & Juszczak, 2009), entre otros. Sin embargo, pocos estudios se han enfocado en el procesado físico de las materias primas para incrementar el valor nutricional de los productos sin gluten. En este sentido se ha descrito el impacto de la distribución del tamaño de partícula de las harinas de arroz sobre la calidad tecnológica y nutritiva de los panes sin gluten, concretamente el impacto sobre la hidrólisis del almidón y consecuentemente el índice de glucemia. (de la Hera et al., 2013; de la Hera, Rosell, & Gomez, 2014).

Otra alternativa que ha ido adquiriendo importancia como enriquecedor de productos de panificación, es la germinación de los granos de arroz (Charoenthaikij et al, 2012; Charoenthaikij et al., 2010a; Charoenthaikij et al. 2010b; Watanabe et al., 2004). En la germinación se producen cambios bioquímicos, nutricionales y sensoriales del grano de arroz. El arroz integral germinado es considerado un grano libre de gluten con un valor nutricional alto. En efecto, durante la germinación, las enzimas hidrolíticas son activadas y estas descomponen a los almidones y proteínas en azúcares simples, proteínas solubles y aminoácidos. Entre las enzimas más determinantes se encuentran la α-amilasa, β-amilasa, α-glucosidasa y proteasas. La α-amilasa y α-glucosidasa son las responsables de degradar los granulos de amilosa e hidrolizan el almidón en oligosacaridos (Xu, Zhang, Guo, & Qian, 2012). Adicionalmente, durante la germinación se produce también la liberación de los minerales internos, haciendo del arroz integral con textura más suave y apetitoso (Ito & Ishikawa, 2004).

En general, la descomposición de los polímeros de alto peso molecular producen la generación de sustancias bio-funcionales. Entre los compuesto bioactivos que se generan durante

la germinación están: el γ-orizanol (Pestana et al., 2009; Shokrzadeh & Ebadi, 2006), el gama aminobutirico (GABA) (Banchuen et al., 2010), compuestos fenólicos, fibra dietética, ácido felúrico (Banchuen et al., 2010), tocotrienoles, magnesio, potasio, zinc y vitamina E, así como el incremento de la actividad antioxidante (Caceres, Martinez-Villaluenga, Amigo, & Frias, 2014; Wu et al., 2013a). Al mismo tiempo, se produce una disminución del ácido fítico, incrementando la disponibilidad de los minerales (Kim et al., 2012). Entre los efectos benéficos de estos compuestos bioactivos se encuentran la regulación de la presión arterial, alivio del dolor y ansiedad, mejora del sueño, características antimutagénicas y anticancerígenas, entre otros (Oh & Oh, 2004; Okada et al., 2000). La acumulación de los compuestos bioactivos durante la germinación depende de la variedad del grano, pH, presencia de aditivos, aireación, temperatura y tiempo durante la fase de remojo y germinación (Islam & Becerra, 2011; Watchararparpaiboon, Laohakunjit, & Kerdchoechuen, 2010; Zhang et al., 2014). La tabla 1 muestra las características funcionales de los componentes nutricionales dela arroz integral germinado.

El obtener granos germinados de alta calidad es difícil debido los efectos sinérgicos de las diferentes variables del proceso de germinación (Capanzana & Buckle, 1997). Muchos estudios han buscado optimizar el proceso de germinación, estableciendo temperaturas y tiempos de remojo y germinado, con el fin de obtener un alto contenido de GABA y orizanol. Se han reportado temperaturas óptimas comprendidas entre 27 y 37°C (Banchuen et al., 2010; Caceres et al., 2014; Moongngarm & Saetung, 2010; Tian, Nakamura, & Kayahara, 2004; Watanabe et al., 2004). Aunque para el arroz en cáscara, temperatura de 30°C demostró un efecto importante en el aumento de GABA, γ-orizanol, proteínas, aminoácidos esenciales, fibra, entre otros (Moongngarm & Saetung, 2010). Caceres et al. (2014) demostraron que la acumulación de GABA se inicia desde el remojo y continúa de una manera dependiente del tiempo durante la germinación. Durante el remojo se induce la actividad de la enzima glutamato descarboxilasa (GAD) que cataliza la c-decarboxilación de ácido L-glutámico a dióxido de carbono y GABA. La actividad de la GAD se incrementa con el tiempo de germinación.

Tabla 1: Ejemplos de actividades funcionales de arroz integral germinado

Nutrientes	Actividad Funcional en el Arroz Integral Germinado
GABA	Efecto hipotensor, Acelerador del Metabolismo en el cerebro, previene dolores de cabeza o depresión posterior, arteriosclerosis cerebral y apoplejía cerebral, previene el desordenes climatéricos, previene el insomnio, irritación mental y activa las funciones renales
Fibra Dietaría	Alivia la constipación, previene el cáncer del colón, regula los niveles de azúcar en la sangre.
Inositol	Acelera el metabolismo de las grasas, previene la arterioesclerosis
Ácido Felúrico	Eliminación de radicales superóxidos, Inhibidor de la melanogénesis
Ácido Fítico	Efecto Antioxidante, Protector de enfermedades cardiovasculares, Previene agregación plaquetaria
Tocotrienoles	Eliminación de radicales superóxidos, protege a la piel de los rayos ultravioleta
Magnesio	Previene enfermedades del corazón
Potasio	Disminuye la presión sanguínea
Zinc	Activa la función reproductiva, previene la arterioesclerosis
Gamma Orizanol	Efecto antioxidante, previene el envejecimiento de la piel, regula los valores del colesterol
Inhibidor de prolilendopeptidasa	Posiblemente previene el Alzheimer

Fuente: Patil & Khan (2011).

La temperatura y el tiempo de germinación no sólo influye en el contenido de compuestos bioactivos, también afectan las características fisicoquímicas de la harina de arroz integral germinada (Wu et al., 2013b; Xu et al., 2012). Capanzana y Buckle (1997) demostraron que el tiempo y temperatura de proceso afecta significativamente la actividad amilasa. La activación de las enzimas alfa amilasa origina la formación de dextrinas de bajo peso molecular, que se han descrito como agentes antienvejecimiento del pan (Gray & Bemiller, 2003; Watanabe et al., 2004); pero una alta actividad alfa amilasa podría producir un pan pegajoso (Gujral & Kumar, 2003). Algunos estudios han demostrado que durante la germinación el contenido total de almidón disminuye, reduciéndose mayoritariamente el contenido de amilosa frente al de amilopectina (Wu et al., 2013a; Xu et al., 2012). Además, se produce un acortamiento de las cadenas de amilopectina y amilosa. Nutricionalmente, la disminución del contenido de amilosa produce un incremento del almidón de digestión lenta (ADL). Durante la germinación, las enzimas hidrolizan la región amorfa del granulo de almidón (constituida principalmente por amilosa), quedando la región cristalina (mayoritaría en amilopectina) que es más difícil de digerir (Xu et al., 2012). En general, durante la germinación se produce una disminución del almidón total (AT), almidón resistente

(AR) y el índice glucémico, debido al uso del almidón como fuente de energía durante la germinación (Swieca, Baraniak, & Gawlik-Dziki, 2013). A diferencia del arroz pulido, el arroz integral germinado puede ser consumido por pacientes con diabetes mellitus por su bajo índice glicémico (Kim et al., 2012). Shallan et al (2010) encontraron que la alimentación de ratones con arroz integral pre-germinado disminuye el contenido de glucosa y lipidos en la sangre.

A pesar de las características saludables del arroz germinado, descritas anteriormente, son limitados los estudios que analizan el contenido de estos compuestos en una matriz alimenticia como el pan. Tecnológicamente, estos cambios que se generan durante la germinación producen una disminución de la viscosidad, disminución de la solubilidad, y aumento del poder de hinchamiento (Capanzana & Buckle, 1997; Xu et al., 2012). Adicionalmente, se ha demostrado que después de 24 horas de germinación la temperatura de gelatinización y la entalpía de gelatinización disminuye (Wu et al., 2013b; Xu et al., 2012). Adicionalmente, en el proceso de panificación, los compuestos funcionales pueden ser degradados durante el amasado, fermentación o horneado. Estudios anteriores, demostraron que el contenido de GABA disminuía durante el proceso de panificación (Lamberts, Joye, Beliën, & Delcour, 2012; Watanabe et al., 2004). Según Lamberts et al. (2012) la levadura consume el GABA como fuente de nitrógeno durante la fermentación.

La harina de arroz integral germinado sólo ha sido utilizada como sustituto parcial entre el 10 y 30% de la harina de trigo (Charoenthaikij et al., 2012; Charoenthaikij et al., 2010a; Charoenthaikij et al., 2010b; Watanabe et al., 2004). No existen reportes sobre el uso total de arroz integral germinado en la elaboración de pan libre de gluten. En parte, el efecto de la germinación puede ser beneficioso en la elaboración del pan, si se compara con el arroz integral, porque en sustituciones parciales mejora la textura del pan e induce mayor formación de gas (Charoenthaikij et al., 2010b). Al utilizar la harina de arroz integral germinado se obtendrían los beneficios de los compuestos bioactivos; que permitiría brindar un alimento funcional tanto para la población de celiacos como a personas con otros tipos de enfermedades. No obstante, otros investigadores demuestran que la sustitución parcial de harina de trigo con harina de arroz germinada produce

cambios en las propiedades reológicas de la masa (Charoenthaikij et al., 2010b; Renzetti & Arendt, 2009; Sivaramakrishnan, Senge, & Chattopadhyay, 2004), además las características sensoriales se ven afectadas ya que disminuye el volumen del pan, se incrementa la firmeza (Gray & Bemiller, 2003; Kadan et al., 2001; Renzetti & Arendt, 2009) y el oscurecimiento del pan. Ohtsubo et al. (2005) elaboraron un pan con 30% de sustitución con harina de arroz integral germinada previamente extruida, indicaron que se obtuvo un mejor sabor, mayor contenido de GABA, azúcares libres y maltosa. Las investigaciones sobre el efecto de la germinación en las características fisicoquímicas del pan son limitadas y se han abordado únicamente sustituciones parciales.

Bibliografía:

Alvarez-Jubete, L., Auty, M., Arendt, E. K., & Gallagher, E. (2009). Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *European Food Research and Technology, 230*(3), 437-445. doi: 10.1007/s00217-009-1184-z

Arendt, E., Moore, M., & Bello, F. D. (2008). Gluten Free Breads. In E. Arendt & F. D. Bello (Eds.), *Gluten Free cereal products and Beverages.* (pp. 289- 319). London: Academic Press

Banchuen, J., Thammarutwasik, P., Ooraikul, B., Wuttijumnong, P., & Sirivongpaisal, P. (2010). Increasing the bio-active compounds contents by optimizing the germination conditions of southern Thai brown rice. *Songklanakarin Journal of Science and Technology, 32*(3), 219-230.

Caceres, P. J., Martinez-Villaluenga, C., Amigo, L., & Frias, J. (2014). Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. *Food Chemistry, 152*, 407-414. doi: 10.1016/j.foodchem.2013.11.156

Capanzana, M. V., & Buckle, K. A. (1997). Optimisation of Germination Conditions by Response Surface Methodology of a High Amylose Rice (Oryza sativa) Cultivar. *LTW Food Science and Technology, 30*, 155-163.

Capriles, V. D., & Arêas, J. A. G. (2014). Novel Approaches in Gluten-Free Breadmaking: Interface between Food Science, Nutrition, and Health. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 871-890. doi: 10.1111/1541-4337.12091

Cauvain, S. (2007). Bread: The product. In S. Cauvain & L. Young (Eds.), *Technology of Bread Making* (Second ed., pp. 1-18). New York: Springer.

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & No, H. K. (2012). Composite wheat-germinated brown rice flours: selected physicochemical properties and bread application. *International Journal of Food Science & Technology, 47*(1), 75-82. doi: 10.1111/j.1365-2621.2011.02809.x

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., No, H. K., & King, J. M. (2010a). Physicochemical properties and consumer acceptance of wheat-germinated brown rice bread during storage time. *Journal of Food Science, 75*(6), S333-339. doi: 10.1111/j.1750-3841.2010.01684.x

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & Tungtrakul, P. (2010b). Germination conditions affect selected quality of composite wheat-germinated brown rice flour and bread formulations. *Journal of Food Science, 75*(6), S312-318. doi: 10.1111/j.1750-3841.2010.01712.x

de la Hera, E., Gomez, M., & Rosell, C. M. (2013). Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. *Carbohydrate Polymers, 98*(1), 421-427. doi: 10.1016/j.carbpol.2013.06.002

de la Hera, E., Martinez, M., & Gómez, M. (2013). Influence of flour particle size on quality of gluten-free rice bread. *LWT - Food Science and Technology, 54*(1), 199-206. doi: 10.1016/j.lwt.2013.04.019

de la Hera, E., Rosell, C. M., & Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food Chemistry, 151*, 526-531. doi: 10.1016/j.foodchem.2013.11.115

FAOSTAT. (2014). Retrieved 30 January 2015, 2015, from http://faostat3.fao.org/home/E

Gray, J., & Bemiller, J. (2003). Bread staling: molecular basis and control. *Comprehensive Reviews in Food Science and Food Safety, 2*(1), 1-21.

Guandalini, S., & Assiri, A. (2014). Celiac disease: a review. *JAMA pediatrics, 168*(3), 272-278.

Guha, M., & Ali, S. Z. (2011). Changes in rheological properties of rice flour during extrusion cooking. *Journal of Texture Studies, 42*(6), 451-458.

Gujral, H. S., Haros, M., & Rosell, C. M. (2003). Starch hydrolyzing enzymes for retarding the staling of rice bread. *Cereal Chemistry, 80*(6), 750-754.

Gujral, H. S., & Kumar, V. (2003). Effect of accelerated aging on the physicochemical and textural properties of brown and milled rice. *Journal of Food Engineering, 59*(2-3), 117-121. doi: 10.1016/s0260-8774(02)00438-7

Han, H. M., Cho, J. H., Kang, H. W., & Koh, B. K. (2012). Rice varieties in relation to rice bread quality. *Journal of Science of Food and Agriculture, 92*(7), 1462-1467. doi: 10.1002/jsfa.4727

Ho, L.-H., Aziz, N. A. A., & Azahari, B. (2013). Physico-chemical characteristics and sensory evaluation of wheat bread partially substituted with banana (Musa acuminata X balbisiana cv. Awak) pseudo-stem flour. *Food Chemistry, 139*(1), 532-539.

INEC. (2010). Sistema Agrolimentario del Arroz. In I. N. d. E. y. Censo (Ed.). Quito

Islam, M. A., & Becerra, J. X. (2011). Analysis of Chemical Components Involved in Germination Process of Rice Variety Jhapra. *Journal of Scientific Research, 4*(1). doi: 10.3329/jsr.v4i1.7598

Ito, S., & Ishikawa, Y. (2004). *Marketing of value- added rice products in japan: germinated brown rice and rice bread.* Paper presented at the FAO Rice Conference, Italy.

Kadan, R., Robinson, M., Thibodeaux, D., & Pepperman, A. (2001). Texture and other physicochemical properties of whole rice bread. *Journal Food Science, 66*(7), 940-944.

Kim, H. Y., Hwang, I. G., Kim, T. M., Woo, K. S., Park, D. S., Kim, J. H., Jeong, H. S. (2012). Chemical and functional components in different parts of rough rice (Oryza sativa L.) before and after germination. *Food Chemistry, 134*(1), 288-293. doi: 10.1016/j.foodchem.2012.02.138

Kim, J.-M., Song, J.-Y., & Shin, M. (2010). Physicochemical properties of high amylose rice starches purified from Korean cultivars. *Starch - Stärke, 62*(5), 262-268. doi: 10.1002/star.200900230

Korus, J., Grzelak, K., Achremowicz, K., & Sabat, R. (2006). Influence of prebiotic additions on the quality of gluten-free bread and on the content of inulin and fructooligosaccharides. *Food Science and Technology International, 12*(6), 489-495.

Korus, J., Witczak, M., Ziobro, R., & Juszczak, L. (2009). The impact of resistant starch on characteristics of gluten-free dough and bread. *Food Hydrocolloids, 23*(3), 988-995. doi: 10.1016/j.foodhyd.2008.07.010

Marco, C., & Rosell, C. M. (2008). Functional and rheological properties of protein enriched gluten free composite flours. *Journal of Food Engineering, 88*(1), 94-103. doi: 10.1016/j.jfoodeng.2008.01.018

Matos, E., & Rosell, C. (2012). Relationship between instrumental parameters and sensory characteristics in gluten-free breads. . *European Food Research and Technology, 235*(1), 107 - 117.

Matos., M., & Rosell., C. (2014). A review: understanding gluten free dough for reaching breads with physical quality and nutritional balance. . *Journal of the Science of Food and Agriculture., 95*(4), 653-661. doi: 10.1002/jsfa.6732

McCarthy, D., Gallagher, E., Gormley, T., Schober, T., & Arendt, E. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry, 82*(5), 609-615.

Miñarro, B., Albanell, E., Aguilar, N., Guamis, B., & Capellas, M. (2012). Effect of legume flours on baking characteristics of gluten-free bread. *Journal of Cereal Science, 56*(2), 476-481. doi: http://dx.doi.org/10.1016/j.jcs.2012.04.012

Moongngarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry, 122*(3), 782-788. doi: 10.1016/j.foodchem.2010.03.053

Noomhorm, A., Bandola, D., & Kongseree, N. (1994). Effect of rice variety, rice flour concentration and enzyme levels on composite bread quality. *Journal of the Science Food and Agriculture, 64*(4), 433-440.

Oh, C.-H., & Oh, S.-H. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *Journal of Medicinal Food, 7*(1), 19-23.

Ohtsubo, K. i., Suzuki, K., Yasui, Y., & Kasumi, T. (2005). Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. *Journal of Food Composition and Analysis, 18*(4), 303-316. doi: 10.1016/j.jfca.2004.10.003

Okada, T., Sugishita, T., Murakami, T., Murai, H., Saikusa, T., Horino, T., . . . Takahashi, T. (2000). Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. *Nippon Shokuhin Kagaku Kogaku Kaishi= Journal of the Japanese Society for Food Science and Technology, 47*(8), 596-603.

Park, C.-E., Kim, Y.-S., Park, K.-J., & Kim, B.-K. (2012). Changes in physicochemical characteristics of rice during storage at different temperatures. *Journal of Stored Products Research, 48*, 25-29.

Patil, S. B., & Khan, M. K. (2011). Germinated brown rice as a value added rice product: A review. *Journal of Food Science and Technology, 48*(6), 661-667. doi: 10.1007/s13197-011-0232-4

Pestana, V. R., Zambiazi, R. C., Mendonça, C. R. B., Bruscatto, M. H., & Ramis-Ramos, G. (2009). Influencia del procesado industrial sobre las características químico-físicas y contenido en lípidos y antioxidantes del salvado de arroz. *Grasas y Aceites, 60*(2), 184-193. doi: 10.3989/gya.075108

Renzetti, S., & Arendt, E. K. (2009). Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. *Journal of Cereal Science, 50*(1), 22-28. doi: 10.1016/j.jcs.2009.02.002

Rosell, C. (2009). Enzymatic manipulation of gluten-free bread. In E. Gallagher. (Ed.), *Gluten-free Food Science and Technology* (pp. 83-98). Oxford, UK.: Wiley-Blackwell Publishing Ltd,.

Rosell, C., & Gómez, M. (2006). Rice. In W. Zhou, Y. H. Hui, I. DeLeyn, M. A. Pagani, C. M. Rosell, J. Selman, and N. Therdthai. (Eds.), *Bakery products: Science and Technology* (pp. 23-133). Ames, Iowa: Blackwell Publishing, ISBN: 9781118792001. DOI: 10.1002/9781118792001..

Rosell, C., & Gómez, M. (2014). Rice. In W. Zhou, Y. H. Hui, I. DeLeyn, M. A. Pagani, C. M. Rosell , J. Selman, & N. Therdthai. (Eds.), *Bakery products: Science and Technology.* (2 ed., pp. 89-106). Ames, Iowa. USA: John Wiley & Sons, Ltd.

Rubio–Tapia, A., Kyle, R. A., Kaplan, E. L., Johnson, D. R., Page, W., Erdtmann, F., Lahr, B. D. (2009). Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology, 137*(1), 88-93.

Sabanis, D., Lebesi, D., & Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *LWT - Food Science and Technology, 42*(8), 1380-1389. doi: 10.1016/j.lwt.2009.03.010

SENPLADES. (2013). Plan Nacional para el Buen Vivir 2013-2017. In S. N. d. P. y. Desarrollo (Ed.). Quito.

Shallan, M., El-Beltagi, H., Mona, A., Amera, T., & Sohir, N. (2010). Effect of Amylose Content and Pre-germinated Brown Rice on Serum Blood Glucose and Lipids in Experimental Animal. *Australian Journal of Basic and Applied Sciences, 4*(2), 114-121.

Shokrzadeh, M., & Ebadi, A. (2006). Study of Antioxidative Activity in Four Kinds of Cultivated Rice Grains of Mazandaran Province (Iran). *Pakistan Journal of Biological Sciences, 9*(14), 2723 -2725.

Sivaramakrishnan, H. P., Senge, B., & Chattopadhyay, P. K. (2004). Rheological properties of rice dough for making rice bread. *Journal of Food Engineering, 62*(1), 37-45. doi: 10.1016/s0260-8774(03)00169-9

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., & Berghofer, E. (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry, 124*(1), 132-140. doi: 10.1016/j.foodchem.2010.05.115

Swieca, M., Baraniak, B., & Gawlik-Dziki, U. (2013). In vitro digestibility and starch content, predicted glycemic index and potential in vitro antidiabetic effect of lentil sprouts obtained by different germination techniques. *Food Chemistry, 138*(2-3), 1414-1420. doi: 10.1016/j.foodchem.2012.09.122

Tananuwong, K., & Malila, Y. (2011). Changes in physicochemical properties of organic hulled rice during storage under different conditions. *Food Chemistry, 125*(1), 179-185.

Tian, S., Nakamura, K., & Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agriculture and Food Chemistry, 52*(15), 4808-4813.

Torres, R., González, R., Sánchez, H., Osella, C., & Torre, M. d. l. (1999). Comportamiento de variedades de arroz en la elaboración de pan sin gluten. *Archivos Latinoamericanos de Nutrición, 49*(2), 162-165.

Watanabe, M., Maeda, T., Tsukahara, K., Kayahara, H., & Morita, N. (2004). Application of Pregerminated Brown Rice for Breadmaking *Cereal Chemistry, 81*(4), 450- 455.

Watchararparpaiboon, W., Laohakunjit, N., & Kerdchoechuen, O. (2010). An improved process for high quality and nutrition of brown rice production. *Food Science and Technology International, 16*(2), 147-158.

Wu, F., Yang, N., Toure, A., Jin, Z., & Xu, X. (2013a). Germinated brown rice and its role in human health. *Critical Reviews of Food Science and Nutrition, 53*(5), 451-463. doi: 10.1080/10408398.2010.542259

Wu, F., Chen, H., Yang, N., Wang, J., Duan, X., Jin, Z., & Xu, X. (2013b). Effect of germination time on physicochemical properties of brown rice flour and starch from different rice cultivars. *Journal of Cereal Science, 58*(2), 263-271. doi: 10.1016/j.jcs.2013.06.008

Xu, J., Zhang, H., Guo, X., & Qian, H. (2012). The impact of germination on the characteristics of brown rice flour and starch. *Journal of Science of Food and Agriculture, 92*(2), 380-387. doi: 10.1002/jsfa.4588

Yu, S., Ma, Y., Menager, L., & Sun, D.-W. (2010). Physicochemical Properties of Starch and Flour from Different Rice Cultivars. *Food and Bioprocess Technology, 5*(2), 626-637. doi: 10.1007/s11947-010-0330-8

Zhang, Q., Xiang, J., Zhang, L., Zhu, X., Evers, J., van der Werf, W., & Duan, L. (2014). Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice. *Journal of Functional Foods, 10*, 283-291. doi: 10.1016/j.jff.2014.06.009

Zhu, L.-J., Liu, Q.-Q., Sang, Y., Gu, M.-H., & Shi, Y.-C. (2010). Underlying reasons for waxy rice flours having different pasting properties. *Food Chemistry, 120*(1), 94-100. doi: 10.1016/j.foodchem.2009.09.076

Zuwariah, I., & Aziah, A. N. (2009). Physicochemical properties of wheat breads substituted with banana flour and modified banana flour. *Journal of Tropical Agriculture and Food. Science, 37*(1), 33-42.

OBJETIVOS Y PLAN DE TRABAJO

OBJETIVOS

El objetivo general de la tesis doctoral es la obtención de panes libres de gluten nutricionalmente mejorados a base de harina de arroz de grano largo integral germinado.

En dicho objetivo general se incluyen los siguientes objetivos particulares:

1. Determinar la viabilidad de uso de seis variedades de arroz de grano largo (INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09 and F50) en el proceso de panificación e identificar las propiedades de la harina que interviene en la calidad del pan libre de gluten.

2. Determinar el efecto del tiempo de germinación en las características fisicoquímicas de la harina de arroz integral y definir el efecto del uso de la harina de arroz integral germinada, como ingrediente principal, en la calidad del pan libre de gluten

3. Establecer efecto de las condiciones de germinación en la características nutricionales (composición proximal, ácido fitico, digestibilidad in vitro de proteína, digestibilidad in vitro de almidón, contenido de componentes bio-activos como GABA y gama-orizanol y compuestos fenólicos) del pan de arroz integral libre de gluten.

PLAN DE TRABAJO

Para la consecución de los objetivos anteriormente propuestos se propone el siguiente plan de trabajo:

- Comparación entre las características fisicoquímicas (propiedades químicas, de hidratación, térmicas y reológicas) de harinas provenientes de seis variedades de arroz de grano largo.

- Fabricación de panes libres de gluten a partir de arroz de grano largo. Determinación de la posible correlación entre las características fisicoquímicas (propiedades químicas, de hidratación, térmicas y reológicas) de la harina de arroz de grano largo con las características (humedad, volumen específico, color y textura) del pan obtenido.

- Análisis de las características fisicoquímicas (propiedades de hidratación, térmicas y reológicas) de harina de arroz integral, arroz de remojo (24 horas) y arroz germinado a 12, 24 y 48 horas.

- Elaboración de panes libres de gluten a partir de harinas integral, remojada y germinada y caracterización de los mismos atendiendo a su volumen específico, textura y color. Análisis de posibles correlaciones las propiedades de la harina de arroz germinada y las características de los panes.

- Análisis de las características nutricionales (análisis proximal, compuestos bioactivos, digestibilidad in-vitro de proteína y almidón) del pan de arroz integral germinado.

Este plan de trabajo se ha estructurado en tres capítulos que han sido origen de sendas publicaciones.

- Physicochemical Properties of Long Rice Grain Varieties in Relation to Gluten Free Bread Quality. *LTW- Food Science and Technology*, 62 (2015) 1203-12102

- Influence of Germination Time of Brown Rice in Relation to Flour and Gluten Free Quality. *Journal of Food Science and Technology* (2015) 1 – 8.

- Effects of Germination on the Nutritive Value and Bioactive Compounds o Brown Rice Bread. *Food Chemistry*, 173 (2015) 298-304

CAPÍTULO 1

PHYSICOCHEMICAL PROPERTIES OF LONG RICE GRAIN VARIETIES IN RELATION TO GLUTEN FREE BREAD QUALITY

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Physicochemical properties of long rice grain varieties in relation to gluten free bread quality

Fabiola Cornejo [a, b], Cristina M. Rosell [a, *]

[a] Institute of Agrochemistry and Food Technology (IATA-CSIC), Avenida Agustín Escardino, 7, Paterna 46980, Valencia, Spain
[b] Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ingeniería Mecánica y Ciencias de la Producción, Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador

Abstract

The aim of this study was conducted to determine the breadmaking potential of six long-grain rice varieties (INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09 and F50) and to identify any flour characteristic governing their breadmaking behavior. Pasting parameters, thermal parameters assessed by differential scanning calorimetry and bread quality parameters (specific volume, color, and crumb texture profile analysis) were assessed. Results confirmed the suitability of long-grain rice varieties for breadmaking. Nevertheless, significant differences were observed in flour properties among varieties. A significant correlation was observed between specific volume of the gluten-free bread (GFB) with swelling power (r=0.71, P<0.01), breakdown viscosity (r=-0.97, P<0.01) and conclusion temperature (Tc) of gelatinization (r=0.81, P<0.05). Moreover, a strong correlation was found between cohesiveness and properties of rice flour such as peak temperature (Tp) (r=-0.96, P<0.001), ΔH (r=0.71 P<0.05) and swelling volume (r=0.82, P<0.05). The quality characteristics of the gluten-free breads made of long-grain rice flour were comparable to those reported in commercial GFB. INIAP 14 and F09 were the most promising varieties for bakery applications. Results suggested that the most important parameters of rice flour when defining breadmaking performance of GFB would be WBC, SP, SV, Tp, Tc and enthalpy.

Key words: rice flour; variety; bread; gluten-free; quality.

1. Introduction

Rice is mainly consumed as cooked rice, but during the last decade the consumption of rice flour has increased due to its application in breadmaking. Rice has unique sensorial and nutritional advantages for developing gluten-free foods. Specifically, rice flour has a neutral flavor, low levels of sodium, easy digestibility, hypoallergenic proteins, and does not contain gluten. These characteristics make rice flour a suitable ingredient for gluten-free bakery products (Marco &

Rosell, 2008). However, features of rice based breads are greatly dependent on rice flour functionality. Physicochemical properties of rice flour, main determinants of its technological functionality, are greatly variable. In fact, these properties are significantly influenced by rice variety (Han, Cho, Kang & Koh, 2012; Sompong, Siebenhandl-Ehn, Linsberger-Martin & Berghofer, 2011; Yu, Ma, Menager & Sun, 2012), storage conditions (Park, Kim, Park & Kim, 2012; Tananuwong & Malila, 2011), particle size of the flour and length of rice grain (de la Hera, Gomez & Rosell, 2013a), processing method (Guha & Ali, 2011), chemical structure and composition (Kim, Song & Shin, 2010; Zhu, Liu, Sang, Gu & Shi, 2010), among others.

Regarding variety, there is a general agreement that rice grain length is a factor that influences the bread quality in gluten-free breads (GFB), although discrepancies about the most convenient type of rice grain have been reported (Kadan, Robinson, Thibodeaux & Pepperman, 2001; Rosell & Gomez, 2006; de la Hera, Martinez & Gómez, 2013b). In general, the size of the grain is related to its amylose content. Long-grain rice contain higher amylose content and gelatinization temperature, as well as more tendency of retrogradation than short-grain rice. Noomhorm, Bandola & Kongseree (1994) demonstrated that rice varieties with low amylose content exhibited low gelatinization temperature and soft gels, which was beneficial for baking. Kadan et al. (2001) reported that combining part of the long-grain variety with 10% of short-grain variety produces a smoother texture in bread. Furthermore, Han et al. (2012) stated that intermediate amylose content and low water absorption capacity of rice gave better rice bread physicochemical properties. Studies had shown that short and medium-grain rice varieties presented better bread textures (Rosell et al., 2006). de la Hera et al., (2013b) showed that short-grain rice produced breads with higher specific volume and lower hardness than long-grain rice. Conversely, few studies demonstrated that long-grain rice could have good breadmaking performance (Han et al., 2012; Kadan et al., 2001; Sivaramakrishnan, Senge, & Chattopadhyay, 2004). In fact, Sivaramakrishnan et al. (2004) reveal that flour of long-grain rice with 3g of hydroxypropylmethylcellulose per 100g of flour presents better texture than flour of short-grain rice. Generally, studies related to rice gluten-free bread have been carried out with commercial rice flour, without controlling the rice type or variety (de la Hera et al., 2013b; Kadan et al., 2001; Rosell et al., 2006). Since there is

not knowledge about the main properties of rice flour governing breadmaking potential, it is necessary to get additional insight on the properties of rice flour from long-grain varieties.

Therefore, the aim of this study was to determine the breadmaking potential of six long-grain rice varieties and to assess any possible flour characteristic (physicochemical and rheological) of their breadmaking behavior.

2. Materials and Method

Six varieties of long-grain rice were selected as representative of the main production in the region. Four of them were from the National Institute of Agricultural Research from Ecuador (INIAP, Boliche, Ecuador): INIAP 14, INIAP 15, INIAP 16 and INIAP 17 and two varieties were from the company PRONACA (Guayaquil, Ecuador): F09 and F50. The average size of the rice grains was 7.2mm±0.1mm. All varieties were harvested between May and December of 2011. All the samples were provided by INIAP.

2.1. Flour production and chemical characterization

The seeds were polish and milled (Cyclotec Sample Mill, Tecator, Hoganas, Sweden) with a 500 μm screen. Considering the already stated relationship between physicochemical properties of starches and their apparent amylose content (AAC), protein and lipid (Gani, Wani, Masoodi & Salim, 2013, Tester & Morrison 1990a, Kim et al. 2010), these parameters have been analyzed. The flour protein, lipid content and moisture content were analyzed following AOAC methods (AOAC 18th 92087 for protein and AOAC18th 922.06 for fats). Moisture content was determined following the ISO method (ISO 712:1998). The AAC of the rice flour was measured following the iodine calorimetric method (Juliano et al., 1981). Spectrophotometer (PerkinElmer, Waltham, USA) measurements were made at 620nm after the above starch-iodine solution was incubated for 20min at ambient temperature. Standard curve was generated using starch reference with 66g

of amylose per 100g of flour from the Megazyme kit K-AMYL 04/06t (Megazyme International Ltd, Wicklow, Ireland). All the analyses were made by triplicate.

2.2. Flour hydration properties

The water holding capacity (WHC) defined as the amount of water retained by the sample without being subjected to any stress was determined by mixing (1.000g ± 0.005g) of flour with distilled water (10ml) and kept at room temperature for 24h. The supernatant was carefully removed with a pipette. WHC was expressed as grams of water retained per gram of solid. The swelling volume (SV) was determined following the method reported by Gularte & Rosell (2011) with slight modification. The swelling volume was calculated by dividing the total volume of the swollen sample after 24h at room temperature by the powder weight of the sample. The water binding capacity (WBC) defined as the amount of water retained by the sample under low-speed centrifugation was determined as described the standard method (AACC, 2010). Samples (1.000g ± 0.005g) were mixed with distilled water (10ml) and centrifuged at 2,000x*g* for 10min. WBC was expressed as grams of water retained per gram of solid. All the analyses were made by triplicate. WHC, SV and WBC were calculated by the equations 1 to 3:

$$\text{WHC (g/g)} = \frac{\text{Weight of sediment after draining supernatant -Sample dry weight}}{\text{Sample weight}} \qquad \text{Eq. 1}$$

$$\text{SV (ml/g)} = \frac{\text{Total volume of swollen sample}}{\text{Sample weight}} \qquad \text{Eq. 2}$$

$$\text{WBC (g/g)} = \frac{\text{Weight of sediment after centrifugation -Sample dry weight}}{\text{Sample weight}} \qquad \text{Eq. 3}$$

2.3. Flour gelling behavior

Water absorption index (WAI), water solubility index (WSI) and the swelling power (SP) of different rice flour gels were determined following the method of Anderson, Conway, Pheiser &

Griffin (1969), with slight modification. Briefly, flour (50.0mg ± 0.1mg) sample was dispersed in 1ml of distilled water and cooked at 90ºC for 15min in a water bath. The cooked paste was cooled to room temperature, and centrifuged at 3,000x*g* at 4°C for 10min (Thermo Scientific, Walthman, USA). The supernatant was decanted for determination of its solid content into an evaporating dish and the sediment was weighed. The weight of dry solids recovered by evaporating the supernatant overnight at 110ºC was determined. Four replicates were made for each sample. WSI, WAI and SP were calculated by the equations 4 to 6:

$$WAI\ (g/g) = \frac{\text{Weight of sediment}}{\text{Sample weight}} \quad \text{Eq. 4}$$

$$WSI\ (g/g) = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Sample weight}} \quad \text{Eq. 5}$$

$$SP\ (g/g) = \frac{\text{Weight of sediment}}{(\text{Sample weight} - \text{Weight of dissolved solids in supernatant})} \quad \text{Eq. 6}$$

For the determination of oil absorption capacity (OAC), the method of Lin et al. (1974) was followed and it was expressed as grams of oil bound per gram of the sample on dry basis. Three replicates were made for each sample. OAC was calculated by the equation 7:

$$OAC\ (g/g) = \frac{\text{Weight of sediment after draining oil}}{\text{Sample weight}} \quad \text{Eq. 7}$$

2.4. Determination of pasting properties of rice flours

Pasting properties of the rice flour were determined using a rapid visco analyser (RVA) (Newport Scientific model 4-SA, Warriewood, Australia) by following ICC standard method No 162 (ICC, 1996). Sample (3g based on 14g of moisture per 100g of flour) was added to 25mL of water. The mixture was heated to 50°C for 1min and then heated to 95°C at a rate of 12.2°C min^{-1}. After holding at 95°C for 2.5min, the mixture was cooled to 50°C at a rate of 11.8°C min^{-1}. The rotational speed of the paddle was maintained at 160rpm through the run, except during the first 10s, when a 960rpm speed was used. Peak viscosity, breakdown, final viscosity and setback (difference between final viscosity and peak viscosity) were evaluated. Three replicates were carried out per sample.

2.5. Assessment of gelatinization parameters of rice flour

Evaluation of gelatinization was performed by using a TA instruments Q-200 differential scanning calorimeter (Newcastle, USA). Deionized water was added to rice flour (3.0mg) in aluminum pan to obtain a flour/water ratio of 1:3 (w/w, dry weight basis). The pans were hermetically sealed and the samples were allowed to stand for one hour at room temperature before analysis. The scanning temperature range was between 20-130°C to have a good assessment of thermal changes of flour. In order to increase accuracy and resolution without loss of sensitivity of results, 5°C min^{-1} heat rate was used. The calibration was made with indium and the thermogram was recorded using an empty pan as reference. The parameters evaluated were the transition temperatures (the onset (T_0), peak (Tp) and conclusion (Tc), gelatinization temperature range (Ig) and the enthalpy of gelatinization (ΔH). In addition, the peak height index (PHI) was calculated by equation 9. PHI provides a numerical value that is descriptive of the relative shape of the endotherm. A tall narrow endotherm has a high PHI than a short one does, even if the energy involved in the transition is the same (Krueger, Knutson, Inglett & Walker, 1987). High PHI value could be related to more structured starch matrix (Correia & Beirão-da-Costa, 2012). All DSC experiments were replicated at least three times.

$$I_g = T_c - T_0 \quad \text{Eq. 8}$$

$$PHI = \frac{\Delta H}{T_p - T_0} \quad \text{Eq. 9}$$

2.6.Breadmaking and evaluation of bread quality

Compressed yeast supplied by LEVAPAN (Guayaquil, Ecuador) and hydroxypropylmethylcellulose (Methocel, K4M) provided by Dow Chemical Company (Michigan, USA) were used for breadmaking. The dough was performed using the recipe reported by Marco et al. (2008) (table 1). Half of the rice flour was mixed with boiling water (half of the water) for 5min in Oster blender model 2700 (Oster, Boca Raton, USA) with dough hooks set at low speed (position 1). The dough was left to rest until the temperature decreased to 30°C. Then, the rest of the flour, the other ingredients and water were added and mixed for 5min, set at low speed (position 1). Later, dough were put into pans and fermented for 40min at 35°C and 85% RH. Finally, the fermented dough was baked for 35min at 175°C. Breads were analyzed after 24h of baking.

Table 1. Gluten-free bread recipe used for breadmaking.

Ingredient	**Percentage in flour basis**
Rice flour	100
Water	110
Dry yeast	3
Salt	1.8
Sugar	3
Vegetal oil	6
Baking improver (HPMC)	2

The analyzed bread characteristics included specific loaf volume, crumb color and crumb texture parameters. The loaf volume was determined by rapeseed displacement, while the specific volume (ml x g^{-1}) was calculated as the ratio of the volume (ml) to the weight (g) of the bread.

The crumb color was determined by the computer vision system (Yam & Papadakis, 2004). The computer vision system station included a light source, a camera (canon SX500 IS, 16 mega pixel, Tokio, Japan) for image acquisition and software (Adobe Photoshop CS5) for image processing and analysis. The software quantify the color of crumb in the CIE-L^* a^* b^* uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (−) to red (+) axis, and b^* indicates hue on a blue (−) to yellow (+) axis. Data from three slices per bread were averaged. Additionally the cylindrical coordinates: hue and Chroma (C^*_{ab}) were defined by the following equations:

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \qquad \text{Eq. 10}$$

$$Hue = \arctan\left(\frac{b^*}{a^*}\right) \qquad \text{Eq. 11}$$

Hue angle is the angle for a point calculated from a^* and b^* coordinates in the color space. Chroma is the quantitative component of the color, which reflected the purity of color in the CIELAB space. Texture measurements in form of texture profile analysis (TPA) (Bourne, 1978), of the breadcrumbs was performed by a Texture Analyzer CT3 (Brookfield, Middleboro, USA). A bread slice of 1cm thickness was compressed up to 50% of its original height at a crosshead speed of 1mm/s with a cylindrical acrylic probe (diameter 25.4mm). From the TPA curves, the following texture parameters were measured: hardness (N), springiness, cohesiveness, resilience and chewiness (N). Hardness was defined by peak force during first compression cycle. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness was defined as a ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Chewiness was obtained by multiplying hardness, cohesiveness and springiness. Resilience was calculated as the area during withdrawal of the penetration, divided by the area of the first penetration.

2.7.Statistical analysis

Standarized skewness and standarized kurtosis analyses were made to verify normal distribution of the data. Multiple sample comparison was conducted to evaluate significant differences among samples by analysis of variance (ANOVA) and multiple range tests. Fisher's least significant differences (LSD) test was used to describe means with 95% confidence ($P<0.05$). Data was also evaluated using Pearson correlation coefficients to establish relationship among variables. Only correlation coefficients (in absolute value) equal or greater than 0.68 were considered meaningful. All statistical analyses were performed using Statgraphics Centurion 16 (Statistical Graphics Corporation, UK).

3. Results and Discussion

3.1. Protein, fat and apparent amylose content

In order to understand the possible role of rice grain composition on the flour functionality, protein, fat and apparent amylose content (AAC) of the rice flour varieties were determined (table 2). There was a significant difference in the chemical composition among all the varieties especially in the protein content, which ranged from 5.4 to 8.0g of protein per 100g of flour. The F09 followed by INIAP 14 and INIAP 17 showed the highest amount of protein. Apparent amylose content varied between 22.7 to 28.1g per 100g of flour. Thus, all varieties could be considered as intermediate to high amylose content, in agreement with values reported for long-grain rice (Wani et al., 2012). The highest value was found in the INIAP 17 and F09. On the other hand, the fat content ranged from 0.5 to 1.0g of fat per 100g of flour. No significant difference in AAC and lipid content were found among INIAP 14, INIAP15 and INIAP 16.

Table 2. Proximate composition of flours from different long-grain rice varieties.

Variety	AAC (g/100g)	Protein (g/100g)	Fat (g/100g)	Moisture content (g/100g)
INIAP 14	22.67±1.88c	7.67±0.10b	0.63±0.03c	12.47±0.03d
INIAP 15	23.74±1.15bc	5.43±0.10e	0.59±0.04c	11.53±0.06c
INIAP 16	24.62±1.53bc	6.23±0.06d	0.60±0.05c	10.62±0.14b
INIAP 17	28.16±2.50a	7.67±0.16b	0.47±0.03d	11.65±0.16c
F09	25.65±1.27ab	8.01±0.02a	0.79±0.02b	10.17±0.02a
F50	23.82±0.94bc	7.04±0.04c	0.95±0.05a	10.18±0.11a

AAC: Apparent Amylose Content.
Values with different letters in the same column are significantly different (P<0.05) (n=3).

3.2. Flour and flour gel hydration properties:

The hydration properties of the rice flours and the rice gels are shown in table 3. No statistical difference in oil absorption capacity (OAC) and water solubility index (WSI) were observed among varieties.

The lowest value of water binding capacity (WBC) was displayed by INIAP 17, which is the variety with the highest amylose content. In fact, some researchers have reported that an increase in amylose level results in reduced water binding (Gani et al. 2013; Iturriaga, Lopez, & Añon, 2004). However, the INIAP 14, which has the lowest amylose content, did not follow that trend, likely other intrinsic factors such as particle size, protein conformation, lipid and protein content and lipid-amylose complex are affecting this capacity (Gani et al., 2013; Tester et al., 1990a). Actually, no correlation was found between WBC with protein, fat and AAC, implying that the synergic effect of these entire factors influence the WBC.

The WBC of the flour has been related to stickiness of rice flour dough, high WBC reduces stickiness and produces stiff dough (Han et al., 2012). The values for WBC were higher than the ones reported by Han et al. (2012), who showed that rice lines with low WBC produce fresh bread with suitable volume and firmness. On the other hand, INIAP 17 showed the highest value of WHC, thus could retard staling in gluten-free bread (Sciarini, Ribotta, León, & Pérez, 2010).

There was no significant difference on SV among INIAP 17, F09 and F50 varieties and among INIAP 14, INIAP 15 and INIAP 16, which presented lower SV value.

INIAP 14, which had the lowest AAC, presented the highest SP value. This result agrees with previous reports describing that high amylose content inhibits swelling power (SP) of cereal starch (Tester & Morrison, 1990b; Singh, Kaur, Sandhu, Kaur & Nishinari, 2006). However, when correlation matrix was carried out, no significant correlation was found between the apparent amylose content and the SP. Therefore, result might be derived from the effect of starch content, protein content, bounding forces and molecular structure of starch, especially of the amylopectin (Kim et al., 2010).

The correlation analysis indicates a positive correlation parameter between SP and WAI (r=0.89, P<0.001) and negative correlation between SP and WBC (r=-0.68, P<0.01) as well as WAI and WBC (r=-0.68, P<0.01). Previous reports indicate that WBC of flours depends on the hydrophilic parts of proteins and carbohydrates (Wani, Sogi, Wani & Gill, 2013), water is absorbed in the amorphous zone of the starch and become swollen prior to any change in the small crystallites facilitating the striping of chains and melting of crystallites (Iturraga et al., 2004). As water increases in amorphous region SP increases (Kim et al., 2010). Presumably, in flours with high WBC, water become less available to hydrate the amorphous region of the starch, during the hydration and gelatinization of the flour; therefore the SP and WAI decrease. The INIAP 14 and INIAP 17 showed high values of WAI and SP. It has been reported that high absorption of water during baking can enhance initial softness and decrease firming of bread (Arendt, Moore & Dal Bello, 2008).

Table 3. Hydration properties of flours and gels from different long-grain rice varieties.

Variety	WBC (g /g)	WHC (g /g)	SV (ml/g)	WAI (g/g)	WSI (g/g) x 100	SP (g/g)	OAC (g/g)
INIAP 14	1.28±0.04cd	1.56±0.10cd	2.99±0.00bc	13.34±0.61a	1.74±0.49a	13.58±0.61a	1.76±0.01a
INIAP 15	1.33±0.07abc	1.48±0.05d	2.79±0.19c	5.04±0.28e	2.23±0.77a	5.16±0.32d	1.73±0.05a
INIAP 16	1.38±0.05a	1.74±0.06b	2.93±0.12bc	6.26±0.81d	2.19±0.59a	6.39±0.84c	1.71±0.01a
INIAP 17	1.23±0.02d	1.95±0.15a	3.13±0.12ab	11.65±0.19b	2.73±0.75a	12.07±0.15b	1.71±0.02a
F09	1.35±0.02ab	1.51±0.08d	3.37±0.23a	5.95±0.38d	1.89±0.42a	6.07±0.38c	1.71±0.01a
F50	1.30±0.02bc	1.70±0.09bc	3.27±0.12a	7.49±0.79c	3.00±0.01a	11.73±0.61b	1.77±0.06a

WBC: Water Binding Capacity, WHC: Water Holding Capacity, SV: Swelling Volume, SP: Swelling Power, WAI: Water Absorption Index, WSI: Water Solubility Index, OAC: Oil Absorption Capacity.
Values with different letters in the same column are significantly different ($P<0.05$), (n=3).

3.3.Pasting Properties of rice flour

The pasting properties and the pasting curves of the long-grain rice varieties are shown in table 4 and figure 1, respectively. INIAP 14 showed the lowest peak viscosity, and INIAP 17 and F09 the highest one. Nevertheless, no significant correlation was found between pasting properties and the amylose content, which agrees with previous report (Sompong et al., 2011). Negative correlation between peak viscosity and WAI was found (r=-0.77, P<0.01). INIAP 17 also showed high final viscosity value, indicating high capacity to form gel, but with high retrogradation tendency due to its high setback value (Gani et al., 2013). Indeed, a significant correlation between final viscosity and AAC was found (r=0.70, P<0.05), although no with the setback. Result that differed from previously reported (Sompong et al., 2011; Gani et al., 2013).

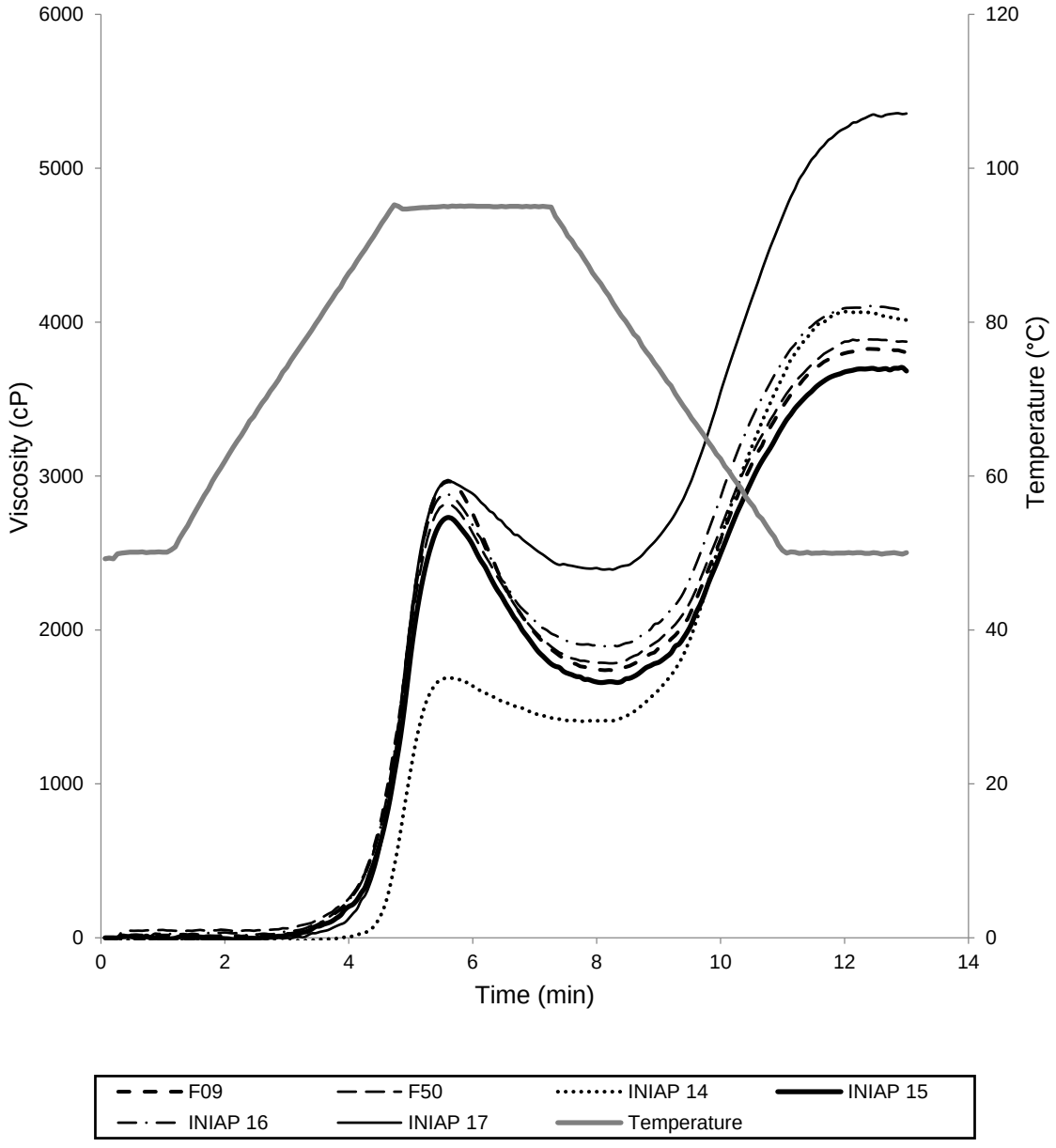
INIAP 14 and INIAP 17 presented lower breakdown viscosity and F09 the highest. The breakdown is caused by the disintegration of gelatinized starch granule structure during continued stirring and heating. Differences in breakdown among rice starches have been related to differences in rigidity of swollen granules (Gani et al., 2013). A negative correlation was found between breakdown viscosity and SP (r=-0.72, P<0.01). Hence, INIAP 17 and INIAP 14 could lead to bread with high specific volume.

Table 4. Pasting Properties of flours from different long-grain rice varieties determined from the RVA plots.

Variety	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
INIAP 14	1692±16d	287±1d	4014±18b	2609±3b
INIAP 15	2731±24c	1083±47b	3683±0d	2035±23d
INIAP 16	2883±10b	989±1b	4079±2b	2184±11c
INIAP 17	2964±29a	580±1c	5356±74a	2972±46a
F09	2971±15a	1237±40a	3803±21c	2069±19d
F50	2822±66b	1040±100b	3874±13c	2092±21d

Values with different letters in the same column are significantly different (P <0.05), (n=3).

Figure 1: Rapid viscoanalyzer plots showing the pasting behavior of flours from different long-grain rice varieties.



1.2. Gelatinization parameters of rice flour

The gelatinization temperatures (onset, To; peak, Tp; and conclusion, Tc), gelatinization enthalpy (ΔH), gelatinization temperature range (Ig) and peak height index (PHI) of rice flours from different varieties are shown in table 5. Significant differences were observed in the thermal properties due to varieties. Gelatinization temperatures and enthalpy were similar to those reported by other authors for rice flour and starch (Han et al., 2012; Iturriaga et al., 2004; Singh et al., 2006). Variation even of 10°C was found among the onset gelatinization temperatures (To) of the INIAP 14 and INIAP 15 and the other varieties.

Table 5. Gelatinization parameters of flours from different long-grain rice varieties determined from the DSC plots.

Variety	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Ig (°C)	PHI
INIAP 14	69.0±0.4a	74.1±0.5ab	80.6±0.4a	12.7±2.7ab	11.7±0.7b	2.44±0.31a
INIAP 15	68.5±1.0a	75.2±0.8a	79.8±2.4a	8.6±1.6b	11.4±2.7b	1.39±0.04c
INIAP 16	60.8±2.0b	74.6±0.2ab	81.6±0.8a	11.7±3.3ab	20.9±2.6a	0.84±0.12d
INIAP 17	59.0±0.8bc	73.7±0.2b	79.5±1.2a	12.9±1.8ab	20.5±0.5a	0.88±0.14d
F09	58.4±2.0c	66.8±0.8c	80.9±0.9a	13.9±3.4ab	22.5±2.9a	1.68±0.19c
F50	58.7±0.4bc	66.4±1.0c	81.3±1.2a	16.0±2.8a	22.6±1.5a	2.07±0.04b

To: Onset Temperature, Tp: Peak Temperature, Tc: Conclusion Temperature, ΔH: Enthalpy of gelatinization, Ig: gelatinization temperature range, PHI: Peak Height Index.
Values with different letters in the same column are significantly different ($P<0.05$), (n=3).

Negative correlation between To and Ig was found ($r=-0.97$, $P<0.001$). INIAP 14 and INIAP 15 showed high values on To and low on Ig. Considering that INIAP 14 and INIAP 15 showed low amylose content, this result agrees with Krueger et al. (1987), who reported that the high the amylopectin content of the starch, the low temperature range of gelatinization. In addition, negative correlation between Tp and SV was found ($r=-0.80$, $P<0.001$). INIAP 14, INIAP 15 and INIAP 16 showed high Tp value, and likely with high resistance to swell.

The enthalpy of gelatinization (ΔH) has been used as indicator of the loss of molecular order within the granule that occurs during gelatinization (Tester et al., 1990 b). INIAP 15 showed the lowest ΔH value, indicating less stability of crystals (Chiotelli & Le Meste, 2002). Peak high index (PHI) provides a numerical value that describes the relative shape of endotherm. INIAP 16 and INIAP 17 showed low peak high index (PHI) values, which can be related to lower structured starch matrix (Kruger et al., 1987). Therefore, INIAP 17 showed low To, PHI and broad Ig that might be due to the presence of irregularly-shaped granules (Kaur, Singh, Sandhu & Guraya 2004). The same analysis could be applied to F09 and F50 varieties. On the other hand, INIAP 14 showed the highest values of To, Tp, ΔH, PHI and narrow Ig, which could indicate high degree of molecular order (Correia et al., 2012; Sandhu, Singh & Kaur, 2004).

Figure 2: DSC plots showing the endothermic curves of flours from different long-grain rice varieties. Legends: Grey line: temperature

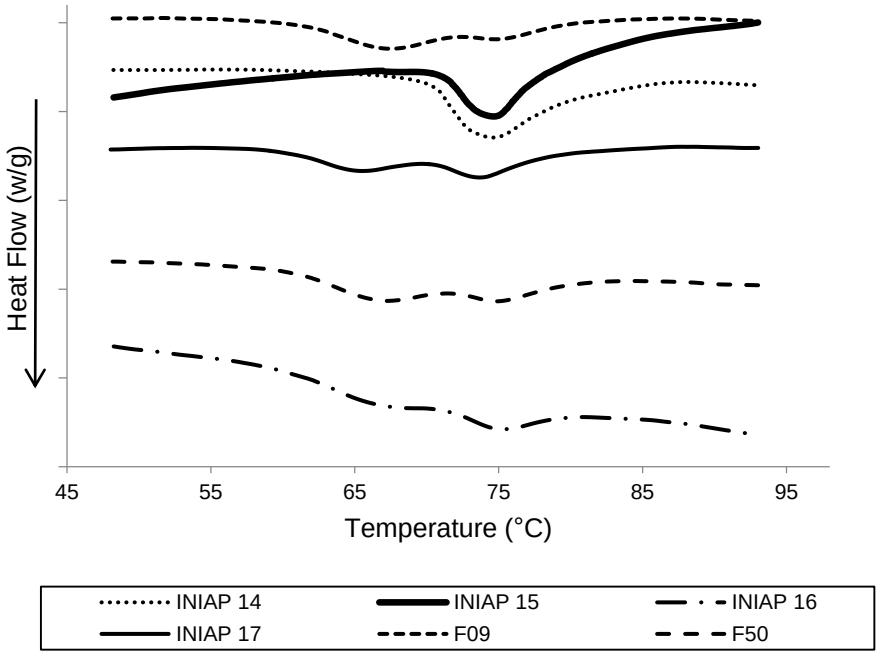
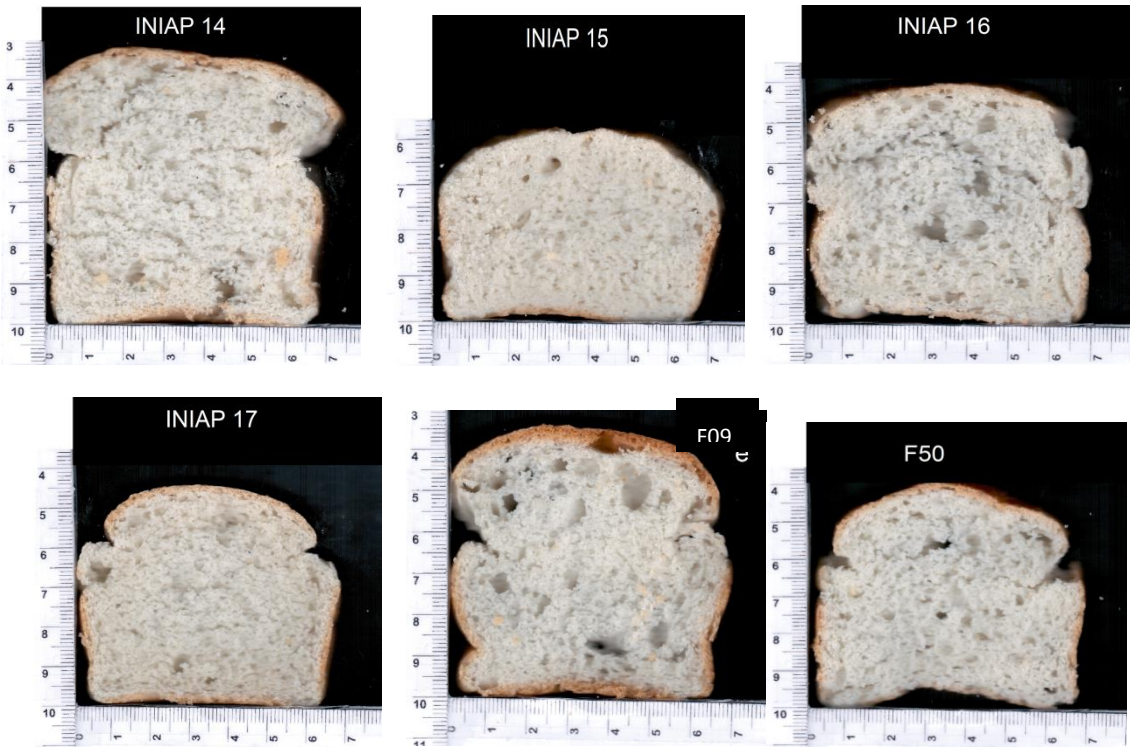


Figure 2 shows the endothermic curves of the rice varieties. INIAP 17, INIAP 16, F09 and F50 showed a shoulder peak; like it has been reported in Thai rice variety due to amylopectin structure composed by two different molecular structures (Kim et al., 2010). No significant correlation was observed between gelatinization parameters and hydration properties, neither with apparent amylose content. Presumably, the presence of protein and lipid in rice flour also influence the gelatinization process, and affect the water- starch bonding (Iturriaga et al., 2004).

3.5 Evaluation of Bread

Figure 3 shows the cross section of breads from all rice flour varieties. INIAP 15 and INIAP 17 showed smaller gas cells, which led to a more compact crumb. F09 had a more flattened surface, indicating lower driving force in the oven, possibly due to weaker mass structure

Figure 3: Cross-section of gluten-free breads made of rice flours from different long-grain varieties. Recipe described in Table 1.



The quality characteristics of the breads obtained from rice varieties are shown in table 6. Specific volume values of gluten-free breads (GFB) ranged from 1.80 to 2.41ml/g, which agrees with previously reported (de la Hera et al., 2013b; Matos & Rosell 2012; Sciarini et al., 2010). A significant correlation was observed between specific volume and SP (r=0.71, P<0.01) and breakdown viscosity (r=-0.97, P<0.01). Matos & Rosell (2013) reported also a negative correlation between specific volume and pasting properties assessed with Mixolab. In fact, INIAP 14 presented high specific volume, high SP and low breakdown viscosity. As was reported before by Han et al. (2012), rice lines with low WBC produce fresh bread with a suitable volume and firmness. Whereas INIAP 14 has low WBC and high specific volume, no correlation was found between both properties. In addition, no correlation was found between amylose content and specific volume of the bread. However, the conclusion temperature (Tc) of gelatinization has a positive correlation (r=0.81, P<0.05) with the specific volume. This result could be related with the time that the bread has to increase the volume within the oven.

Table 6. Technological characteristics of GFB made of rice flour from different long-grain varieties.

Variety	Specific Volume (ml/g)	*L**	*a**	*b**	Chroma	Hue angle (°)
INIAP 14	2.35±0.14a	74.00±0.00a	-3.77±0.58a	18.33±1.55b	18.71±1.08bc	-78.63±2.18a
INIAP 15	2.09±0.02c	72.00±1.00c	-2.67±0.58a	21.00±0.00a	23.82±4.62a	-83.51±1.44b
INIAP 16	2.28±0.03b	72.67±0.58bc	-2.67±0.58a	18.67±0.58b	18.86±0.54bc	-81.85±1.87ab
INIAP 17	1.80±0.02d	73.67±1.15ab	-3.00±0.00a	21.00±0.00a	22.53±2.29ab	-82.30±0.74ab
F09	2.41±0.19a	74.67±0.58a	-3.67±0.58a	18.00±1.00b	18.38±0.89c	-78.41±2.29a
F50	2.24±0.14bc	74.00±0.00a	-3.33±1.15a	19.33±1.53b	19.65±1.34bc	-80.05±3.92ab

Values with different letters in the same column are significantly different (P <0.05), (n=3).

The color of the crumb has been also an important parameter for characterizing GFB. *L** value indicates the lightness of the crumb. Rice flour based breads give a very white crumb, which differs from the wheat flour based crumbs that are yellowish. The *L** *r*ange (74 to 72) agreed with the ones reported before (Matos et al., 2012). Lower crumb

luminosity (65) was reported by Marco et al. (2008) when using short-grain rice flour in the same GFB recipe. The range of *a** values were indicating hue on green axis, as was obtained with short-grain rice flour (Marco et al., 2008). On the other hand, the positive range of *b** values were within the ones reported for commercial breads, showing hue on yellow axis (Matos et al., 2012). Values of *b** ranged from 18 to 21, whereas with short-grain rice flour was around 8, likely due to differences in rice polishing degree (Marco et al., 2008). All the samples showed a negative hue angle that reflected yellow-greenish hue. INIAP 15 presented the major intensity of yellow component, indicated by chroma value.

Significant differences were observed in the crumb texture properties among rice varieties (table 7). Crumb hardness ranged from 3.5N to 10.8N, thus softer crumbs than those reported in commercial GFB (Matos et al., 2012), but harder than GFB made with short-grain rice flour (1.96 ±0.19N) (Marco et al., 2008). INIAP 14, F09 and F50 showed lower hardness values and INIAP 17 the highest one. The range of springiness in the studied rice varieties were in the lower limit of the ranges reported before (Marco et al., 2008; Matos et al., 2012), showing their fragility and tendency to crumble when sliced (McCarthy, Gallagher, Gormley, Schober & Arendt, 2005). High springiness values are preferred because it is related to the freshness and elasticity of the bread. Not only springiness but also resilience characterizes the loss of elasticity, because it indicates the ability of a material to return to its original shape after stressing (Onyango, Mutungi, Unbehend & Lindhauer, 2011). With the exception of INIAP 17, all the INIAP varieties showed significant lower resilience values than F09 and F50. In fact, all varieties showed lower values than the one reported for GFB from long-grain rice (Kadan et al., 2001) and close to the 0.26 resilience value reported for GFB from short-grain rice (Marco et al., 2008). These results are in the upper level of the result of Matos et al. (2012) in commercial GFB. Chewiness of the rice varieties ranged between 8.97 to 20.26N, those values were within the upper level of commercial GFB (Matos et al., 2012). INIAP 17 showed significantly higher value.

Table 7. Texture profile analysis of crumbs of GFB made of rice flours from different long-grain varieties.

Variety	Hardness (N)	Resilience	Springiness	Chewiness (N)	Cohesiveness
INIAP 14	5.88±1.79cd	0.23±0.02c	0.73±0.06abc	8.97±2.31c	0.48±0.05bc
INIAP 15	9.67±2.06ab	0.20±0.03c	0.68±0.05c	14.95±2.97b	0.44±0.05c
INIAP 16	7.16±1.31bc	0.21±0.03c	0.70±0.00bc	11.05±2.31bc	0.45±0.04c
INIAP 17	10.84±3.21a	0.29±0.05b	0.75±0.07abc	20.26±6.11a	0.54±0.08b
F09	3.85±0.82d	0.30±0.01ab	0.80±0.00a	9.04±1.87c	0.62±0.01a
F50	3.54±1.06d	0.35±0.02a	0.75±0.06ab	9.16±2.62c	0.67±0.03a

Values with different letters in the same column are significantly different (P<0.05), (n=3).

F09 and F50 presented the highest cohesiveness value, which was desirable because it forms a bolus rather than disintegrates during mastication (Onyango et al., 2011). Cohesiveness characterizes the extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the material. A strong correlation was found between cohesiveness and properties of rice flour such as Tp (r=-0.96, P<0.001), ΔH (r=0.71 P<0.05) and SV (r=0.82, P<0.05).

Therefore, results confirmed that long-grain rice varieties are suitable for obtaining gluten free breads, but rice variety had a significant impact on the GFB characteristics. Among the varieties tested, INIAP 14 and F09 gave the best GFB features, moreover considering specific volume and crumb texture properties.

4. Conclusion

Rice flour from short-grain has been recommended for making gluten-free bread, but present results show that flour from long-grain rice is suitable for making gluten-free bread, having similar characteristics than previously reported GFB made from short-grain rice. Likely, discrepancies could be attributed to the wide use of commercial rice flours instead of using specific rice varieties. Results obtained with six different long- grain rice varieties confirmed their suitability for breadmaking performance. Significant differences

were observed within varieties. Results suggested that the most important parameters of rice flour when defining breadmaking performance of GFB would be WBC, SP, SV, Tp, Tc and enthalpy. Technological parameters (hydration and pasting) would not be able to predict specific volume or hardness. Nevertheless, regarding crumb cohesiveness and resilience, it would be advisable to select flours with high swelling value, and enthalpy but low gelatinization peak temperature. In addition, no correlation has found between WBC with hardness and specific volume of the GFB. Flour properties such as SV, Tp and ΔH were strongly related to cohesiveness value, F09 and F50 showed the highest cohesiveness value. Also, high SP and low breakdown viscosity of the rice flour, showed in INIAP 14, are related to high specific volume of the GFB. Previous reports supported that short rice grain was the only suitable for gluten-free breadmaking. Nonetheless, this study indicated that the length of the rice is not a determining factor for breadmaking, and long-grain rice could be used for bakery, indeed it seems that synergic effect of intrinsic factors such as particle size, protein conformation, lipid and protein content and lipid-amylose complex and starch structure that could affect the properties of rice flour. In addition, results suggest that by selecting specific varieties of rice it would be possible to improve the baking performance of the rice flours, and presumably breeding could also be a good tool to obtain new promising varieties for baking.

Acknowledgments

Authors acknowledge the financial support of Spanish Scientific Research Council (CSIC), the Spanish Ministry of Economy and Sustainability (Project AGL2011-23802), and the Generalitat Valenciana (Project Prometeo 2012/064). F. Cornejo acknowledges the financial support of National Secretary of High Education, Science, Technology and Innovation (SENESCYT, Ecuador). National Autonomous Institute of Agricultural Research from Ecuador (INIAP) for providing the BR cultivars, Metrological Testing and Material Laboratory of ESPOL (LEMAT, Ecuador) and CIBE (Ecuador) for the laboratory facilities.

References

Anderson, R., Conway, H., Pheiser V. & Griffin, E. (1969). Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today, 14*, 4-12.

Arendt, E., Moore, M., & Dal Bello, F. (2008). Gluten-Free Breads. In: E. Arendt, & F. Dal Bello (Eds.), *Gluten-Free cereal products and Beverages* (pp. 289- 319). London: Academic Press.

Bourne, M.C. (1978). Texture profile analysis. *Food Technology, 32(7)*, 62–66.

Correia, P., & Beirão-da-Costa, M. L. (2012). Effect of drying temperatures on starch-related functional and thermal properties of chestnut flours. *Food and Bioproducts Processing, 90*(2), 284-294.

Chiotelli, E., & Le Meste, M. (2002). Effect of small and large wheat starch granules on thermomechanical behavior of starch. *Cereal Chemistry 79*(2), 286-293.

de la Hera, E., Gomez, M., & Rosell, C. M. (2013a). Particle Size Distribution of Rice Flour Affecting the Starch Enzymatic Hydrolysis and Hydration Properties. *Carbohydrate Polymers, 98*(1), 421-427.

de la Hera, E., Martinez, M., & Gómez, M. (2013b). Influence of flour particle size on quality of gluten-free rice bread. *LWT-Food Science and Technology, 54* (1), 199-206.

Gani, A., Wani, S. M., Masoodi, F. A., & Salim, R. (2013). Characterization of rice starches extracted from Indian cultivars. *Food Science and Technology International, 19*(2), 143-152.

Guha, M., & Ali, S. (2011). Changes in rheological properties of rice flour during extrusion cooking. *Journal of Texture Studies, 42*(6), 451-458.

Gularte, M. A., & Rosell, C. M. (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymers, 85*(1), 237-244.

Han, H. M., Cho, J. H., Kang, H. W., & Koh, B. K. (2012). Rice varieties in relation to rice bread quality. *Journal of the Science of Food and Agriculture, 92*(7), 1462-1467.

Iturriaga, L., Lopez, B., & Añon, M. (2004). Thermal and physicochemical characterization of seven argentine rice flours and starches. *Food Research International, 37*, 439-447.

Juliano, B., Perez, C., Blakeney, A., Castillo, T., Kongseree, N., Laignelet, B., Lapis, E., Murty, V., Paule, C., & Webb, B. (1981). International Cooperative Testing on the Amylose Content of Milled Rice. *Starch - Stärke, 33*(5). 157–162

Kadan, R., Robinson, M., Thibodeaux, D., & Pepperman, A. (2001). Texture and other Physicochemical Properties of Whole Rice Bread. *Journal of Food Science, 66*(7), 940–944.

Kaur, M., Singh, N., Sandhu, K. S., & Guraya, H. S. (2004). Physicochemical, morphological, thermal and rheological properties of starches separated from kernels of some Indian mango cultivars (Mangifera indica L.). *Food Chemistry, 85*, 131-140.

Kim, J. M., Song, J. Y., & Shin, M. (2010). Physicochemical properties of high amylose rice starches purified from Korean cultivars. *Starch/Stärke, 62*, 262–268.

Krueger, B. R., Knutson, C. A., Inglett, G. E., & Walker, C. E. (1987). A differential scanning calorimetric study on the effect of annealing on gelatinization behavior of corn starch. *Journal of Food Science, 52*(3), 715-718.

Marco, C., & Rosell, C. (2008). Breadmaking performance of protein enriched, gluten-free breads. *European Food Research and Technology, 227*(4), 1205-1213.

Matos, M. E., & Rosell, C.M. (2012). Relationship between instrumental parameters and sensory characteristics in gluten-free breads. *European Food Research and Technology, 235*(1), 107-117.

Matos, M. E., & Rosell, C.M. (2013) Quality indicators of rice-based gluten-free bread-like products: relationships between dough rheology and quality characteristics. *Food Bioprocess Technology 6*, 2331–2341.

McCarthy, D. F., Gallagher, E., Gormley, T. R., Schober, T. J., & Arendt, E. K. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry*, *82*(5), 609-615.

Noomhorm, A., Bandola, D. C., & Kongseree, N. (1994). Effect of rice variety, rice flour concentration and enzyme levels on composite bread quality. *Journal of the Science of Food and Agriculture*, *64*(4), 433-440.

Onyango, C., Mutungi, C., Unbehend, G., & Lindhauer, M. G. (2011). Modification of gluten-free sorghum batter and bread using maize, potato, cassava or rice starch. *LWT-Food Science and Technology*, *44*(3), 681-686.

Park, C., Kim, Y., Park, K., & Kim, B. (2012). Changes in physicochemical characteristics of rice during storage at different temperatures. *Journal of Stored Products Research*, *48*, 25-29.

Rosell, C., & Gómez, M. (2006). Rice. In: Y. H. Hui, H. Corke, I. De Leyn, W. K. Nip, & N. Cross (Eds.), *Bakery Products: Science and Technology* (pp. 123–133). Oxford: Blackwell Publishing,

Sandhu, K. S., Singh, N., & Kaur, M. (2004). Characteristics of different corn types and their grain fractions: physicochemical, thermal, morphological and rheological properties of starches. *Journal of Food Engineering*, *64* (1), 119–127

Sciarini, L. S., Ribotta, P. D., León, A. E., & Pérez, G. T. (2010). Influence of gluten-free flours and their mixtures on batter properties and bread quality. *Food and Bioprocess Technology*, *3*(4), 577-585.

Singh, N., Kaur, L., Sandhu, K. S., Kaur, J., & Nishinari, K. (2006). Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food hydrocolloids*, *20*(4), 532-542.

Sivaramakrishnan, H. P., Senge, B., & Chattopadhyay, P. K. (2004). Rheological properties of rice dough for making rice bread. *Journal of Food Engineering, 62*(1), 37-45.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., & Berghofer, E. (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry, 124*(1), 132-140.

Tananuwong, K., & Malila, Y. (2011). Changes in physicochemical properties of organic hulled rice during storage under different conditions. *Food Chemistry, 125*(1), 179-185.

Tester, R., & Morrison, W. (1990a). Swelling and Gelatinization of Cereal Starches. I. Effects of Amylopectin, Amylose, and Lipids. *Cereal Chemistry 67*(6),551-557.

Tester, R., & Morrison, W. (1990b). Swelling and Gelatinization of Cereal Starches. II. Waxy Rice Starches. *Cereal Chemistry 67*(6), 558-563.

Wani, A. A., Singh, P., Shah, M. A., Schweiggert-Weisz, U., Gul, K., & Wani, I. A. (2012). Rice Starch Diversity: Effect on Structural, Morphological, Thermal, and Physicochemical Properties—A Review. *Comprehensive Reviews in Food Science and Food Safety, 11*(5), 417-436.

Wani, I. A., Sogi, D. S., Wani, A. A., & Gill, B. S. (2013). Physico-chemical and functional properties of flours from Indian kidney bean (Phaseolus vulgaris L) cultivars. *LWT-Food Science and Technology, 53* (1), 278-284.

Yam, K. L., & Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Journal of Food Engineering, 61*(1), 137-142.

Yu, S., Ma, Y., Menager, L., & Sun, D. W. (2012). Physicochemical properties of starch and flour from different rice cultivars. *Food and Bioprocess Technology, 5*(2), 626-637.

Zhu, L., Liu, Q., Sang, Y., Gu, M., & Shi, Y. (2010). Underlying reasons for waxy rice flours having different pasting properties. *Food Chemistry, 120*(1), 94

CAPITULO 2

INFLUENCE OF GERMINATION TIME OF BROWN RICE IN RELATION TO FLOUR AND GLUTEN FREE BREAD QUALITY

J Food Sci Technol
DOI 10.1007/s13197-015-1720-8

ORIGINAL ARTICLE

Influence of germination time of brown rice in relation to flour and gluten free bread quality

Fabiola Cornejo · Cristina M. Rosell

Abstract

The effect of germination time on physicochemical characteristics of brown rice flour and its effect on gluten free bread qualities have been investigated**.** Germination was carried out at 28°C and 100% RH for 12, 24 and 48 hours; brown rice and soaked brown rice was also analyzed. Significant changes on hydration and pasting properties of brown rice flour were found during germination. The starch degradation by enzyme activity could be evidenced with the decrease in viscosity and water binding capacity (WBC). No significant effect in specific volume, humidity and water activity of the gluten free bread was found as germination time increase, but a significant softness of the crumb was obtained. However, at 48 hours of germination, the intense action of α amylase could result in excessive liquefaction and dextrinisation, causing inferior bread quality. Overall, germinated rice flour showed appropriate functionality for being used as raw ingredient in gluten free breadmaking.

Key words: germinated brown rice, gluten free bread; quality.

1. Introduction

Rice flour is one of the elected cereals for obtaining gluten free baked goods when those are addressed to gluten intolerant patients. Many studies have been focused on making rice flour based leavened baked gluten free products, and specifically bread (GFB) (Rosell and Gomez 2014). Rice flour is the side product of the rice milling industry but flour properties are definitive in the gluten free bread quality, as has been reported when assessing the impact of rice variety (Han et al. 2012), and particle size of the flour and length of rice grain (de la Hera et al. 2013). Having in mind gluten free bread quality, even treatments as extrusion has been proposed for improving rice flour breadmaking functionality (Martinez et al. 2014). In general, GFB are made from refined flours, which have lost very important nutritional compounds like fibers, vitamins and minerals.

Nevertheless, endogenous constituents of the rice grain cannot be put back when using refined rice flour, resulting in a detrimental effect on the nutritional quality of the food.

Germinated brown rice flour (GBRF) and germinated glutinous brown rice flour have been used as functional food ingredients in breadmaking (Charoenthaikij et al. 2010a; 2010b; 2012; Watanabe et al. 2004), due to its high content in bioactive compounds such as γ-aminobutiric acid and antioxidants such as phenolic compounds, γ-oryzanol and vitamin E (Cáceres et al. 2014). The benefits of these bioactive compounds include regulation of blood pressure and heart rate, alleviation of pain and anxiety, inhibition of cancer cell proliferation, and protection for oxidative stress (Oh and Oh 2004). Despite their nutritional benefits, germinated brown rice flour, pregerminated brown rice flour and germinated glutinous brown rice flour have been only used for partial substitution (10-30%) of wheat flour in breadmaking (Charoenthaikij et al. 2010a; 2010b; 2012; Watanabe et al. 2004). This practice produces changes in the rheological properties of the batter, modifying dough handling, besides an improvement of crumb texture, increase of gas production during fermentation and retard bread staling (Charoenthaikij et al. 2010b; Watanabe et al. 2004). The late benefit likely related to the alfa-amylase action forming low molecular weight dextrin that could inhibit amylopectin retrogradation (Gray and Bemiller 2003). In addition, germination could produce an excessive liquefaction and dextrinisation of starch granule, producing 'wet sticky crumb' (Hallén et al. 2004), because of that it is necessary to define the right germination time for optimizing flour breadmaking features. Nevertheless, no attempts have been made in using germinated rice brown flour for obtaining gluten free bread.

The aim of this study was to analyze the effect of germination time in physicochemical characteristics of the flour and the effect of use GBRF as primary ingredient of GFB on bread quality.

2. Material and Methods

Commercial certified brown rice cultivar INIAP 15 was provided by the National Institute of Agricultural Research from Ecuador (INIAP). Seeds were harvest between May and December 2011. The gluten-free bread formulations also contained compressed yeast (LEVAPAN, Lessafre, Madrid, Spain) and hydroxypropylmethylcellulose Methocel K4M obtained from Dow Chemical Company (Michigan,USA).

2.1.Germination and Flour preparation

Brown rice was sterilized with 0.1% sodium hypochlorite solution (1:5 w/v) for 30 min, and then rinsed with distilled water. Afterwards, rice was soaked in distilled water (seed water ratio, 1/5, w/v) for 24 h at 28±1 ºC. Soaking water was drained and seeds were placed in plastic trays containing moist filter and were also covered with moist filter paper. The filter papers were kept wet by capillarity. Germination was carried out at 28 ±1°C and 100% relative humidity under darkness for 12, 24 and 48 hours. After germination, seeds were dried at 50±1°C for 24 hours. Once dried, seeds were ground until a diameter inferior to 1mm with a cyclone mill (UDY Corporation, USA). Five treatments were analyzed, brown rice flour as reference, soaked rice flour and germinated for 12, 24 and 48 hour rice flour. Two sets of samples were prepared for each treatment.

2.2.Flour hydration properties

The water holding capacity (WHC) defined as the amount of water retained by the sample without being subjected to any stress was determined mixing 1.000g ± 0.001g of flour with distilled water (10 ml) and kept at room temperature for 24 h. WHC was expressed as grams of water retained per gram of solid. The swelling volume (SV) was determined following the method reported by Gularte and Rosell (2011) with slight modification. Samples (1g ± 5mg) were placed in a graduated cylinder and mixed with distilled water (10 ml), then kept at room temperature for 24 h. The swelling volume was calculated by dividing the total volume of the swollen sample by the original dry weight of the sample. The water binding capacity (WBC) defined as the amount of water retained

by the sample under low-speed centrifugation was determined as described the standard method (AACC 2010). Samples (1.000g ± 0.001g) were mixed with distilled water (10 ml) and centrifuged at 2000x*g* for 10min. WBC was expressed as grams of water retained per gram of solid. All the analyses were made in triplicate.

2.3.Flour Gel hydration properties

Water absorption index (WAI), water solubility index (WSI) and the swelling power (SP) of different rice flour fractions were determined following the method of Anderson et al. (1969) with slight modification. Briefly, flour (50.0mg ± 0.1mg) sample was dispersed in 1 ml of distilled water in an Eppendorf tube using a wire rod and cooked at 90 ºC for 15 min in a water bath. The cooked paste was cooled with ice to room temperature, and then centrifuged at 3000x *g* at 4ºC for 10 min. The weight of dry solids was recovered by evaporating the supernatant overnight at 110 ºC. Four replicates were made for each sample. WSI, WAI and SP were calculated by the equations 1 to 3:

$$WAI\ (g/g) = \frac{\text{Weight of sediment}}{\text{Sample weight}} \qquad \text{Eq. 1}$$

$$WSI\ (g/100g) = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Sample weight}}\ x\ 100 \qquad \text{Eq. 2}$$

$$SP\ (g/g) = \frac{\text{Weight of sediment}}{(\text{Sample weight} - \text{Weight of dissolved solids in supernatant}} \qquad \text{Eq. 3}$$

For the determination of oil absorption capacity (OAC), the method of Lin et al. (1974) was followed. Briefly, flour sample (100.0mg ± 0.2mg) was mixed with 1 ml of vegetable oil. The content was stirred for 1 min with a wire rod to disperse the sample in the oil. After a period of 30 min in the vortex mixer, tubes were centrifuged at 3000 x *g* and at 4ºC for 10 min. The supernatant was carefully removed with a pipette and tubes were inverted for 25 min to drain the oil prior to re-weighing. The oil absorption capacity

was expressed as grams of oil bound per gram of the sample on dry basis. Three replicates were made for each sample. OAC was calculated by the equation 4:

$$OAC\ (g/g) = \frac{\text{Weight of sediment after draining oil}}{\text{Sample weight}} \quad \text{Eq. 4}$$

2.4.Determination of pasting properties of rice flours

Pasting properties of the rice flour were determined using a rapid viscoanalyser (RVA) (Newport Scientific model 4-SA, Warriewood, Australia) by following ICC standard method No 162 (ICC 1996). Sample (3 g based on 14% moisture) was added to 25 mL of water. The suspension was heated at 50 °C for 1 min and then heated up to 95 °C at 12°C/min. After holding at 95 °C for 2.5 min, the suspension was cooled to 50 °C at 12°C/min. The rotational speed of the paddle was maintained at 160-rpm throughout the run, except during the first 10 s, when a 960-rpm speed was used. Peak viscosity, breakdown, final viscosity and setback (difference between final viscosity and peak viscosity) were evaluated.

2.5.Breadmaking and evaluation of bread quality

The dough was prepared using the formula of Marco and Rosell (2008). Half of the rice flour was mixed with boiling water (half of the water) in a Brabender Farinograph (Duisburg, Germany) for five minutes. The dough was left to rest until the temperature decreased to around 30 °C. Afterwards, the rest of the flour and water, besides the other solid ingredients, were added and mixed in a Brabender Farinograph (Duisburg, Germany) for 5 min. Then, dough was transferred (180 g) to pans and fermented for 40 min at 35°C and 85% RH. Finally, the fermented dough was baked 35 min at 175°C. Loaves were cooled down at room temperature for one hour and then packed in polyethylene pouches. Further analysis was carried out after 24h of baking.

Bread quality properties included specific loaf volume, height/width ratio of the slices, crumb color and its texture including hardness, springiness, resilience, chewiness

and cohesiveness. The loaf volume was determined by rapeseed displacement, while the specific volume (mL g−1) of the bread was calculated as the ratio of the volume (mL) to the weight (g) of the bread.

The crumb color was determined by the computer vision system (Yam and Papadakis 2004). The computer vision system station included a light source, a camera (canon SX500 IS, 16 mega pixel) and software (Adobe Photoshop CS5) for image processing and analysis. The texture profile analysis (TPA) of the breadcrumbs was performed by a Texture Analyzer CT3 (Brookfield, Middleboro, USA). A bread slice of 1-cm-thickness was compressed up to 50% of its original height at a crosshead speed of 1 mm/s with a cylindrical acrylic probe (diameter 25.4 mm).

2.6.Statistical analysis

Standarized skewness and standarized kurtosis analyses were made to verify normal distribution of the data. Multiple sample comparison was conducted to evaluate significant differences among samples by analysis of variance (ANOVA) and multiple range tests. Fisher's least significant differences (LSD) test was used to describe means with 95% confidence ($P<0.05$). Data was also evaluated using Pearson correlation coefficients to establish relationship among variables. Only correlation coefficients (in absolute value) equal or greater than 0.68 were considered meaningful. All statistical analyses were performed using Statgraphics Centurion 16 (Statistical Graphics Corporation, UK).

3. Results and Discussions

3.1.Flour and flour gel hydration properties:

The Table 1 shows the hydration properties of brown rice, soaked and germinated brown rice flour and their gels. It can be seen that there was a decreasing trend on the hydration properties of the flour (WBC, WHC and SV) as germination time increased, although that decrease was only significant in the case of WBC. During germination,

enzyme activities like the α-amylase increases, which led to starch degradation and subsequent increase of small dextrins and fermentable sugar (Islam and Becerra 2012). The starch degradation might induce the release of the water entrapped within the starch granule, and that effect was even more dramatic as the starch hydrolysis proceeds and thus at extended germination, reducing the flour hydration properties. Additionally, the released sugars from starch hydrolysis during germination could form crosslinks between starch chains in the amorphous regions of a starch granule, which restricted starch swelling (Baek et al. 2004) also they could interact with water hindering the water available for starch hydration (Peroni-Okita et al. 2013). The water binding capacity (WBC) and water holding capacity (WHC) are important properties for bakery process, and become even more essential for making gluten free baked goods. Texture or consistency of dough depends on water absorption leading to body thickening and viscosity (Aguilera et al. 2011). High water absorption reduces stickiness and produces stiff dough (Han et al. 2012). At bread level, Han et al. (2012) found that rice lines with low water absorption produce fresh bread with a suitable volume and firmness. Presumably, that effect could explain texture improvement observed when partial replacement of wheat flour with germinated rice and pregerminated rice (Charoenthaikij et al. 2010b; Watanabe et al. 2004). It is interesting to notice that WHC of the soaked sample was significantly lower than that of samples germinated for 12 hour and 24 hours. Again, this result could be explained due to α amylase action that takes some time to degrade intact starch granules, changing its structure. Hydration properties of the flour gel (WAI and SP) were not significantly affected as germination time increases, with the exception of 48H germination. This could be also explained by the change produced in the starch structure and the presence of released sugars that will interact with the starch forming more compact gels. In contrast, water soluble index (WSI) increased as the germination time increases, which confirmed the action of enzymes during germination and thus the release of water soluble compounds. The oil absorption capacity (OAC) remained constant until 24 hours of germination and only increased after 48 hours germination.

Table 1. Hydration properties of brown, soaked and germinated rice flour and their gel.

Treatment	WBC (g/g)	WHC (g/g)	SV (ml/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)	OAC (g/g)
Brown Rice	1.52±0.03^{a}	1.68±0.14^{a}	3.10±0.00^{a}	11.64±0.21^{a}	0.03±0.00^{c}	12.02±0.23^{a}	1.59±0.01^{b}
Soaked	1.39±0.04^{b}	1.23±0.05^{c}	3.03±0.06^{a}	9.72±0.31^{b}	0.05±0.01^{b}	10.27±0.35^{b}	1.63±0.02^{b}
12H GF	1.35±0.02^{b}	1.43±0.07^{b}	3.00±0.00^{a}	9.87±0.22^{b}	0.06±0.01^{b}	10.63±0.52^{b}	1.63±0.07^{b}
24H GF	1.26±0.04^{c}	1.55±0.18^{ab}	2.43±0.12^{b}	9.95±0.25^{b}	0.05±0.01^{bc}	10.46±0.28^{b}	1.60±0.01^{b}
48H GF	1.02±0.04^{d}	0.89±0.01^{d}	2.49±0.00^{b}	5.65±0.31^{c}	0.15±0.03^{a}	6.46±0.55^{c}	1.71±0.02^{a}

WBC: Water Binding Capacity, WHC: Water Holding Capacity, SV: Swelling Volume, SP: Swelling Power, WAI: Water Absorption Index, WSI: Water Solubility Index, OAC: Oil Absorption Capacity. Values with different letters in the same column are significantly different (P<0.05).

3.2.Flour Pasting Properties

The pasting plots and the recorded pasting properties of brown rice, soaked and germinated brown rice flour are shown in figure 1 and table 2, respectively. A progressive reduction of the viscosity during heating and cooling was observed as the germination proceeded, which agree with previous reports (Charaenthakij et al. 2012). Soaking induced a significant decrease of the viscosity after reaching the maximum viscosity during heating, which resulted in much lower viscosity after cooling. It seems that soaking process was enough to activate amylases and their activity became evident after starch gelatinization and during cooling where amylose recrystallization occurs. After 12 hours germination (12H) only slight increase in the peak viscosity was detected, likely due hydrolysis products were washed out after soaking. Despite intact granules are less susceptible to amylase action, giving sufficient time starch can be degraded and sugars are released (Dura et al. 2014). At 48 hour of germination the viscosity plot was drastically reduced during heating and cooling, owing to extensive degradation of starch granules. It might be expected that at this degree of degradation flour would not be suitable for breadmaking. Therefore, it seems that germination induced enzyme activation and that might respond to an exponential curve, as revealed the great action on the starch after 48H germination. The reduction of starch content with a simultaneous increase of

reducing sugars content during germination have been previously reported (Charaenthakij et al. 2012; Wu et al. 2013).

Figure 1 Pasting curves of brown, soaked and germinated rice flour.

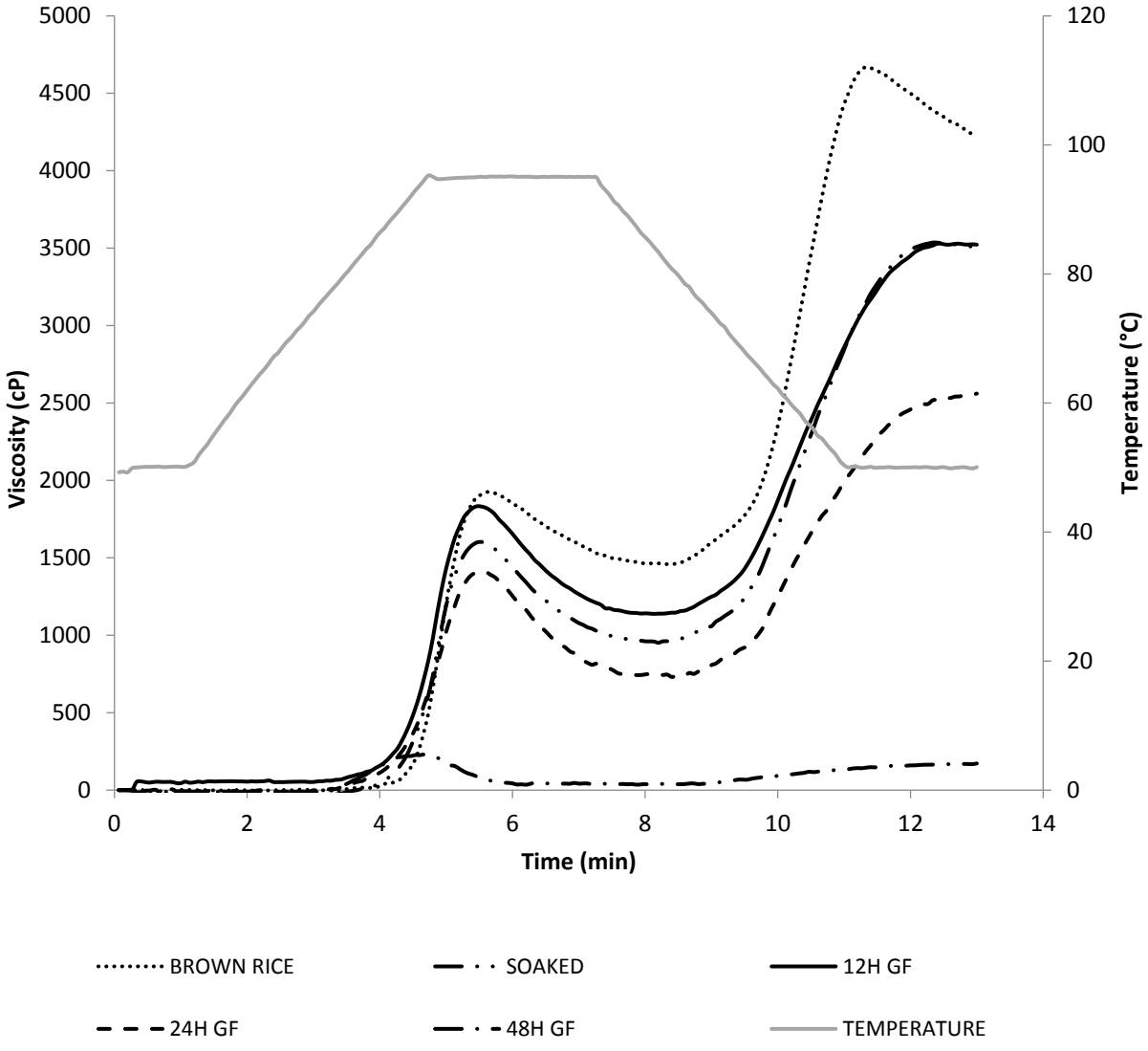


Table 2. Pasting properties of brown, soaked and germinated rice flour.

Treatment	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
Brown Rice	1926±4[a]	468±24[c]	4222±78[a]	2295±43[a]
Soaked	1602±4[c]	651±14[b]	3500±9[b]	1897±13[b]
12H GF	1833±38[b]	697±7[a]	3522±30[b]	1689±9[c]
24H GF	1409±6[d]	683±9[ab]	2560±3[c]	1151±3[d]
48H GF	229±1[e]	200±6[d]	172±11[d]	58±12[e]

Values with different letters in the same column are significantly different (P<0.05).

Peak viscosity, breakdown, setback and final viscosity decreased with germination due to degradation of the starch by the enzyme activity (Charoenthaikij et al 2009; 2012, Mäkinen et al. 2013; Wu et al. 2013). The action of α amylase changes the structure of the starch molecule breaking down the polymers chains and reducing its ability to bind water and increase the viscosity. The setback value reflects the degree of retrogradation of amylose (Gani et al. 2013). Thus, germination reduces the ability of amylose to retrograde, which might be beneficial in breadmaking to reduce the gluten free bread tendency to stale during storage.

It is interesting to notice that breakdown, related to starch cooking stability (Rojas et al. 1999), increased until 12 hours of germination. Previous studies about germination reported a breakdown decrease due to this process (Charoenthaikij et al 2012; Mäkinen et al. 2013; Wu et al. 2013). However, those studies did not analyzed soaking and the first stage of germination (after 12 hours of germination). A high breakdown demonstrates the ease of starch granules to be broken upon heating after the maximum swelling at the peak viscosity (Rojas et al. 1999). These results could demonstrate that during soaking and the first hours of germination starch granule was more susceptible to breaking, likely due to starch annealing or internal structure organization.

3.3.Evaluation of Bread Quality:

Figure 2 displays cross section bread slices from brown, soaked and germinated rice flour. As germination time increased the crumb structure showed more elongated gas cells

and of increasing size, which occurred up to 24 hours germination (24H). Mäkinen et al. (2013) observed a reduction of batter viscosity with the use of malted cereal, which enhanced α amylase activity, and those batters led to breads with more open crumb. Intermediate or lower paste viscosities could favor expansion of batters during baking resulting in large specific volume and more open crumbs (Renzetti and Arendt 2009). Indeed, the increase in amylase activity and sugar content due to germination produced faster increase of the batter volume during fermentation allowing the formation of large holes in the center of the crumb. However, no significant correlation was found among specific volume and pasting properties. Actually, at 48 hour of germination (48H GB) the crumb became deteriorated due to an excess of amylase activity, which, as it was discussed before in the pasting properties, induced complete degradation of starch. In fact, the presence of high level of α amylase could result in excessive liquefaction and dextrinisation, causing an inferior quality of bread described by the term 'wet sticky crumb' (Hallen et al. 2004). In addition, it can be observed that as germination time increased the surface became flattened; really at 24 hours of germination it was more concave. This result suggests that as the germination time increased the fermentation time should be decreased because fermentable sugars released from α amylase action speed up the leavening. Fermentation was carried out at fixed time for comparing flours behavior, but even crumb structure revealed an over-fermentation in breads obtained from germinated flour.

Quality characteristics of gluten free bread from brown, soaked and germinated rice flour are shown in table 3. No significant differences were found in the humidity, water activity and specific volume between samples, implying that germination does not produce significant changes in these bread characteristic. Although breads showed a significant higher specific volume than the bread obtained from non-germinated flour. A strong correlation was found between humidity and pasting properties such as peak viscosity ($r=0.92$ $P<0.001$), breakdown ($r=0.74$ $P<0.01$), final viscosity ($r=0.89$ $P<0.01$) and setback ($r=0.86$ $P<0.01$) as well as humidity and WHC ($r=0.73$ $P<0.01$), although those could not be related with bread quality properties. A negative correlation has been

reported between dough consistency at cooling and specific volume of rice based gluten free breads (Matos et al. 2013) and a positive correlation between apparent viscosity and loaf volume (Sabanis et al. 2009), but no correlation was observed when germinated rice flour was used as raw material.

No significant effect was observed on the geometry (width/height) of the breads due to the time of germination, although a tendency to increase with the germination time was envisaged. A strong correlation was found between width/height and SV ($r=0.79$ $P<0.001$). The specific volume values of the samples ranged from 1.5 to 2.3 ml/g, these results are in agreement with the ones reported for rice GFB (de la Hera et al. 2013; Matos and Rosell 2012; Marco and Rosell 2008b).

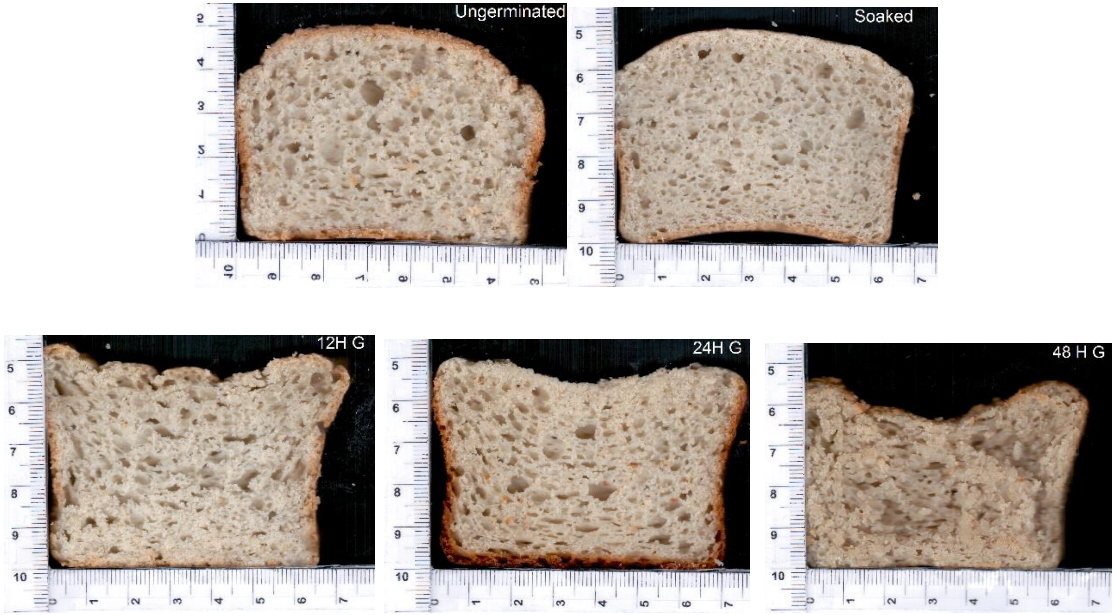
Generally rice gluten free breads are rather pale due to the refined flour use in their production, because of that crumb color becomes of importance when developing this type of baked products. Cie$L^*a^*b^*$ scale was used to characterize crumb color. L^* value showing the lightness of the crumb (table 3) underwent a significant reduction as the germination time increased, which is desirable because GFB tends to have lighter color than wheat bread. The L^* values ranged from 54.9 to 73.5, which are within the lower values reported in commercial gluten free rice breads (Matos and Rosell 2012). All the samples, except soaked flour, showed positive values for a^*, which are associated with reddish color, also b^* positive values were found, indicating yellowish tone. Indeed, a^* and b^* values increased as the germination time was extended. Those parameters showed higher values than the ones reported in commercial gluten free rice bread (Matos and Rosell 2012). All the samples showed a positive hue angle that reflected yellow-orange hue. Chroma values associated with the purity of color were higher than the ones obtained with commercial gluten free breads, which revealed its higher purity of color related to major intensity of the yellow component. In fact, Charoenthaikij et al (2010a) reported an increase of yellowness of bread with the addition of germinated brown rice flour to wheat flour. Hence, the increase of the chroma value could be due to the increase of reducing sugar during germination.

Table 3. Quality characteristics of gluten free brown rice bread from brown, soaked and germinated rice flour.

Treat-ment	Specific Volume (ml/g)	Humidity (%)	Aw	Width/ Height	*L**	*a**	*b**	Chroma	Hue angle (°)
Brown Rice	1.52±0.06^{b}	50.46±2.98	0.97±0.00	1.49±0.19^{a}	73.54±1.33^{a}	0.34±2.74^{bc}	37.85±2.79^{ab}	37.93±2.87^{ab}	89.53±3.91^{ab}
Soaked	2.28±0.04^{a}	50.08±1.39	0.98±0.01	1.36±0.03^{b}	66.35±7.78^{b}	-0.35±1.93^{c}	35.12±3.44^{b}	35.17±3.49^{b}	90.35±2.91^{a}
12H GF	1.99±0.45^{a}	50.46±1.72	0.98±0.00	1.42±0.09^{ab}	67.82±5.49^{b}	0.71±0.85^{bc}	35.82±3.03^{b}	35.84±3.04^{b}	88.90±1.28^{ab}
24H GF	2.14±0.33^{a}	49.98±0.75	0.98±0.00	1.49±0.10^{a}	65.57±4.96^{b}	1.42±1.17^{ab}	38.53±3.88^{ab}	38.57±3.86^{ab}	87.82±1.81^{bc}
48H GF	2.07±0.08^{a}	42.30±3.71	0.97±0.01	1.51±0.10^{a}	54.89±2.21^{c}	2.44±2.70^{a}	40.67±3.81^{a}	40.83±3.71^{a}	86.27±3.87^{c}

Values with different letters in the same column are significantly different (P<0.05).

Figure 2 Gluten free bread slices from brown, soaked and germinated brown rice flour.



Significant differences were observed in the crumb texture properties among grain treatments (table 4). The hardness values were lower than 10 N, that are inferior to those reported in commercial GFB (Matos and Rosell 2012), but still harder than wheat bread with hydroxypropylmethylcellulose (1.96 ±0.19 N) reported by Bárcenas and Rosell (2005). Considering that GFB present high crumb hardness due to their complex formulation, these values are sought after (Matos and Rosell, 2011). As the germination time increased the hardness of the crumb decreased, the degradation of starch during germination could cause a decrease of crumb hardness, probably due to the formation of thinner cell walls that led to softer crumbs. Also, Charoenthaikij et al. (2010a) found that a partial substitution with germinated brown rice flour reduces hardness of wheat bread compared to brown rice flour.

Table 4. Analysis of crumb texture of gluten free brown rice bread from brown, soaked and germinated rice flour.

Treatment	Hardness (N)	Resilience	Springiness	Chewiness (N)
Brown Rice	6.64 ± 2.56^{b}	0.29 ± 0.03^{a}	0.94 ± 0.35^{a}	14.73 ± 5.78^{a}
Soaked	9.16 ± 1.48^{a}	0.21 ± 0.05^{b}	0.82 ± 0.30^{ab}	13.94 ± 5.53^{a}
12H GF	5.37 ± 2.39^{bc}	0.15 ± 0.02^{c}	0.67 ± 0.05^{bc}	5.25 ± 2.91^{b}
24H GF	4.42 ± 0.55^{c}	0.17 ± 0.03^{c}	0.72 ± 0.09^{bc}	5.68 ± 0.82^{b}
48H GF	2.13 ± 0.65^{d}	0.15 ± 0.03^{c}	0.56 ± 0.10^{c}	2.77 ± 1.88^{b}

Values with different letters in the same column are significantly different ($P<0.05$).

High springiness values are preferred because it is related to the freshness and elasticity of the bread. As the germination time increased, the springiness value decreased, indicating an increase in fragility and tendency to crumble when is sliced (McCarthy et al. 2005). Indeed, after 12 hours of germination springiness values were lower than the ones reported before in gluten free rice bread (Marcos and Rosell 2008; Matos and Rosell 2012). These low values can be a limiting factor for the use of germinated brown rice for GFB. The lowest value was found at 48 hours of germination; actually the bread was too

fragile that was difficult to cut. The intense action of α amylase after 48 hour of germination could result in excessive liquefaction and dextrinisation, causing inferior bread quality (Hallen et al. 2004). Not only springiness but also resilience characterizes the loss of elasticity, because it indicates the ability of a material to return to its original shape after a stress (Onyango et al. 2011). Resilience and chewiness values decreased with the germination time. These values agree with those reported previously in commercial GFB (Matos and Rosell 2012).

The reduction of hardness and chewiness could be also related to the results found by Renzetti and Arendt (2009) when using protease treatment to improve the baking quality of brown rice bread. The formation of low molecular proteins and carbohydrates by germination yielded lower batter consistency and paste viscosity besides a decrease in WBC, overall they might improve texture as previously reported (Charoenthaikij et al. 2010b; Watanabe et al. 2004).

Thus, it is important to bear in mind a reformulation when a germinated brown rice is used as raw material, and it would be advisable to use brown rice up to 24 hours of germination to develop GFB. Higher germination time could be used as a partial substitute for increasing nutritional value of GFB.

4. Conclusions

Germinated rice flour showed appropriate functionality for being used as raw ingredient in gluten free breadmaking. The germination time of the rice has a significant effect on flour properties and the resulting bread quality. Specifically, flours obtained after 24 hours of germination led to an improvement in bread texture, which might be ascribed to the increase of amylase activities as well as starch degradation, which agrees with hydration and pasting results. Also bread color improved as a result of non-enzymatic browning reaction. However, excessive germination deteriorated the product as a result of extensive amylolysis. Germinated rice flour of more than 24 hours of germination was not suitable for breadmaking.

Acknowledgments

Authors acknowledge the financial support of Spanish Scientific Research Council (CSIC), the Spanish Ministry of Economy and Sustainability (Project AGL2011-23802), and the Generalitat Valenciana (Project Prometeo 2012/064). F. Cornejo acknowledges the financial support of Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT, Ecuador). Instituto Nacional de Investigaciones Agropecuarias from Ecuador (INIAP) is thanked for providing the BR cultivars and Biotechnological Research Center of Ecuador (CIBE, Ecuador) for the laboratories facilities.

References

Aguilera Y, Estrella I, Benitez V, Esteban R, Martín-Cabrejas M (2011) Bioactive phenolic compounds and functional properties of dehydrated bean flours. *Food Research International*, 44:774-780.

Anderson R, Conway H, Pheiser V, Griffin E (1969) Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today*, 14: 4-12.

Baek M, Yoo B, Lim S (2004) Effects of sugars and sugar alcohols on thermal transition and cold stability of corn starch gel. *Food Hydrocolloid*, 18: 133-142.

Bárcenas M, Rosell C (2005) Effect of HPMC addition on the microstructure, quality and aging of wheat bread. *Food Hydrocolloid*, 19: 1037-1043.

Cáceres P, Martinez-Villaluenga, C, Amigo L, Frias J (2014) Maximizing the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. *Food Chemistry*, 152: 407-414.

Charoenthaikij P, Jangchud K, Jangchud A. Piyachomkwan K, Tungtrakul P, Prinyawiwatkul W (2009) Germination conditions affect physicochemical properties of germinated brown rice flour. *Journal of Food Science*, 74: C658-C665.

Charoenthaikij P, Jangchud K, Jangchud A, Prinyawiwatkul W, Tungtrakul P (2010a) Germination conditions affect selected quality of composite wheat-germinated rown rice flour and bread formulations. *Journal of Food Science,* 75: 312 – 318.

Charoenthaikij P, Jangchud K, Jangchud A, Prinyawiwatkul W, No H, King J (2010b). Physicochemical properties and consumer acceptance of wheat-germinated brown rice bread during storage time. *Journal of Food Science,* 75: 333 – 339.

Charoenthaikij P, Jangchud K, Jangchud A, Prinyawiwatkul W, No H (2012). Composite wheat–germinated brown rice flours: selected physicochemical properties and bread application. *International Journal of Food Science and Technology,* 47: 75-82.

De La Hera E, Martinez M, Gómez M (2013). Influence of flour particle size on quality of gluten-free rice bread. *LWT-Food Science and Technology,* 54: 199-206.

Dura A, Blaszczak W, Rosell, C (2014). Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymer* 101: 837– 845.

Gani A, Wani S, Masoodi F, Salim R. (2013). Characterization of rice starches extracted from Indian cultivars. *Food Science and Technology International,* 19: 143-152.

Gomez M., Talegón M, De La Hera E. (2013). Influence of mixing on quality of gluten-free bread. *Journal of Food Quality*, 36: 139-145.

Gray J, Bemiller J (2003). Bread staling: molecular basis and control. *Comprehensive Review of Food Science,* 2: 1-21.

Gularte M, Rosell, C. (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymer,* 85: 237-244.

Hallen E, Ibanoglu S, Ainsworth P (2004). Effect of fermented/germinated cowpea flour addition on the rheological and baking properties of wheat flour. *Journal of Food Engineering,* 63:177–184.

Han H, Cho J, Kang H, Koh B. (2012). Rice varieties in relation to rice bread quality. *Journal of the Science of Food Agriculture,* 92: 1462-1467

Islam M, Becerra J, (2012). Analysis of chemical components involved in germination process of rice variety Jhapra. *Journal of Scientific Research,* 4: 251-262.

Lin M, Humbert E, Sosulski F. (1974). Certain functional properties of sunflower meal products. *Journal Food Science*, 39: 368-370.

Mäkinen O, Zannini E, Arendt E (2013). Germination of Oat and Quinoa and Evaluation of the Malts as Gluten Free Baking Ingredients. *Plant Foods for Human Nutrition*, 68: 90–95.

Marco C, Rosell C (2008). Breadmaking performance of protein enriched, gluten-free breads. *European Food Research Technology*, 227: 1205-1213.

Martinez M, Oliete B, Román L, Gómez M. (2014). Influence of the addition of extruded flours on rice bread quality. *Journal of Food Quality* 37: 83–94.

Matos M, Rosell C. (2011). Chemical composition and starch digestibility of different gluten free breads. *Plant Foods for Human Nutrition*, 66: 224-230.

Matos M, Rosell C. (2012). Relationship between instrumental parameters and sensory characteristics in gluten-free breads. *European Food Research Technology*, 235: 107-117.

Matos M, Rosell C. (2013). Quality indicators of rice based gluten-free bread-like products: relationships between dough rheology and quality characteristics. *Food Bioprocess Technology*, 6: 2331–2341.

Mccarthy D, Gallagher E, Gormley T, Schober T, Arendt E. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry* 82: 609-615.

Oh C, Oh S. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *Journal of Medicinal Food* 7: 19-23

Onyango C, Mutungi C, Unbehend G, Lindhauer M. (2011). Modification of gluten-free sorghum batter and bread using maize, potato, cassava or rice starch. *LWT-Food Science and Technology* 44: 681-686.

Peroni-Okita F, Cardoso M, Agopian R, Louro R, Nascimento J, Purgatto E, Tavares M, Lojolo F, Cordenunsi B. (2013). The cold storage of green bananas affects the starch degradation during ripening at higher temperature. *Carbohydrate Polymer* 96: 137-147.

Renzetti S, Arendt E. (2009). Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. *Journal of Cereal Science* 50: 22-28.

Rojas J, Rosell C, Benedito C (1999). Pasting properties of different wheat flour-hydrocolloid systems. *Food Hydrocolloid* 13: 27-33.

Rosell C, Gómez M (2014) Rice. In: Zhou W, Hui Y, DeLeyn I , Pagani M, Rosell C, Selman J, Therdthai P (eds) Bakery products: Science and Technology, 2nd Edn. Wiley, Iowa. pp 89-106.

Sabanis D, Lebesi D, Tzia C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. LWT-Food Sci Technol 42: 1380–1389.

Watanabe M., Maeda T., Tsukahara K, Kayahara H, Morita N(2004). Application of pregerminated brown rice for breadmaking. *Cereal Chemistry* 81: 450-455.

Wu F, Chen H, Yang N, Wang J, Duan X, Jin Z, Xu X. (2013). Effect of germination time on physicochemical properties of brown rice flour and starch from different rice cultivars. *Journal of Cereal Science* 58: 263-271.

Yam K, Papadakis S. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Journal of Food Engineering*, 6: 137-142.

CAPITULO 3

EFFECTS OF GERMINATION ON THE NUTRITIVE VALUE AND BIOACTIVE COMPOUNDS OF BROWN RICE BREAD

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of germination on the nutritive value and bioactive compounds of brown rice breads

Fabiola Cornejo [a,b,1], Patricio J. Caceres [b,c,1], Cristina Martínez-Villaluenga [c], Cristina M. Rosell [a,*], Juana Frias [c]

[a] Institute of Agrochemistry and Food Technology (IATA-CSIC), Avenida Agustín Escardino, 7. Paterna 46980, Valencia, Spain
[b] Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ingeniería Mecánica y Ciencias de la Producción, Campus Gustavo Galindo Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador
[c] Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

Abstract

The effect of germination conditions on the nutritional benefits of germinated brown rice flour (GBR) bread has been determined. The proximate composition, phytic acid, *in vitro* protein digestibility and *in vitro* enzymatic hydrolysis of starch, glucose and starch content, as well as the most relevant bioactive compounds (GABA, γ-oryzanol and total phenolic compounds) and antioxidant activity of breads prepared with GBR at different germination conditions was determined. When comparing different germination times (0 h, 12 h, 24 h, 48 h), germination for 48 h provides GBR bread with nutritionally superior quality on the basis of its higher content of protein, lipids and bioactive compounds (GABA and polyphenols), increased antioxidant activity and reduced phytic acid content and glycaemic index, although a slight decrease in *in vitro* protein digestibility was detected. Overall, germination seems to be a natural and sustainable way to improving the nutritional quality of gluten-free rice breads.

***Keywords*:** Brown rice, germination, nutritive value, gluten free.

1. Introduction

In the last decade, the use of brown rice (BR) has broadened not only in the common diet, but also in diet of people with celiac disease or allergies to typical cereals. In addition, the germination of BR grains provides higher nutritional and functional values since they are associated with the quality and quantity of their nutrients, biologically active compounds and antioxidant potential. Currently consumers demand natural foods and sprout products have become increasingly popular among people interested in improving and maintaining their health status by changing dietary habits. In this scenario, sprouted BR grains are excellent examples of functional food, because besides their nutritive value they lower the risk of various diseases and/or exert health promoting effects.

Germinated brown rice (GBR) is considered as gluten-free grain characterized by an excellent nutrient profile and germination enhances sharply the content of bioactive compounds such as GABA (γ-aminobutyric acid), phenolic compounds, γ-oryzanol and the antioxidant activity (Caceres el al., 2014). For instance, while the consumption of rice is associated with diabetes mellitus due to its high glycaemic index, GBR takes a leading role against diabetics and at the same time, a reduction on phytic acid is achieved enhancing mineral availability (Kim et al., 2012).

Scientific research supports the beneficial effects of these bioactive compounds, which includes regulation of blood pressure and heart rate, alleviation of pain and anxiety, improves sleeplessness and the autonomic disorder associated to menopausal or presenile period, suppresses liver damage, inhibits cancer cell proliferation and protects against oxidative stress (Oh & Oh 2004). In Japan, GBR was launched to the market in 1995. Since then, GBR is increasing its popularity within the Japanese population and, simultaneously, numerous derived food products have increased. Consequently, the use of GBR as a functional ingredient has focused the attention of researchers addressing the study on changes in nutritional composition and bioactivity. Thus, an increasing trend is focusing on their use in the formulation of high quality of health products. In this scenery, GBR is used as a raw material for obtaining different food products, like GBR balls, soup, bread, doughnuts, cookies and rice burger (Ito and Ishikawa, 2004).

Bread is a staple food in many parts of the world providing most calories of the diet. Bread is mostly prepared from wheat flour that it is the constraint for celiac patients, lifelong disorder with a prevalence of 1% of the world population. The only acceptable treatment is the restriction of gluten from the diet and, therefore, GBR bread is an attractive healthy alternative for this group of patients. The availability of palatable BR-containing gluten-free products would represent a significant advance towards ensuring an adequate intake of nutrients and bioactive compounds mostly in subjects with celiac

disorder but also in general consumers. Accordingly, developing bread based on GBR with desirable nutritional quality providing bioactive compounds is worthy of investigation.

To date, experimental GBR breads have been characterized with adequate instrumental and sensory attributes (Cornejo & Rosell, 2014). However, to our knowledge, investigations on the effect of germination conditions on the nutritive composition of bread-made BR are very limited. Therefore, the aim of the present study was to assess the proximate composition, phytic acid, *in vitro* protein digestibility and *in vitro* enzymatic hydrolysis of starch, glucose and starch content, as well as the most relevant bioactive compounds (GABA, γ-oryzanol and total phenolic compounds) and antioxidant activity of breads prepared with GBR at different germination conditions.

2. Materials and Methods

2.1. Materials

Commercial certified BR cultivar INIAP 15 was provided by the National Institute of Agricultural Research from Ecuador (INIAP). Seeds were harvest between May and December 2011. The gluten-free bread formulations also contained compressed yeast (LEVAPAN, Lessaffre, Valladolid, Spain) and hydroxypropylmethylcellulose (Methocel K4M) obtained from Dow Chemical Company (Michigan, USA).

2.2. Germination and flour preparation

Brown rice was sterilized with 0.1% sodium hypochlorite solution (1:5 w/v) for 30 min, and then rinsed with distilled water. Afterwards, rice was soaked in distilled water (seed water ratio, 1/5, w/v) for 24 h at 28±1 ºC. Soaking water was drained and rinsed seeds were placed in plastic trays containing moist filter and covered with moist filter paper. The filter papers were kept wet by capillarity. Germination was carried out at 28 ±1°C and 100% relative humidity under darkness for 12, 24 and 48 hours. Germination

period was selected on the basis of preliminary assays where nutritional pattern was followed in parallel to technological functionality of flours. After germination, seeds were dried at 50±1°C for 24 hours. Once dried, seeds were ground with a diameter inferior to 1mm with cyclone mill (UDY Corporation, USA). Brown rice flour was also obtained for comparison purposes, besides flour from soaked rice without germination. Two sets of samples were prepared for each treatment.

2.3. Bread preparation

The dough was performed using the recipe of Marco & Rosell (2008). Half of the rice flour was mixed with boiling water (half of the water) and mixed for five minutes. The dough was left to rest until the temperature decreased to 30 °C. Then, the rest of the flour, the other ingredients and water were added and mixed for 5 min. Later, the dough was put into pans and fermented for 40 min at 35 °C and 85% RH. Finally, the fermented dough was baked for 35 min at 175 °C. The bread was analysed after 24h of baking. Bread samples were coded BR for breads made with unprocessed BR flour, Pre-GBR for breads made with soaked brown rice and GBR preceded with germination time for those germinated brown rice flour (as example, 12h GBR for GBR germinated for 12 h).

2.4. Nutritional composition

Chemical composition of gluten-free breads was determined following AOAC (2005) methods and they include: moisture (method 925.10), ash (method 923.03), fat (method 922.06) and protein (method 920.87). The carbohydrate content of the samples was calculated by difference, subtracting 100 g minus the sum of grams of moisture, protein, fat and ash. The components were converted to food energy using conversion factors (4.0 kcal g-1 for proteins and carbohydrates and 9.0 kcal g-1 for fats) (FAO, 2003).

2.5. Determination of phytic acid

An accurate photometrical Haug and Lantzsch´s determination of phytic acid phosphorus was used (Reichwald and Hatzack, 2008) with some modifications. 1 mL of

HCl 1M was added to 50 mg of sample in an airtight stopper vial and heated for 1 hour in glycerol bath at 80°C under constant agitation at 10 x *g*. The mixture was then cooled to room temperature and centrifuged at 10,621 x *g* for 5 min and 0.250 mL of the supernatant was diluted with 1 mL of distilled water. An aliquot of 0.4 mL of sample, standard (phytic acid solution in 0.2 M HCl) or blank (0.2M HCl) were added to 0.8 mL of ferric solution (0.05 g of $FeCl_3$ in 500 mL of 0.2 M HCl) in an airtight stopper vial and was heated for 1 hour in glycerol bath at 80 °C with agitation at 10 x *g.* The mixture was cooled in ice bath for 15 minutes and centrifuged at 10,621 x *g* for 5 minutes at room temperature. Aliquot of 0.6 mL of the supernatant was added to 0.8 mL of the complexing reagent (0.5 g of 2,2´-bipiridine and 65 µL of thioglycolic acid dissolved in 50 mL of 0.2 M HCl) and absorbance was read at 540nm using a microplate reader (BioTek Instruments, Winooski, VT, USA) controlled by the Gene 5TM software version 1.1. (BioTek Instruments).

2.6. In vitro protein digestibility

The *in vitro* protein digestibility of the samples was determined by the modified method of Hsu et al. (1977). Briefly, 50 ml of aqueous protein suspension having 6.25 mg protein/ml was prepared. Then, samples were placed in a 37 ºC water bath and the pH was adjusted to 8.00 using 0.1 M NaOH and/or 0.1 M HCl, while stirring. Trypsin at a concentration of 1.6 mg/ml was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1M NaOH and/or 0.1M HCl. Five millilitres of enzyme solution were then added to the protein suspension, which was kept stirred at 37 ºC. The trypsin had an activity of 13,766 BAEE units/mg proteins. The pH drop was recorded along 15 s after enzyme addition and at one minute intervals for 10 min. The enzyme solution was always freshly prepared before each series of experiments. The percent protein digestibility (Y) was calculated by using Eq. (1) (Hsu et al., 1977): $Y = 210.464 – 18.1x$ (1), where x is the change in pH after 10 min.

2.7. In vitro starch digestibility and expected glycaemic index

Starch digestibility of bread was determined by dried samples, following the method described by (Dura et al., 2014) with minor modifications. Briefly, for free sugars removal, powder sample (0.1 g) suspended in 2 mL of 80% ethanol was kept in a shaking water bath at 85 °C for 5 min, and then centrifuged for 10 min at 1000× *g*. The remaining pellet was incubated with porcine pancreatic α-amylase (6 U/mL) (Type VI-B, ≥10 units/mg solid, Sigma Chemical, St. Louis, USA) in 10 mL of 0.1 M sodium maleate buffer (pH 6.9) in a shaking water bath at 37 °C. Aliquots of 200 μL were withdrawn during the incubation period and mixed with 200 μL of ethanol (96%, w/w) to stop the enzymatic reaction and the sample was centrifuged at 10,000 × *g* for 5 min at 4 °C. The precipitate was washed twice with 50% ethanol (200 μL) and the supernatants were pooled together and kept at 4 °C for further glucose enzymatic release.

Supernatant (100 μL) was diluted with 850 μL of 0.1 M sodium acetate buffer (pH 4.5) and incubated with 50 μL amyloglucosidase (33 U/mL) at 50 °C for 30 min in a shaking water bath. After centrifuging at 2000 × *g* for 10 min, supernatant was kept for glucose determination.

The glucose content was measured using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland). The absorbance was measured using an Epoch microplate reader (Biotek Instruments, Winooski, USA) at 510 nm. Starch was calculated as glucose (mg) × 0.9. The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, 120, 150, and 180 min). Replicates (n = 4) were carried out for each determination. A non-linear model established by Goñi et al (1997) was applied to describe the kinetics of starch hydrolysis. The first order equation (2) has the form: $C=C_{\infty}(1-e^{-kt})$ (2) , where C corresponds to the percentage of starch hydrolyzed at time *t*, C_{∞} is the equilibrium percentage of starch hydrolyzed after 180 min, *k* is the kinetic constant and *t* is the time (min). The parameters C_{∞} and *k* were estimated for each treatment.

Using the hydrolysis curve (0–180 min), hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the area of standard material obtained for white bread. The expected glycemic index (eGI) was calculated using the equation described by Grandfeldt et al. (1992): eGI = 8.198 + 0.862HI.

2.8. *Determination of γ-aminobutyric acid (GABA)*

γ-Aminobutyric acid (GABA) content was determined by HPLC as described in Caceres at al. (2014). 50 μL aliquot of concentrated water-soluble extract and 10μL allyl-L-glycine solution (Sigma-Aldrich) used as internal standard were derivatized with 30 μL phenyl isothiocyanate (PITC 99%, Sigma-Aldrich) and dissolved in mobile phase A for GABA analysis. An Alliance Separation Module 2695 (Waters, Milford, USA), a photodiode array detector 2996 (Waters) and an Empower II chromatographic software (Waters) were used as chromatographic system. 20μL of sample were injected into a C18 Alltima 250 x 4.6 mm i.d., 5 μm size (Alltech) column equipped with a same filling guard column (Alltech), both thermostatted at 30 ºC. The chromatogram was developed at a flow rate of 1.0 mL/min by eluting the sample with mobile phase A (0.1 M ammonium acetate pH 6.5) and mobile phase B (0.1 M ammonium acetate, acetonitrile, methanol, 44/46/10, v/v/v, pH 6.5) as in Caceres et al. (2014). Samples were independently analyzed in triplicate and results were expressed as mg GABA/100 g.

2.9. *Determination of γ-oryzanol*

The analysis of γ-oryzanol in rice samples was performed according to Moongngarm et al. (2010) by extraction in methanol, filtration, concentration and ulterior recovering in methanol to be analysed by HPLC. The system consisted in an Alliance Separation Module 2695 (Waters, Milford, USA), a photodiode array detector 2996 (Waters) setted at 325 nm wavelengh and Empower II software (Waters). 20μL were injected into a C18 column (150 x 3.9 mm i.d., 5 μm size, Waters) and mobile phase (1.0 mL/min) was eluted consisting in solvent A (acetonitrile), solvent B (methanol) and solvent C (bi-distilled water) for 50 min as follows: isocratic flow 60% A, 35% B and 5%

C for first 5 min, gradient flow 60% A and 40% B to 8 min keeping it at isocratic flow to 10 min, and then gradient flow 22% A and 78% B to min 20 to maintain isocratically to 35 min, changing to initial conditions to 45 min, isocratic conditions that were kept to equilibrate column to 50 min. γ-Oryzanol in rice samples was identified by retention time and spiking the sample with a standard solution of γ-oryzanol from bran rice (Cymit, Spain) and the purity of peaks was confirmed comparing the spectra and by MS analysis. γ-Oryzanol content was quantified by percentage of peak area according to the calibration curve prepared γ-oryzanol standard solutions. Replicates were independently analyzed and results were expressed in mg γ-oryzanol/100 g.

2.10. *Determination of total phenolic content*

The Folin-Ciocalteu method was used for determination of total phenolic content (TPC) according to Caceres et al., (2014). The absorbance was measured at 739 nm using a microplate reader (Synergy HT, BioTek Instruments) and TPC were quantified by external calibration using gallic acid (Sigma-Aldrich) as standard. Samples were independently analyzed in triplicate and results were expressed as mg of gallic acid equivalents (GAE) per 100g.

2.11. *Determination of oxygen radical absorbance capacity (ORAC)*

Antioxidant activity was determined by the method of oxygen radical absorbance capacity by fluorescence using an automatic multiplate reader (BioTek Instruments) at λ_{exc} 485 nm and λ_{em} 520 nm as described recently in Caceres at al., (2014). Individual samples were analysed in triplicated and results were expressed as mg of Trolox equivalents (TE)/100g.

2.12. Statistical Analysis

Standardized skewness and standardized kurtosis analyses were made to verify normal distribution of the data. Multiple sample comparison was conducted to evaluate significant differences among samples by analysis of variance (ANOVA) and multiple range tests. Fisher's least significant differences (LSD) test was used to describe means

with 95% confidence (P<0.05). All statistical analyses were performed using Statgraphics Centurion 16 (Statistical Graphics Corporation, UK).

3. Results and Discussion

3.1. Effect of soaking and germination time on nutritional properties of BR bread

The chemical composition of gluten free bread from BR and non-germinated BR showed no significant difference, with exception of ash content that was significantly lower in the bread from soaked flour likely due to the loss of minerals during washing (Table 1). The chemical composition of the gluten free breads agrees with values reported by Matos & Rosell (2011) in commercial gluten free breads. It can be seen that germination increased the protein content and decreased the carbohydrate, but that effect was independent on the germination time of the grains. In addition, a progressive reduction of ash content was observed with the germination time. Regarding the fat content, it was observed a progressive decrease up to 24 hours germination, but after that a significant increase was observed. There was a significant increase of free glucose content as germination proceeded, likely due to sugars released during germination. In fact, some researches had found a reduction of starch content and an increase of reducing sugar content during germination due to degradation of the starch by the enzyme activity (Charoenthaikij et al 2012, Xu et al 2012). During germination, enzymes become active and the α-amylase activity increases, acting on starch degradation, and in consequence increasing the amount of small dextrin and fermentable sugars. Despite fermentable sugars are used by yeast during bread fermentation, results revealed that significant differences were observed ascribed to the flour used.

Table 1. Proximate composition, energy, free glucose and phytic acid content of gluten-free bread from raw (BR), pre-germinated (Pre-GBR) and germinated brown rice (GBR) at different times.

Treatment	Moisture (g/100g)	Total Protein (g/100g)	Fat (g/100g)	Carbohydrates (g/100g)	Ash (g/100g)	Energy (Kcal)	Free Glucose (g/100g)	Phytic acid (g/100g)
BR	49.77±2.15a	6.03±0.05c	6.96±0.05b	74.19±0.91a	2.85±0.01a	214±9b	0.29±0.02d	1.09±0.05c
Pre-GBR	50.08±1.40a	6.12±0.04c	6.74±0.04c	74.20±1.01a	2.42±0.03d	213±6b	0.31±0.02d	0.82±0.06a
12h GBR	50.46±1.72a	8.14±0.21a	6.50±0.06d	72.45±1.18b	2.65±0.04b	210±7b	0.39±0.03c	0.82±0.08a
24h GBR	49.98±0.75a	8.01±0.08ab	5.58±0.03e	73.74±0.55ab	2.52±0.03c	209±3b	0.52±0.04b	0.95±0.02b
48h GBR	44.45±1.49b	7.81±0.12b	7.72±0.04a	72.49±0.51b	2.35±0.05e	230±1a	0.97±0.02a	0.81±0.02a

Values with different letters in the same column are significantly different ($P<0.05$).

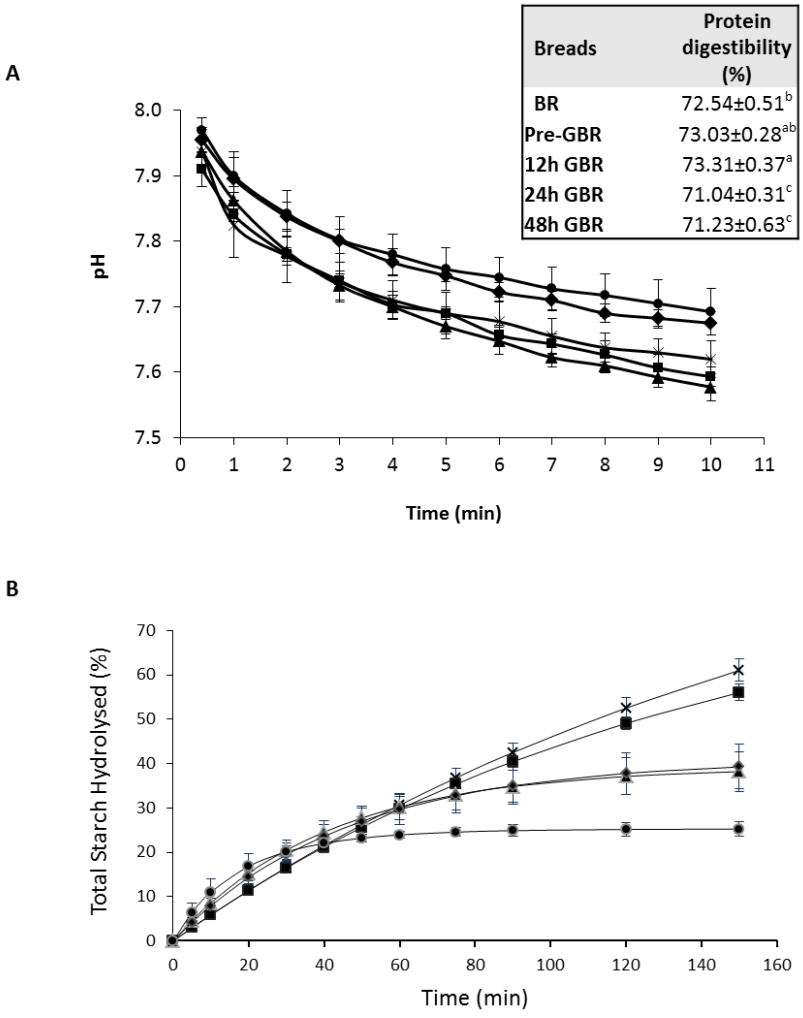
A reduced phytic acid content was observed in bread when BR was submitted to steeping and germination processes ($P \leq 0.05$) (Table 1). A higher phytic acid reduction was reached at 12 and 48 h of BR germination (25%) than at 24 h (13%) ($P \leq 0.05$). Lower phytic acid content observed in bread from pre-germinated and GBR could be explained by leaching of this compound into the soaking water and activation of endogenous phytase activity during germination that provides myoinositol and phosphoric acid for seedling growth (Albarracín et al., 2013). Phytic acid has the ability to chelate minerals (iron, zinc, magnesium and calcium) and affects negatively the absorption of amino acids, proteins, and starch (Oatway et al., 2001). Previous studies have demonstrated that reduced phytic acid content achieved by rice soaking and germination treatment lead to improved protein digestibility and mineral diazability (Albarracín et al., 2013). Therefore, germination of BR provides bread with better nutritional quality on the basis of its reduced phytic acid content compared to control bread. On the other hand, there has been increasing evidences that phytic acid may display health benefits reducing cholesterol levels in the diabetic KK mice (Lee et al., 2005) and exerting antioxidant and anticarcinogen effects (Schlemmer et al., 2009).

3.2. Effect of soaking and germination time on in vitro protein digestibility of BR bread

Considering that germination activates enzymes like amylases, proteases and so on, protein digestibility was tested to determine if germination might improve protein digestibility of the resulting breads. Germination affected *in vitro* protein digestibility (Figure 1, panel A), inducing an increase that was significant in breads obtained from rice after 12 hours germination (12h GBR), but further germination led to a significant reduction in protein digestibility. Bread samples 24h GBR and 48h GBR showed slower decline in pH compared with other treatments (Figure 1, panel A). It has been reported that BR germination increases the albumin and decreased the globulin and gliadin content, improving the protein bioavailability (Zheng et al., 2007). In addition, germination increases the amount of free amino acid, especially GABA content (Veluppillai et al., 2009). Divergences with the results obtained in the present study might be attributed to the participation of lysine containing proteins in the non-enzymatic browning (Maillard)

reaction during baking that is more accentuated in breads obtained from flours with extended germination (Cornejo & Rosell, 2014). In addition, the high temperature during baking could produce crosslinks between amino acids forming more rigid structures that reduce protein digestibility. Indeed, Lamberts et al. (2012) demonstrated that GABA was largely involved in Maillard reactions during baking, resulting in GABA trace levels in wheat bread samples.

Figure 1. *In vitro* digestibility of proteins (A) and starch (B) of gluten free bread from raw (BR), pre-germinated (Pre-GBR) and germinated brown rice (GBR) at different times (12, 24 and 48 h). BR (*), Pre-GBR (■),12h GBR (▲), 24h GBR (♦),48h GBR (●). Values with different letters in the table inset are significantly different ($P \leq 0.05$).



3.3. Effect of soaking and germination time on in vitro starch digestibility of BR bread

The *in vitro* starch digestibility curves of gluten free breads are shown in Figure 1 (panel B). In general, it can be observed that soaking and germination influenced the starch hydrolysis of the gluten free bread. Presumably, germination gives some resistance to starch granules likely due to the annealing that could undergo during soaking and drying. This result agrees with Xu et al. (2012) findings in germinated BR flour. They attributed the reduction of the digestion of starch to the presence of more crystalline starch structure after germination, due to the fact that enzymes hydrolyses first the amorphous region that are ease to digest (Dura et al., 2014). In addition, considering that baking is a thermal treatment, Chung et al. (2012) demonstrated that hydrothermal treatment in GBR, reduce the starch digestibility. They attributed this effect to structural changes induced by heat-moisture treatment that provoked rigidity of starch granules and molecules, which are less susceptible to the action of digestive enzymes. No significant difference could be observed between 12h GBR and 24h GBR, but the effect was even more accentuated after 48 hour of germination, slowing down the starch hydrolysis.

The parameters extracted from the regression curves of the recorded *in vitro* starch digestibility are shown in Table 2. The end point values (C_∞) obtained in the hydrolyzed process reflected the concentration at the equilibrium point. The C_∞ value of BR gluten free bread was within the values reported in other gluten free breads (Matos & Rosell, 2011; de la Hera et al., 2014). A significant reduction of C_∞ where found with germination, which reflected decreased digestibility of starch granules, indicating that germination led to less accessible or more resistant starch granules. In addition, *k* value significantly increased as germination time increases, reflecting structural differences (Butterworth et al., 2012; Dura et al., 2014). Presumably, the action of α-amylase during germination changes the internal structure of the starch molecule making it more difficult to digest, as suggested Xu et al. (2012) and Chung et al. (2012). An increase of *k* value by germination could be nutritionally unfavourable due to low *k* values are related to a slow diffusion of pancreatic amylase into the starch granule as digestion proceeds.

However, these *k* values are even lower than the ones reported by Matos & Rosell (2011) obtained in some commercial gluten free breads.

Table 2. Kinetics parameters of the in vitro starch digestibility and estimated glycemic index of gluten-free bread from raw (BR), pre-germinated (Pre-GBR) and germinated brown rice (GBR) at different times.

Treatment	C_∞ (g/100g)	k (min^{-1})	H_{90} (g/100g)	HI	eGI
BR	96.81±1.58a	0.006±0.001c	36.66±1.56b	60.21±3.89a	60.10±3.35a
Pre-GBR	81.23±4.56b	0.007±0.001c	44.84±1.05a	56.63±1.93a	57.01±1.66a
12h GBR	39.29±4.84c	0.025±0.005b	32.65±3.68b	47.04±5.53b	48.74±4.77b
24h GBR	40.88±5.46c	0.022±0.001b	32.86±6.03b	46.42±5.61b	48.22±4.84b
48h GBR	25.27±1.63d	0.041±0.006a	26.15±0.68c	34.30±0.91c	37.76±0.79c

C_∞: equilibrium concentration of starch hydrolysed after 180 min, K: kinetic constant, H90: starch hydrolysis at 90 min, HI: Hydrolysis index, eGI: estimated glycaemic index. Values with different letters in the same column are significantly different (P<0.05).

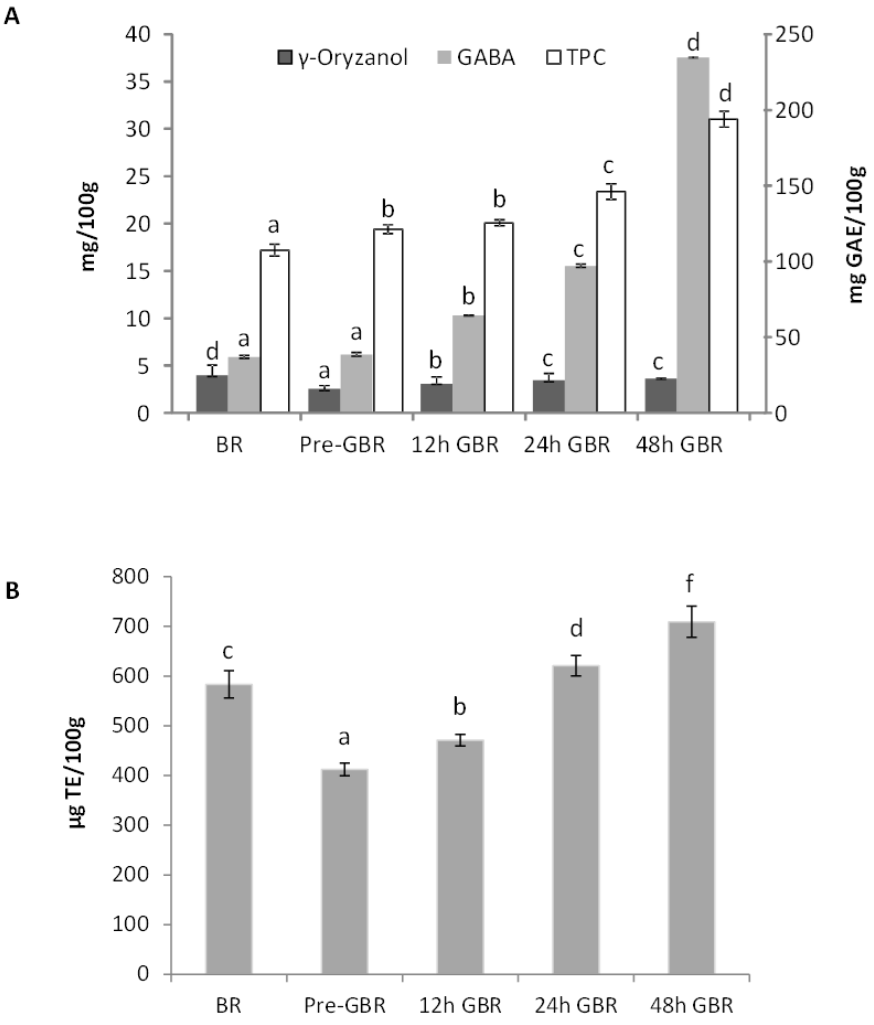
The hydrolysis index (HI) as well as the estimated glycaemic index (eGI) were significantly reduced with germination (Table 2), leading to breads with medium to low eGI. Indeed, the values of HI and eGI were lower than the ones reported for gluten free breads (Matos & Rosell, 2011; de la Hera et al., 2014). Usually, rice gluten free breads are expected to have higher GI (>70), due to the fact that this kind of breads are mainly starchy foodstuff (Matos & Rosell, 2011). However, the varieties of the rice, as well as dough preparation, influence the *in vitro* starch digestibility (Frei et al., 2003; de la Hera et al., 2014). The significant reduction of glycaemic index induced by the rice germination might be associated to the internal changes in the starch granules during germination. Low glycaemic index values are considered favourable to health, especially as a tool to prevent diseases where glycaemic control plays an important role, such as obesity, diabetes and hyperlipidemia.

3.4. Effect of soaking and germination time on the content of bioactive compounds and antioxidant activity of BR bread

The content of γ-oryzanol, GABA and TPC in BR bread (control) was 3.98, 5.92, 121.23 mg/100g d.m., respectively (Figure 2A). Breads from pre-germinated BR and GBR showed lower γ-oryzanol content than control breads ($P \leq 0.05$). Comparison of GBR breads showed that extended germination time (24 and 48 h) brought about increased γ-oryzanol content in breads although levels reached were lower than those found in control bread ($P \leq 0.05$). Our results agree with studies showing a reduced γ-oryzanol concentration in pre-GBR and GBR (Kiing et al., 2009). This effect could be attributed to increased feruloyl esterase activity involved in the hydrolysis of esters of phenolic acids such as γ-oryzanol (esters of trans-ferulic acid) that results in the release of ferulic acid as it has been previously reported in barley (Sancho et al., 1999). On the contrary, several studies have shown that pre-germination for 48 h and germination of BR bring about increased levels of γ-oryzanol (Moongngarm and Khomphiphatkul, 2011). These differences indicate that effect of soaking and germination processes on γ-oryzanol content depends on many factors such as BR cultivar and processing conditions (time, temperature, water pH) (Kiing et al., 2009). The content of γ-oryzanol in breads from pre-GBR and GBR was lower than that found in their respective flours (11 and 14 mg/100g d.m., respectively) (unpublished data). These results indicate that baking led to noticeable γ-oryzanol losses likely due to its thermal degradation and its hydrolysis during dough fermentation by feruloyl esterase activity of *Sacharomyces cerevisiae* that results in the release of ferulic acid (Coghe et al., 2004). γ-Oryzanol is also hydrolyzed upon gastrointestinal digestion into free sterol and ferulic acid by cholesterol esterases (Mandak and Nyström, 2012). Therefore, the reported biological activity of γ-oryzanol is likely due to free ferulic acid released during digestion. Few clinical studies has been performed so far to support the beneficial effect of ferulic acid in humans, however, results from these studies confirmed the potentially important role of ferulic acid in free radical-induced diseases (Alzheimer´s disease, cancer, cardiovascular diseases, diabetes

mellitus and skin disease) observed in preclinical research (Mancuso and Santangelo, 2014).

Figure 2. GABA, γ-oryzanol and total polyphenols content (TPC) (A); and antioxidant activity (B) of gluten free breads from brown rice (BR), pre-germinated brown rice (Pre-GBR) and germinated brown rice for 12 (12h GBR), 24 (24h GBR) and 48 h (48h GBR). Error bars indicate standard deviation. Different letters indicate significant differences ($P \leq 0.05$, LSD test)



Regarding GABA content, breads from pre-GBR were similar to control bread (Figure 2A). Germination markedly improved GABA content in bread, this effect being significantly greater with extended germination time ($P \leq 0.05$). Breads from 48h GBR showed 6 times higher GABA than control bread ($P \leq 0.05$). These results agree with a previous study reporting a time-dependent GABA accumulation during germination of BR (Caceres et al., 2014; Charoenthaikij et al., 2010). GABA accumulation initiates in the soaking process (Caceres et al., 2014; Charoenthaikij et al., 2010) and continues during germination due to the increased activity of glutamate decarboxilase that catalyses the decarboxilation of L-glutamic via GABA shunt pathway (Scott-Taggart y col., 1999). GABA concentration of pre-GBR and GBR breads was lower than that observed by our group in pre-germinated (28 ºC for 24h) and germinated (28 ºC for 48 h) flours from Ecuadorian BR cultivars (8.0-16.7 mg/100 g d.m. and 70.8-83.1 mg/100 g d.m., respectively) (Caceres et al., 2014). This observation indicate that GABA concentration decreases during BR bread making in consistency with previous studies (Watanabe et al., 2004). GABA losses during bread making are attributed to its consumption during yeast fermentation or amino acid degradation in Maillard browning reactions during baking as reported by Lamberts et al. (2012). Human intervention studies have shown that a daily intake of 10-20 mg of GABA is able to prevent pre-hypertension (Inoue et al., 2003). Therefore, a daily consumption of 100 g of bread from GBR for 48 h containing 37.5 mg of GABA would provide enough GABA to display the health benefits observed in previous studies (Inoue et al., 2003).

Total phenolic content was higher in breads from pre-GBR and GBR than control bread ($P \leq 0.05$) (Figure 2A). Similarly to GABA, TPC was noticeably improved in GBR breads with germination time ($P \leq 0.05$). Breads from GBR for 48 h showed 1.5 times higher total phenolic concentration than control bread ($P \leq 0.05$). These results agree with previous studies on grains germination (Caceres et al., 2014; Moongngarm & Saetung, 2010) and are directly related to the induction of enzymes involved in the phenylpropanoid pathway and in the degradation of the cell wall polysaccharides and proteins that cause the release of bound phenolics (He et al., 2011). This is supported by

Tian, Nakamura, and Kayahara (2004) who showed a significant increase in free ferulic, *p*-coumaric and sinapic acids and as well as insoluble but hydrolysable phenolic compounds, together with decreases in the hydroxycinnamate sucrose esters in GBR.

Antioxidant activity of bread was (583 µg TE /100 g d.m.) was reduced when BR was submitted to the steeping process ($P \leq 0.05$) (Figure 2B). BR germination for 12 h slightly increased ORAC values of pre-GBR bread although antioxidant activity was not improved compared to control bread. Interestingly, increased antioxidant activity was observed in bread compared with control when BR was germinated for longer time (24 and 48h). These results could be ascribed to the biosynthesis of compounds with antioxidant activity to keep a balance of the redox homeostasis during germination and to the hydrolysis of bound phenolics due to polysaccharide cell-wall degradation (He et al., 2011). TPC and γ-oryzanol content were positively correlated with ORAC (r^2 =0.8614 and 0.7627, respectively) which supports this hypothesis. Besides radical-scavenging activity, several studies have demonstrated that phenolic compounds and γ-oryzanol may also display their antioxidant effects acting as hydrogen and electron donors and through indirect antioxidant mechanisms such as up-regulation of antioxidant genes and down-regulation of oxidative stress genes markers (Ismail et al., 2010). The use of 48 h GBR as raw material for bread making is recommended as it provides higher antioxidant activity for a better protection against oxidative stress which is linked with the development of several chronic diseases.

4. Conclusions

This study shows that germination of BR is a natural way of improving the nutritional quality of gluten-free rice breads. Brown-rice germination for 48 h provides bread with nutritionally superior quality on the basis of its higher content of protein, lipids and bioactive compounds (GABA and polyphenols), increased antioxidant activity and reduced phytic acid content and glycaemic index.

Acknowledgments

Authors acknowledge the financial support of Spanish Scientific Research Council (CSIC), the Spanish Ministry of Economy and Sustainability (Project AGL 2010-16310 and AGL2011-23802), and the Generalitat Valenciana (Project Prometeo 2012/064). F. Cornejo and P. Caceres acknowledge the financial support of National Secretary of High Education, Science, Technology and Innovation (SENESCYT, Ecuador). National Autonomous Institute of Agricultural Research from Ecuador (INIAP) is thanked for providing the BR cultivars.

References

Albarracín, M., González, R.J., & Drago, S.R. (2013) Effect of soaking process on nutrient bio-accessibility and phytic acid content of brown rice cultivar. *LWT - Food Science and Technology, 53* (1), 76-80.

Butterworth, P. J., Warren, F. J., Grassby, T., Patel, H., & Ellis, P. R. (2012). Analysis of starch amylolysis using plots for first-order kinetics. *Carbohydrate Polymers, 87*, 2189–2197.

Caceres, P. J., Martinez-Villaluenga, C., Amigo, L., Frias, J. Maximizing the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. *Food Chemistry, 152,* 407-414.

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & Tungtrakul, P. (2010). Germination Conditions Affect Selected Quality of Composite Wheat-Germinated Brown Rice Flour and Bread Formulations. *Journal of Food Science, 75* (6), 312 – 318.

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & No, H. (2012). Composite wheat–germinated brown rice flours: selected physicochemical properties and bread application. *International Journal of Food Science & Technology, 47*(1), 75-82.

Chung, H. J., Cho, D. W., Park, J. D., Kweon, D. K., & Lim, S. T. (2012). *In vitro* starch digestibility and pasting properties of germinated brown rice after hydrothermal treatments. *Journal of Cereal Science, 56*(2), 451-456.

Coghe, S., Benoot, K., Delvaux, F., Vanderhaegen, B., & Delvaux, F.R. (2004). Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: indications for feruloyl esterase activity in *Saccharomyces cerevisiae. Journal of Agricultural and Food Chemistry, 52,* 602-608.

Cornejo, F., & Rosell C.M. (2014). Influence of germination time of brown rice in relation to flour features and gluten free bread quality. *Journal of Food Quality*. Submitted.

de la Hera, E., Rosell, C.M., Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food Chemistry, 151*, 526-531.

Dura, A., Blaszczak, W., & Rosell, C.M. (2014). Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers, 101*, 837– 845

FAO Food and Nutrition, Paper 77. (2003). Food energy – methods of analysis and 429 conversion factors, Food and Agriculture Organization of the United Nation, Rome.

Frei, M., Siddhuraju, P., & Becker, K. (2003). Studies on the *in vitro* starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chemistry*, *83*(3), 395-402.

Goni, I., Garcia Alonso, A., & Saura Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*, 427–437.

Granfeldt, Y., Björck, I., Drews, A., & Tovar, J. (1992). An *in vitro* procedure based on chewing to predict metabolic responses to starch in cereal and legume products. *European Journal of Clinical Nutrition, 46*, 649–660.

He, D., Han, C., Yao, J., Shen, S., & Yang, P. (2011). Constructing the metabolic and regulatory pathways in germinating rice seeds through proteomic approach. *Proteomics*, *11*, 2693-2713.

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for 455 estimating protein digestibility. *Journal of Food Science, 42*, 1269-1273.

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., et al. (2003). Blood-pressure-lowering effect of a novel fermented milk containing aminobutyric acid (GABA) in mild hypertensives. *European Journal of Clinical Nutrition, 57*, 490–495.

Ismail, M., Al-Naqeeb, G., Mamat, W.A.A.B., Ahmad, Z. (2010) Gamma-oryzanol rich fraction regulates the expression of antioxidant and oxidative stress related genes in stressed rat's liver. *Nutrition and Metabolism, 7*, art. no. 23.

Ito S, Ishikawa Y. (2004). Marketing of value-added rice products in Japan: germinated brown rice and rice bread. FAO Rice Conference 04/CRS.7. http://www.hatsuga.com/DOMER/english/en/GBRRB.html.

Kiing, S.-C., Yiu, P.-H., Rajan, A., Wong, S.-C. (2009). Effect of germination on γ-oryzanol content of selected sarawak rice cultivars. *American Journal of Applied Sciences, 6* (9), 1658-1661.

Lamberts, L., Joye, I. J., Beliën, T., & Delcour, J. A. (2012). Dynamics of γ-aminobutyric acid in wheat flour bread making. *Food Chemistry*, *130*(4), 896-901.

Lee, S.H., Park, H.J., Jung, H. J., Cho, S. M., Cho, Y. S., Lilleho, H. S. (2005). Effects of dietary phytic acid on serum and hepatic lipid levels in diabetic KK mice. *Nutrition Research, 25*, 869–876.

Mancuso, C., Santangelo, R. (2014). Ferulic acid: Pharmacological and toxicological aspects. *Food and Chemical Toxicology, 65*, 185-195.

Mandak, E., & Nyström, L. (2012). The effect of *in vitro* digestion on steryl ferulates from rice (Oryza sativa L.) and other grains. *Journal of Agricultural and Food Chemistry, 60*, 6123-6130.

Marco, C., & Rosell, C.M. (2008). Breadmaking performance of protein enriched, gluten-free breads. *European Food Research and Technology, 227*(4): 1205-1213.

Matos, E., & Rosell, C.M. (2011) Chemical composition and starch digestibility of different gluten-free breads. *Plant Foods for Human Nutrition, 66*(3), 224-230.

Moongngarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry 122*, 782-788.

Moongngarm, A., Khomphiphatkul, E. (2011). Germination time dependence of bioactive compounds and antioxidant activity in germinated rough rice (*Oryza sativa* L.). *American Journal of Applied Sciences 8*, 15-25.

Oatway, L., Vasanthan, T., Helm, J.H. (2001). Phytic acid. Food Reviews International, 17, 419-431.

Oh, C., & Oh, S. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *Journal of Medicinal Food, 7*(1): 19-23.

Reichwald, K., & Hatzack, F. (2008). Application of a modified Haug and Lantzsch method for rapid and accurate photometrical phytate determination in soybean, wheat, and maize meals. *Journal of Agricultural and Food Chemistry, 56*, 2888-2891.

Sancho, A.I., Faulds, C.B., Bartolomé, B., Williamson, G. (1999). Characterisation of feruloyl esterase activity in barley. *Journal of the Science of Food and Agriculture, 79* (3), 447-449.

Schlemmer, U., Frølich, W., Prieto, R.M., & Grases, F. (2009). Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition and Food Research, 53*, 330-375.

Scott-Taggart, C.P., Van Cauwenberghe, O. R., McLean, M. D., & Shelp, B. J. (1999). Regulation of γ-aminobutyric acid synthesis in situ by glutamate availability. *Physiologia Plantarum, 106,* 363-369.

Tian, S., Nakamura, K., & Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. *Journal of Agricultural and Food Chemistry, 52,* 4808-4813.

Veluppillai, S., Nithyanantharajah, K., Vasantharuba, S., Balakumar, S., & Arasaratnam, V. (2009). Biochemical changes associated with germinating rice grains and germination improvement. *Rice Science, 16,* 240–242.

Watanabe M., Maeda T., Tsukahara K., Kayahara H. & Morita N. (2004). Application of pre-germinated brown rice for breadmaking. *Cereal Chemistry*, *81*(4), 450-455.

Xu J., Zhang H., Guo X. & Qian H. (2012). The impact of germination on the characteristics of brown rice flour and starch. *Journal of the Science of Food and Agriculture*, *92*(2): 380-387.

Zheng, Y. M., Li, Q., and Ping, H. (2007). Effects of germination on protein and amino acid composition in brown rice. *Journal of Chinese Cereals Oils Association*, *22*, 7–1.

DISCUSIÓN GENERAL

La celiaquía es una enfermedad que afecta al 1% de la población mundial (Guandalini & Assiri, 2014). En la última década se ha incrementado el interés científico no sólo en desarrollar alimentos libres gluten, sino también que sean nutritivos y funcionales (Capriles & Arêas, 2014). La presente tesis exploró el desarrollo de panes sin gluten con una harina funcional como harina de arroz integral germinado, utilizando materia prima ecuatoriana. Las variedades de arroz ecuatorianas son variedades de grano largo y extra largo, siendo un requerimiento del consumidor ecuatoriano. Científicamente, esta característica no ha sido considerada apropiada para el desarrollo de panes libre de gluten, pero estudios previos han establecido dicha afirmación sin validar su veracidad sobre distintas variedades de arroz.

En la primera etapa de la tesis, se analizó las características funcionales de seis variedades de arroz de grano largo, y su potencialidad para ser usadas en el desarrollo de panes sin gluten. Las variedades estudiadas incluyeron INIAP 14, INIAP 15, INIAP 16, INIAP 17, F09, F50, que son consideradas de alto contenido de amilosa (23- 28%) (Wani et al., 2012). Estas variedades fueron seleccionadas debido a que son las más comercializadas en el Ecuador. El estudio comprendió el análisis de las propiedades fisicoquímicas de la harina de arroz y físicas del pan de arroz sin gluten. Entre las propiedades fisicoquímicas de la harina se estudió las características bromatológicas (contenido de amilosa, proteína, lípidos y humedad), propiedades de hidratación (capacidad de retención de agua (CRA), capacidad de ligación de agua (CLA), capacidad de absorción de aceite (CAO) volumen de hinchamiento (VH), poder de hinchamiento (PH), índice de absorción de agua (IAA), índice de solubilidad en agua (ISA)), las características reológicas (utilizando el analizador de viscosidad rápida (RVA)), y las propiedades térmicas (temperaturas de transición (To, Tp, Tc), rango de gelatinización (Ig), entalpía de gelatinización (ΔH)).

Respecto a las variedades, la variedad INIAP 17 presentó el mayor valor de CRA, lo cual podría contribuir al retardo en el envejecimiento del pan sin gluten (Sciarini, Ribotta, León, & Pérez, 2010). En INIAP 14 e INIAP 17 se encontraron valores altos de IAA y PH que podrían ser positivos en el proceso de panificación por estar relacionados con el incremento de la suavidad del pan (Arendt, Moore, & Bello, 2008). La INIAP 14 presentó el mayor volumen específico de pan, un bajo CLA incrementa el valor de PH, y se obtuvo alto valor de volumen específico en el pan (Han, Cho, Kang, & Koh, 2012). Según el análisis térmico la INIAP 14, a diferencia de la INIAP 17, F09 y F50, muestra valores altos de To, Tp, ΔH, PHI y un angosto Ig, que estaría relacionado con un alto grado de orden molecular (Correia & Beirão-da-Costa, 2012).

En general, no se observó correlación entre el contenido de amilosa y las propiedades fisicoquímicas de la harina de arroz y del pan. Algunos estudios relacionan el contenido de amilosa con CLA (Gani, Wani, Masoodi, & Salim, 2013; Iturriaga, Lopez, & Añon, 2004), y la amilosa con el PH (Singh, et al., 2006; Tester & Morrison, 1990). Cabe recalcar que estas investigaciones fueron realizadas en almidones de arroz y no en harina. Por lo tanto, los resultados indicarían que factores intrínsecos (el contenido de proteína y lípidos, el tamaño de partícula y la estructura del almidón) y la sinergia entre ellos inciden en la propiedades fisicoquímicas de las harinas (Kim, Song, & Shin, 2010). Se presume que la presencia de lípidos y proteínas en la harina influye en el proceso de gelatinización y afecta a la unión agua-almidón (Iturriaga et al., 2004). Estos resultados deberían ser corroborados con estudios más profundos de la estructura del granulo de almidón.

Por otro lado, se observó una correlación positiva entre PH y IAA (r=0.89, P<0.001) y una correlación negativa entre PH y CLA (r=-0.68, P<0.01) y entre IAA y CLA (r=-0.68, P<0.01) este resultado indicaría que harinas con alta CLA producen la disminución del agua disponible para que sea absorbida en la zona amorfa del granulo de

almidón, durante la hidratación y la gelatinización; reduciendo el PH y el IAA (Iturriaga et al., 2004; Kim et al., 2010). Respecto al pan de arroz sin gluten, no se encontró correlación entre CLA con la dureza y el volumen específico del pan. Las propiedades de la harina como VH, Tp y ΔH tuvieron una correlación alta con la cohesividad de la miga de pan. Adicionalmente, se encontró una correlación positiva (r=0.81, P<0.05) entre la Tc y el volumen específico del pan que podría estar relacionado con el impulso en el horno del pan. Los resultados demostraron que las características térmicas y de hidratación no predicen el comportamiento de las características del pan sin gluten como volumen específico y textura.

En general, los valores de volumen específico de los panes sin gluten con arroz de grano largo concuerdan con valores de panes comerciales (Matos & Rosell, 2012a) y estudios anteriores (de la Hera, Martinez, & Gómez, 2013; Sciarini et al., 2010). En cuanto a la textura, los valores de elasticidad y resiliencia fueron más bajos que los observados en otros estudios (Marco & Rosell, 2008; Matos & Rosell, 2012b), indicando una tendencia a que la miga se desmorone cuando se corta, lo cual debería corregirse con mejoras en la formulación.

Con este estudio se demostró que la variedad de arroz tiene una influencia significativa en las características del pan obtenido. Este resultado resaltaría la importancia de creación de variedades para fines industriales, en programas de mejoramiento se arroz. Además, se puede concluir que el tamaño del grano no es factor significativo en las características del pan.

Una vez demostrado que las variedades de arroz de grano largo pueden utilizarse para desarrollar pan sin gluten con características comerciales, se avanzó para conseguir una mejora nutritiva del mismo mediante el uso de arroz integral germinado. En esta etapa se utilizó la variedad INIAP 15, por ser la más comercializada, lo que facilitaría una industrialización posterior. El estudio comprendió el análisis de cuatro etapas de

germinación: remojo, germinación por 12, 24 y 48 horas. Como blanco se analizó el pan con harina de arroz integral. Entre los análisis realizados se consideró las propiedades fisicoquímicas de las harinas (propiedades de hidratación y propiedades reológicas) y del pan (textura, color, composición proximal, volumen específico), así como las características nutricionales del pan (digestibilidad *in vitro* de almidones y proteínas, así como componentes nutritivos). El estudio de componentes nutritivos del pan fue realizado por el grupo de investigación de ICTAN- CSIC.

Respecto a las propiedades de hidratación de la harina, se observó una tendencia a la reducción; siendo significativa la reducción de la CLA. Este fenómeno podría atribuirse a la acción de las enzimas durante la germinación que provocaron la degradación del almidón, alterando su estructura e incrementando los azúcares fermentables. Estos azúcares fermentables pueden formar enlaces entrecruzados con las cadenas de almidón en la zona amorfa del granulo, lo que impide el hinchamiento del grano (Baek, Yoo, & Lim, 2004). Además estos azúcares pueden interaccionar con el agua, restringiendo el agua libre para el hinchamiento del grano. Este incremento de azúcares fermentables también produce un incremento en el ISA.

Es importante destacar que a las 48 horas de germinación, propiedades como CLA, CRA, IAA, PH, se redujeron marcadamente y se produjo un incremento significativo del ISA. Esto indicaría que la degradación del granulo de almidón es paulatina hasta las primera 24 horas de germinación y a las 48 horas, se muestra una destrucción casi total del granulo de almidón. El contenido de azúcares fermentables fue significativamente mayor que el contenido de almidón, haciendo más soluble la harina (Cornejo et al., 2015; Charoenthaikij et al., 2012; Wu et al., 2013). Este hecho, también afectó las propiedades reológicas de la harina. Se pudo observar que el registro de viscosidad a las 48 horas de germinación, fue drásticamente reducido comparando con los otros tiempos de germinación. A este nivel de germinación, la degradación de los gránulos de almidón en la harina de arroz fue tal, que se hizo inviable su utilización para panificación. En efecto, la miga se volvió pegajosa y húmeda, por la alta actividad de la

α- amilasa. Por tanto, la germinación indujo la activación exponencial de enzimas como la α-amilasa, localizándose la mayor actividad a las 48 horas de germinación.

El incremento de la actividad α -amilasa durante las primeras 24 horas de germinación tuvo un efecto positivo en el pan sin gluten. Se pudo observar un incremento en el volumen de pan a medida que se incrementaba el tiempo de germinación. El rango de volumen específico se encontró entre 1.5 y 2.3 ml/g, que fue comparable a panes de arroz sin gluten reportados en estudios anteriores (de la Hera et al., 2013; Marco & Rosell, 2008; Matos & Rosell, 2012a). Por otra parte, se observó que a medida que se incrementó el tiempo de germinación la superficie del pan se mostró más plana hasta el punto de llegar a ser cóncava. Posiblemente, como consecuencia del mayor contenido de azúcares fermentables, se produjo una fermentación excesiva de la masa, dado que se utilizó un tiempo constante de fermentación. Por consiguiente, a medida que se incrementa el tiempo de germinación, el tiempo de fermentación de la masa debería reducirse. Adicionalmente, se observó un oscurecimiento no enzimático de la miga por la presencia de los azúcares reductores (producto de la germinación), haciendo al pan germinado más parecido a los panes integrales. Igualmente, se presentó una mejora de textura, la dureza del pan disminuyó a medida que se incrementó el tiempo de germinación, lo cual es adecuado en panes sin gluten. Sin embargo, los valores de resiliencia y elasticidad disminuyeron con la germinación, indicando mayor fragilidad de la miga al cortarse. Este defecto también se observó en las variedades de arroz de grano largo, por lo que se debería proceder a reformular los panes para subsanarlo.

Como se mencionó anteriormente, la germinación incrementa el valor nutricional del pan sin gluten. La digestibilidad de proteína se incrementó hasta las 12 horas de germinación y posteriormente se redujo. Estudios anteriores indicaron un incremento de la digestibilidad proteica con el tiempo de germinación (Zheng, Li, & Ping, 2007). La divergencia con los resultados obtenidos en el presente estudio podría explicarse por las diversas reacciones que ocurren durante el proceso de panificación en las cuales están

implicadas las proteínas. En primer lugar, la lisina presente en algunas proteínas podría actuar en las reacciones no enzimáticas durante el horneado, limitando la digestibilidad de las proteínas que contengan este aminoácido. En efecto, Lamberts et al. (2012) demostraron que el GABA actúa en las reacciones de Maillard durante el horneado. Adicionalmente, las altas temperaturas de horneado pueden producir entrecruzamiento entre aminoácidos formando estructuras más rígidas que reducen la digestibilidad de la proteína.

Adicionalmente, también se observó una reducción en la digestibilidad de los gránulos de almidón, indicando que la germinación indujo la formación de almidones más resistentes. Según Xu et al. (2012), durante la germinación las enzimas hidrolizan la parte amorfa del granulo de almidón quedando un granulo más cristalino y difícil de digerir. Los resultados reflejaron además un cambio en la estructura del granulo de almidón, debido al incremento del valor *K* con el tiempo de germinación. Aunque nutritivamente no es favorable el incremento del valor *K*, puesto que significa una aceleración de la velocidad de digestibilidad del almidón, los valores obtenidos fueron inferiores a los de panes comerciales (Matos & Rosell, 2012b). Otro factor positivo que se encontró fue la reducción del índice glucémico.

Por tanto, las propiedades nutritivas de la harina de arroz integral germinado son aconsejables para el desarrollo de panes sin gluten. Sin embargo, a diferencia de otros estudios que recomiendan el uso de harinas germinadas con tiempo de germinación superiores a 48 horas (Charoenthaikij et al., 2010; Watanabe et al., 2004), se ha demostrado que las propiedades tecnológicas de la harina tras periodos prolongados de germinación no son recomendables para panificación. A pesar de que los panes con harinas germinadas durante periodos inferiores a 24 horas no presentaron tan altos contenidos de GABA, compuestos fenólicos y actividad antioxidante como los panes de 48 horas, estos contenidos fueron significante altos y representaron un incremento en la funcionabilidad nutricional del pan sin gluten. Harinas germinadas durante más de 48 horas podrían ser utilizadas como ingredientes para sustituciones parciales para el

desarrollo de panes. Esta investigación abre la posibilidad de realizar estudios posteriores de validación nutricional y económica sobre la utilización de harina germinada durante periodos inferiores a 24 horas como ingrediente primario del pan o bien utilizar harinas con germinaciones más prolongadas como sustituto parcial del cereal primario en la elaboración de pan.

Bibliografía

Arendt, E., Moore, M., & Bello, F. D. (2008). Gluten Free Breads. In E. Arendt & F. D. Bello (Eds.), *Gluten Free cereal products and Beverages.* (pp. 289- 319). London: Academic Press

Baek, M. H., Yoo, B., & Lim, S. T. (2004). Effects of sugars and sugar alcohols on thermal transition and cold stability of corn starch gel. *Food Hydrocolloids, 18*(1), 133-142. doi: 10.1016/s0268-005x(03)00058-4

Capriles, V. D., & Arêas, J. A. G. (2014). Novel Approaches in Gluten-Free Breadmaking: Interface between Food Science, Nutrition, and Health. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 871-890. doi: 10.1111/1541-4337.12091

Cornejo, F., Caceres, P. J., Martínez-Villaluenga, C., Rosell, C. M., & Frias, J. (2015). Effects of germination on the nutritive value and bioactive compounds of brown rice breads. *Food Chemistry, 173*, 298-304.

Correia, P., & Beirão-da-Costa, M. L. (2012). Effect of drying temperatures on starch-related functional and thermal properties of chestnut flours. *Food and Bioproducts Processing, 90*(2), 284-294. doi: 10.1016/j.fbp.2011.06.008

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & No, H. K. (2012). Composite wheat-germinated brown rice flours: selected physicochemical properties and bread application. *International Journal of Food Science & Technology, 47*(1), 75-82. doi: 10.1111/j.1365-2621.2011.02809.x

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., & Tungtrakul, P. (2010). Germination conditions affect selected quality of composite wheat-

germinated brown rice flour and bread formulations. *Journal of Food Science, 75*(6), S312-318. doi: 10.1111/j.1750-3841.2010.01712.x

de la Hera, E., Martinez, M., & Gómez, M. (2013). Influence of flour particle size on quality of gluten-free rice bread. *LWT - Food Science and Technology, 54*(1), 199-206. doi: 10.1016/j.lwt.2013.04.019

Gani, A., Wani, S. M., Masoodi, F. A., & Salim, R. (2013). Characterization of rice starches extracted from Indian cultivars. *Food Science and Technology International, 19*(2), 143-152. doi: 10.1177/1082013212442189

Guandalini, S., & Assiri, A. (2014). Celiac disease: a review. *JAMA pediatrics, 168*(3), 272-278.

Han, H. M., Cho, J. H., Kang, H. W., & Koh, B. K. (2012). Rice varieties in relation to rice bread quality. *Journal of Science of Food and Agriculture, 92*(7), 1462-1467. doi: 10.1002/jsfa.4727

Iturriaga, L., Lopez, B., & Añon, M. a. (2004). Thermal and physicochemical characterization of seven argentine rice flours and starches. *Food Research International, 37*(5), 439-447. doi: 10.1016/j.foodres.2003.12.005

Kim, J.-M., Song, J.-Y., & Shin, M. (2010). Physicochemical properties of high amylose rice starches purified from Korean cultivars. *Starch - Stärke, 62*(5), 262-268. doi: 10.1002/star.200900230

Lamberts, L., Joye, I. J., Beliën, T., & Delcour, J. A. (2012). Dynamics of γ-aminobutyric acid in wheat flour bread making. *Food Chemistry, 130*(4), 896-901. doi: 10.1016/j.foodchem.2011.08.004

Marco, C., & Rosell, C. M. (2008a). Breadmaking performance of protein enriched, gluten-free breads. *European Food Research and Technology, 227*(4), 1205-1213.

Matos, M. E., & Rosell, C. M. (2012a). Quality Indicators of Rice-Based Gluten-Free Bread-Like Products: Relationships Between Dough Rheology and Quality Characteristics. *Food and Bioprocess Technology, 6*(9), 2331-2341. doi:10.1007/s11947-012-0903-9

Matos, E., & Rosell, C. (2012b). Relationship between instrumental parameters and sensory characteristics in gluten-free breads. . *European Food Research and Technology,, 235*(1), 107 - 117.

Sciarini, L. S., Ribotta, P. D., León, A. E., & Pérez, G. T. (2010). Effect of hydrocolloids on gluten-free batter properties and bread quality. *International Journal of Food Science & Technology, 45*(11), 2306-2312. doi: 10.1111/j.1365-2621.2010.02407.x

Singh, N., Kaur, L., Sandhu, K. S., Kaur, J., & Nishinari, K. (2006). Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloids, 20*(4), 532-542. doi: 10.1016/j.foodhyd.2005.05.003

Tester, R., & Morrison, W. (1990). Swelling and gelatinization of cereal starches. II. Waxy rice starches. . *Cereal Chemistry, 67*(6).

Wani, A. A., Singh, P., Shah, M. A., Schweiggert-Weisz, U., Gul, K., & Wani, I. A. (2012). Rice Starch Diversity: Effects on Structural, Morphological, Thermal, and Physicochemical Properties-A Review. *Comprehensive Reviews in Food Science and Food Safety, 11*(5), 417-436. doi: 10.1111/j.1541-4337.2012.00193.x

Watanabe, M., Maeda, T., Tsukahara, K., Kayahara, H., & Morita, N. (2004). Application of Pregerminated Brown Rice for Breadmaking *Cereal Chemistry, 81*(4), 450-455.

Wu, F., Chen, H., Yang, N., Wang, J., Duan, X., Jin, Z., & Xu, X. (2013). Effect of germination time on physicochemical properties of brown rice flour and starch from different rice cultivars. *Journal of Cereal Science, 58*(2), 263-271. doi: 10.1016/j.jcs.2013.06.008

Xu, J., Zhang, H., Guo, X., & Qian, H. (2012). The impact of germination on the characteristics of brown rice flour and starch. *Journal of the Science of Food and Agriculture, 92*(2), 380-387. doi: 10.1002/jsfa.458

Zheng, Y. M., Li, Q., & Ping, H. (2007). Effects of germination on protein and amino acid composition in brown rice. *Journal of Chinese Cereals Oils Association*, 22, 7–11.

CONCLUSIONES

El trabajo de investigación demostró que el tamaño del grano de arroz no es un factor determinante en las características fisicoquímicas de panes libres de gluten. No se evidenció una correlación entre las propiedades de hidratación y reológicas de la harina con el volumen específico y dureza de miga de los panes. Los resultados también revelaron una diferencia significativa en las propiedades fisicoquímicas de panes provenientes de diferentes variedades. En consecuencia, se sugeriría que la selección de una variedad de arroz específica podría mejorar la capacidad de panificación de la harina.

Por otro lado, se demostró que la harina de arroz integral germinada puede ser utilizada como ingrediente funcional en el desarrollo de panes libres de gluten. El tiempo de germinación es un factor determinante en las propiedades de la harina y del pan obtenido. A medida que se incrementa el tiempo de germinación la calidad nutricional y funcional de la harina se incrementa. Sin embargo, a pesar de que a 48 horas de germinación la harina es nutricional y funcionalmente superior, las características fisicoquímicas del pan sin gluten no son apropiadas, debido a un exceso de actividad alfa amilasa y a una degradación del granulo de almidón. A las 24 horas de germinación las características del pan, en especial la textura y el volumen específico, son comparables con panes sin gluten comerciales. En esta etapa la actividad alfa amilasa sería beneficiosa para el proceso de panificación. En consecuencia, se recomienda el uso de harina de arroz germinada hasta 24 horas de germinación como ingrediente principal de panes sin gluten y a mayores tiempos de germinación se recomendaría combinarla con otros tipos de harina.

En general, el desarrollo de este trabajo de investigación permitió obtener un pan si gluten con propiedades funcionales que benefician no solo a pacientes con celiaquía sino también a pacientes con enfermedades como diabetes, hipertensión, cáncer, estrés, entre otros. Además, el desarrollo de pan de arroz germinado permitirá establecer una tecnología para la industrialización del arroz, beneficiando al sector arrocero del Ecuador. Finalmente, la información proporcionada sobre las características fisicoquímicas y

tecnológicas de las variedades de arroz desarrolladas por INIAP, permitirá a la institución desarrollar variedades genéticas con fines tecnológicos.

ABREVIATURAS

ΔH	Enthalpy of gelatinization
ACC	Apparent Amylose Content
BR	Brown Rice
C_∞	Equilibrium concentration of starch hydrolysed after 180 min,
CAA	Capacidad de absorción de agua
CAO	Capacidad de absorción de aceite
CLA	Capacidad de ligación de agua
CLA	Capacidad de ligación de agua
CRA	Capacidad de retención de agua
DSC	Dynamic Scanning Calorimetry
eGI	Estimated glycaemic index
GBR	Germinated Brown Rice
GFB	Gluten Free Bread
H90	Starch hydrolysis at 90 min.
HI	Hydrolysis index
IAA	Índice de Absorción de Agua
Ig	Gelatinization temperature range
ISA	Índice de solubilidad en agua
K	Kinetic constant in Starch Hydrolysis
OAC	Oil Absorption Capacity

ORAC	Oxygen radical absorbance capacity
PH	Poder de hinchamiento
PHI	Peak height index
RVA	Rapid Visco Analyzer
SP	Swelling Power
SV	Swelling Volume
T_0	Onset gelatinization temperature
Tc	Conclusion gelatinization temperature
Tp	Peak gelatinization temperature
TPA	Texture Profile Analysis
VH	Volumen de hinchamiento
WAI	Water Absorption Index
WBC	Water Binding Capacity
WHC	Water Holding Capacity
WSI	Water Solubility Index