PhD Thesis

HIGH HYDROSTATIC PRESSURE AS A TECHNOLOGICAL TOOL FOR THE DEVELOPMENT OF GLUTEN-FREE FLOURS WITH IMPROVED NUTRITIONAL AND TECHNO-FUNCTIONAL PROPERTIES

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PROGRAMA DE DOCTORADO EN CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL:

El tratamiento de alta presión hidrostática como herramienta tecnológica para el desarrollo de harinas sin gluten con mejores propiedades nutricionales y tecno-funcionales.

Presentada por Ángel Luis Gutiérrez de la Fuente para optar al grado de
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“Two roads diverged in a wood, and I—
I took the one less travelled by,
And that has made all the difference.”

Robert Frost

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RESUMEN

En los últimos años se ha incrementado el interés del consumidor por productos horneados con un perfil nutricional más balanceado. En particular, se han aplicado diversas estrategias sobre el pan por ser uno de los alimentos más consumidos. Este producto se ha considerado habitualmente como un alimento de alta densidad energética y con un perfil nutricional limitado, debido al predominio del almidón entre sus componentes. Las estrategias para la mejora de este tipo de productos pasan por el empleo de harinas integrales y/o enriquecidas, que ofrecen una mayor variedad y un mejor balance de compuestos nutricionales. Sin embargo, suele ser habitual que este tipo de productos presenten una menor aceptabilidad sensorial que sus homólogos elaborados con harinas refinadas. Esta realidad se acentúa, sobre todo, en los productos horneados sin gluten, en los que, de cara a solventar los déficits nutricionales propios de la población celíaca, se ha incidido especialmente en las estrategias de enriquecimiento nutricional de los productos horneados. En la actualidad, además de los constantes esfuerzos para solventar las limitaciones tecnológicas asociadas a la falta de gluten, existe una tendencia a disminuir o suprimir el uso de aditivos con efecto estructurante. En este contexto, están proliferando las investigaciones orientadas al desarrollo de ingredientes alternativos, como son las harinas modificadas físicamente, cuyas propiedades tecno-funcionales contribuyen a la estructuración de la masa en formulaciones complejas, como es el caso de aquellas que incorporan harinas integrales, o las destinadas a la elaboración de productos horneados sin gluten.

En la presente tesis, se realiza un estudio de diversas alternativas de interés en la industria de productos horneados sin gluten, basadas en la inclusión de ingredientes de alto valor nutricional y funcional sin comprometer la calidad sensorial del producto final enfatizando, sobre todo, en la obtención, la caracterización y la utilización de nuevos ingredientes obtenidos mediante la tecnología de las altas presiones hidrostáticas (APH). Dicha técnica y su aplicación en el campo de los productos horneados, circunscrita al desarrollo de ingredientes modificados físicamente, es descrita en profundidad en la parte introductoria de este documento.

De manera complementaria, en el apartado de resultados y discusión se presentan una serie de capítulos que describen el uso de esta tecnología para dar lugar a harinas de alto valor tecno-funcional y nutricional, a partir de diversas fuentes vegetales adecuadas para su inclusión en formulaciones de productos horneados sin gluten. El empleo de dicha tecnología en el contexto de este trabajo, se fundamentó en la aplicación de este tratamiento tecnológico sobre granos enteros, diferenciándose de las estrategias utilizadas con anterioridad, basadas en su aplicación sobre suspensiones de almidón o harinas en agua. De manera complementaria, en esta tesis se presenta además el aprovechamiento de un subproducto derivado del procesado del grano entero de trigo

sarraceno para la obtención de harina, como es el pericarpio del grano, también llamado cascarilla.

En la fase inicial de la tesis se estudió el efecto del tratamiento APH sobre una matriz de grano entero de trigo sarraceno, un pseudocereal de elevado valor nutricional intrínseco. Para ello, se aplicaron presiones de 600 MPa durante 30 min, evaluándose el impacto de otros factores derivados del tratamiento, como el empleo de un pre-tratamiento de remojo previo al tratamiento APH sobre el grano en condiciones de exceso de agua, durante 4 horas a 40ºC, o la aplicación de la presión mediante diferentes ciclos de tratamiento. Las harinas integrales obtenidas, sobre todo aquellas procedentes de los granos con el tratamiento de remojo previo, mostraron cambios tanto a nivel microestructural, como en sus propiedades térmicas, con un descenso significativo en la entalpía de gelatinización. Además, en los perfiles de empastado se observó una disminución del valor de los principales parámetros de viscosidad estudiados, mientras que, a nivel nutricional, se produjo un incremento en su contenido fenólico.

En los siguientes capítulos, se profundizó en el análisis de otros factores propios del procesado con APH, como la presión o el tiempo de tratamiento, a fin de determinar su impacto en las propiedades tecno-funcionales y nutricionales de las harinas integrales resultantes. Partiendo de la experiencia y resultados obtenidos en el capítulo anterior, tras aplicar también un pre-tratamiento de remojo previo, se llevó a cabo un diseño experimental basado en el estudio de dos niveles de presión (300/600 MPa) y tres de tiempo (0/5/15 min), sobre una matriz de grano entero de trigo sarraceno. Los resultados mostraron diferentes comportamientos en la respuesta tecno-funcional de las harinas en función de las diferentes condiciones de tratamiento. Particularmente, se observó un descenso en la capacidad de absorción de agua para aquellas tratadas a 300 MPa, mientras que esta variable aumentó al incrementar ese nivel de presión. Además, la muestra tratada en las condiciones de máxima intensidad (15 min, 600 MPa) mostró un descenso en la entalpía de gelatinización, siendo este resultado contrario al observado para la harina procedente de granos tratados a la menor intensidad (0 min, 300 MPa). También se observó una disminución gradual del carácter elástico de los geles elaborados con dichas harinas a medida que aumentaba la intensidad del tratamiento. Dentro de las harinas tratadas, las resultantes del tratamiento más intenso (600 MPa y 15 min) mostraron el mayor contenido fenólico y capacidad antioxidante.

El efecto del tratamiento APH también se evaluó sobre el grano entero de arroz provisto de cascarilla (arroz paddy), ya que ésta es una de las fuentes vegetales más utilizadas en las formulaciones de productos horneados sin gluten. En este caso, los factores de estudio fueron la aplicación de un pretratamiento de remojo previo (en las mismas condiciones que en los estudios anteriores) y el tiempo de tratamiento (5/15/30 min) manteniéndose, en todos los casos, un nivel de presión de 600 MPa. De forma análoga a los resultados anteriores, se observó que aquellas harinas resultantes de aplicar un tratamiento de remojo previo se caracterizaron por una mayor capacidad de absorción de agua y termo-estabilidad en sus perfiles de empastado. También se observó que las muestras tratadas a tiempos superiores a 5 min tuvieron un mayor contenido fenólico y de vitaminas del grupo B, concretamente tiamina y piridoxina.

En esta tesis también se evaluó el comportamiento en panificación de las harinas resultantes del tratamiento APH. Para ello, en base a los resultados obtenidos para ambas matrices, se seleccionaron las condiciones de tratamiento que condujeron a un mayor impacto sobre las propiedades funcionales y nutricionales de las harinas y que, simultáneamente, fueran más fácilmente implementables a nivel industrial. Concretamente, se aplicó un pretratamiento previo de remojo del grano seguido de un procesado APH de 15 min y 600 MPa obteniéndose, en todos los casos, harinas integrales modificadas. Dichas harinas fueron utilizadas en una formulación base de pan sin gluten elaborado con harina refinada de arroz, estudiándose el impacto de aplicar diferentes porcentajes de sustitución (15, 30, 50 y 70 %). A efectos comparativos, y para valorar la influencia de dichas harinas modificadas, se efectuaron elaboraciones que incluyeron, en las mismas proporciones, una harina integral nativa de trigo sarraceno o de arroz. En los ensayos efectuados y en comparación con las fórmulas que incluían harina nativa, aquellas elaboraciones que contenían la harina tratada dieron lugar a mayores volúmenes específicos del pan y a menores valores de dureza de miga.

Finalmente, la cascarilla de trigo sarraceno, subproducto generado del procesamiento del grano para la obtención de granos pelados y harinas, fue evaluada como ingrediente alimentario para su incorporación en formulaciones de pan sin gluten. Este subproducto posee un elevado valor nutricional debido a su alto contenido en fibra y en compuestos bioactivos, por lo que su uso como ingrediente podría mejorar el perfil nutricional de los productos en los que se emplee, favoreciendo la economía circular como modelo de producción sostenible. Para este estudio, se utilizaron dos fracciones de cascarilla de distinto tamaño de partícula (D_{50} = 62,7 μm y 307 μm) que se aplicaron en dos dosificaciones distintas (3 y 6 %, p/p) para garantizar las declaraciones nutricionales "fuente de fibra" y "alto contenido en fibra" según el Reglamento (CE) Nº 1924/2006 de 20 de diciembre, relativo a las declaraciones nutricionales y de propiedades saludables en los alimentos. Entre los resultados obtenidos, destacó el importante impacto de este ingrediente sobre el valor nutricional del pan sin gluten resultante, ya que, a pesar del reducido porcentaje de adición, las elaboraciones resultantes mostraron un elevado contenido en fibra dietética (6,5-10,4 % p/p) y experimentaron un incremento significativo en su contenido fenólico y su capacidad antioxidante. En términos de calidad del pan, aquellas elaboraciones que incorporaron las partículas de menor tamaño (D_{50} = 62,7 μm) en porcentajes de adición del 3% (p/p), no dieron lugar a una merma en las propiedades físicas y sensoriales del producto, observándose una mayor aceptación global del mismo con respecto al resto de elaboraciones que contenían partículas de cascarilla de trigo sarraceno.

A tenor de estos resultados obtenidos en esta tesis, se constata la capacidad de la tecnología APH para la obtención de harinas modificadas de elevado valor añadido, caracterizadas por unas propiedades tecno-funcionales y nutricionales adaptadas a los requerimientos de la industria de elaboración de productos de panificación sin gluten y del colectivo celiaco. Además, mediante control de los parámetros de procesamiento en los tratamientos APH, se puede lograr la modulación de la respuesta tecno-funcional de las harinas resultantes. Entre las condiciones estudiadas, resulta de especial interés la aplicación del tratamiento APH de 600 MPa a tiempos mayores de 5 min sobre granos que han sido sometidos previamente a un tratamiento de remojo previo. Los panes elaborados con las harinas resultantes de aplicar estas condiciones, incluso con el menor porcentaje de sustitución estudiado (15%, p/p), mostraron una mayor calidad física y sensorial que sus análogos elaborados con harinas integrales sin tratar.

ABSTRACT

In recent years, consumer interest in bakery products with a more balanced nutritional profile has increased. In particular, various strategies have been applied to bread as one of the most widely consumed foods. This product has usually been considered as an energy-dense food with a limited nutritional profile, due to the predominance of starch among its components. Strategies to improve this type of products include the use of whole grain and/or enriched flours, which offer greater variety and a better balance of nutritional compounds. However, it is common for such products to have lower sensory acceptability than their counterparts made from refined flours. This is particularly relevant for gluten-free baked goods, where, in order to overcome the nutritional deficits of the coeliac population, special emphasis has been placed on strategies for nutritional enrichment of baked goods. Nowadays, in addition to the constant efforts to overcome the technological limitations associated with the lack of gluten, there is a trend to reduce or eliminate the use of additives with a structuring effect. In this context, research is growing into the development of alternative ingredients, such as physically modified flours, whose techno-functional properties contribute to the structuring of dough in complex formulations, such as those incorporating wholemeal flours, or those intended for the production of gluten-free baked goods.

In the present thesis, a study is made of various alternatives of interest in the gluten-free baked goods industry, based on the inclusion of ingredients of high nutritional and functional value without compromising the sensory quality of the final product, emphasising particularly on the collection, characterisation and use of new ingredients obtained using high hydrostatic pressure (HHP) technology. This technique and its application in the field of baked goods, limited to the development of physically modified ingredients, is described in depth in the introductory part of this document.

In addition, in the section on results and discussion, a series of chapters are presented to describe the use of this technology to produce flours of high techno-functional and nutritional value from various plant sources suitable for inclusion in gluten-free baked product formulations. The use of this technology in the context of this work was focused on the application of this technological treatment on whole grains, differing from the strategies used previously, based on its application on starch or flour suspensions in water. In a complementary approach, this thesis also presents the use of a by-product derived from the processing of the whole buckwheat grain to obtain flour, which is the pericarp of the grain, also known as hull.

In the first phase of the thesis, the effect of APH treatment on a buckwheat whole grain, a pseudocereal with a high inherent nutritional value, was studied. For this purpose, pressures of 600 MPa were applied for 30 min, and the impact of other treatment factors, such as the use of a pre-treatment of soaking of the grain under water excess for 4 hours at 40°C or the application of the pressurethrough different treatment cycles, were evaluated. The whole flours obtained, especially those from the pre-soaked grains, showed changes in both microstructural and thermal properties, with a significant decrease in the enthalpy of gelatinisation. In addition, a decrease in the values of the main viscosity parameters studied was observed in the pasting profiles, while at the nutritional level, there was an increase in their phenolic content.

In the following chapters, other factors specific to HHP processing, such as pressure or processing time, were further analysed in order to determine their impact on the techno-functional and nutritional properties of the resulting whole flours. Based on the experience and results obtained in the previous chapter, after applying a pre-soaking pre-treatment, an experimental design was carried out involving the study of two levels of pressure (300/600 MPa) and three levels of time (0/5/15 min), on a buckwheat whole grain matrix. The results showed different behaviours in the techno-functional response of the flours depending on the different treatment conditions. In particular, a decrease in water absorption capacity was observed for those treated at 300 MPa, while this variable increased as the pressure level increased. In addition, the sample treated at the maximum intensity conditions (15 min, 600 MPa) showed a decrease in the enthalpy of gelatinisation, contrary to the result observed for that flour sample from grains treated at the lowest intensity (0 min, 300 MPa). A gradual decrease in the elasticity of the gels made with these flours was also observed as the intensity of the treatment increased. Among the treated flours, those resulting from the most intense treatment (600 MPa and 15 min) showed the highest phenolic content and antioxidant capacity.

The effect of the HHP treatment was also evaluated on the paddy rice grain matrix, as rice is one of the most commonly used vegetable sources in gluten-free baked goods formulations. In this case, the study factors were the application of a pre-soaking treatment (under the same conditions as in the previous studies) and the treatment time (5/15/30 min) maintaining in all circumstances, a pressure level of 600 MPa. Similarly to the previous results, it was observed that those flours resulting from a pre-soaking treatment were characterised by a higher water absorption capacity and thermo-stability in their pasting profiles. It was also observed that samples treated at times longer than 5 min had a higher phenolic and B vitamins content, namely thiamine and pyridoxine.

This thesis also evaluated the baking behaviour of those flours resulting from HHP treatment. Based on the results obtained for both matrices, the treatment conditions that had the greatest impact on the functional and nutritional properties of the flours and that they should also be feasible at industrial level, were selected. Namely the application of a soaking pre-treatment of the grain and following the HHP processing conditions for 15 min and at 600 MPa, obtaining, in all cases, modified whole flours.

These flours were used on a basic gluten-free bread formulation made with refined rice flour, and the impact of applying different replacement percentages (15, 30, 50 and 70 %) was studied. For comparative purposes, and to assess the influence of these modified flours, baking products was also carried out by including, in the same proportions as above, a native whole buckwheat or rice flour. In the tests conducted, and in comparison with the formulations containing native flour, those products containing the treated flour resulted in higher specific bread volumes and lower crumb hardness values.

Finally, buckwheat hulls, a by-product generated from the processing of the grain into peeled kernels (groats) and flours, were evaluated as a food ingredient for incorporation into gluten-free bread formulations. This by-product has a high nutritional value due to its high content in fibre and bioactive compounds, so its use as an ingredient could improve the nutritional profile of the products in which it is used, favouring the circular economy as a sustainable production model. For this study, two different husk fractions of different particle size (D_{50} = 62.7 µm and 307 µm) were used and applied at two different rates (3 and 6 % w/w) to ensure the nutrition claims "source of fibre" and "high fibre" according to Regulation (EC) No 1924/2006 of 20 December 2006 on nutrition and health claims made on foods. In terms of bread quality, those products that incorporated the smaller particles (D_{50} = 62.7 µm) at an addition rate of 3% (w/w) did not result in a decrease in the physical and sensory properties of the product, with a higher overall acceptance of the product compared to the other ones made containing buckwheat hull particles.

On the basis of these results, this thesis confirms the capacity of HHP technology to obtain modified flours with high added value, characterised by techno-functional and nutritional properties adapted to the requirements of the gluten-free bakery industry and the celiac community. Furthermore, by controlling the processing parameters in APH treatments, it is possible to modulate the techno-functional response of the resulting flours. Among the conditions studied, the application of the HHP treatment of 600 MPa at times longer than 5 min on grains that have previously undergone a soaking treatment is of particular interest. Breads made with the flours resulting from these conditions, even with the lowest percentage of substitution studied (15 % w/w), showed a higher physical and sensory quality than their analogues made with untreated whole flours.

CONTENTS

I. INTRODUCTION 1

1.1. Background 2

1.2. HHP Technology: Principles, Fundamentals and Processing 4

1.3. Impact of HHP Treatments on the Main Biopolymers of Starchy Raw Materials 4

1.3.1. Effect of HHP Treatments on Starch 4

1.3.2. Effect of HHP Treatments on Protein 6

1.4. Impact of HHP Treatments on Techno-Functional Properties of Starch and Protein Biopolymers 6

1.4.1. Effect of HHP Treatments on Techno-Functional Properties of Starch 6

1.4.2. Effect of HHP Treatments on Techno-Functional Properties of Proteins 9

1.5. Impact of HHP Treatment in Complex Matrices 12

1.6. Impact of HHP Treatments on Dough Properties and Bread Quality 17

1.7. High Hydrostatic Pressure (HHP) as A Strategy to Enhance the Nutritional Value of Food Matrices 21

1.8. Valorization of by-products as heath promoting ingredients in gluten-free baking products 22

II. OBJETIVOS 25

II. OBJETIVES 29

III. MATERIALS AND METHODS 32

3.1 Materials 33

3.2 Experimental designs 33

3.3 High hydrostatic pressure treatment (HHP) and flour obtention 34

3.4 Methods 36

3.4.1 Particle size distribution of flours 36

3.4.2 Flour colour analysis 36

3.4.3 Scanning electron microscopy (SEM) 36

3.4.4 Techno-functional properties 36

3.4.5 Pasting properties 37

3.4.6 Thermal properties 37

3.4.7 Rheological properties of gels 37

3.4.8 Nutritional properties 38

3.4.8.1 Vitamin content 38
3.4.8.2 Mineral content 38
3.4.8.3 Total phenol content (TPC) and total antioxidant capacity (TAC) 38
3.4.9 Dough formulation and breadmaking procedure 39
3.4.10 Rheological properties of doughs 40
3.4.11 Evaluation of bread quality 40
3.4.12 Proximal Composition of Bread Samples 41
3.4.13 Sensory evaluation 41
3.4.14 Statistical analysis 41

IV. RESULTS AND DISCUSSION 42

CHAPTER 1:

4.1 Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles. 44
4.1.1 Particle size distribution of flours 44
4.1.2 Scanning electron microscopy (SEM) 46
4.1.3 Flour colour analysis 47
4.1.4 Functional properties. 48
4.1.5 Pasting properties 49
4.1.6 Rheological properties of gels 51
4.1..7 Thermal properties. 54
4.1.8 Total phenol and total antioxidant capacity 57
4.1.9 Minerals content 58

CHAPTER 2:

4.2 Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time. 61
4.2.1 Particle size distribution of the flours 61
4.2.2 Colour of the flours 62
4.2.3 Functional properties 62
4.2.4 Pasting properties 64
4.2.5 Thermal properties 67
4.2.6 Rheological properties of gels 70
4.2.7 Phenol content (TPc) and total antioxidant capacity (TAC) of flours 71

4.2.8 Rheological properties of doughs 73
4.2.9 Bread quality evaluation 76
4.2.10 Phenol content (TPc) and total antioxidant capacity (TAC) of breads 81
4.2.11 Sensory properties 83

CHAPTER 3:

4.3 Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours. Study of pre-soaking treatment and holding time as processing factors. 87

4.3.1 Granulometry and flour colour characteristics. 87
4.3.2 Scanning electron microscopy 88
4.3.3 Functional properties 91
4.3.4 Pasting properties 93
4.3.5 Rheological properties of gels 95
4.3.6 Nutritional properties of the flours 97
4.3.6.1 Vitamin and mineral content 97
4.3.6.2 Total phenol content and antioxidant capacity 100
4.3.7 Rheological properties of the doughs 102
4.3.8 Bread quality properties 105
4.3.9 Sensory evaluation 112

CHAPTER 4:

4.4 Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread 116

4.4.1. Rheological Properties of Doughs 116
4.4.2. Proximal Composition of Bread Samples 117
4.4.3. Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC) 118
4.4.4. Bread Microstructure 120
4.4.5. Bread Quality Evaluation 122
4.4.6. Sensory Evaluation 126

V. CONCLUSIONES 128
V. CONCLUSIONS 131

VI. REFERENCES 134

VII. APÉNDICE 154

LIST OF TABLES

I. INTRODUCTION

Table 1.1. Technological changes in starch promoted by high-hydrostatic-pressure treatments. 8

Table 1.2. Technological changes in proteins promoted by high-hydrostatic-pressure treatments. 11

Table 1.3. Technological changes on diverse, complex matrices (flours and grains) promoted by HHP treatments. 15

Table 1.4. Impact of HHP-treated ingredients on dough properties and bread quality. 19

IV. RESULTS AND DISCUSSION

CHAPTER 1: **Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles**

Table 4.1.1. Granulometry, colour difference, hydration, emulsion and foaming properties of flour samples obtained from native and HHP-treated buckwheat grains. 45

Table 4.1.2. Flour chromatic parameters and size dispersion of flour samples obtained from native and HHP-treated buckwheat grains. 48

Table 4.1.3. Pasting and rheology parameters of flour samples obtained from native and HHP-treated buckwheat grains. 53

Table 4.1.4. Thermal properties of flour samples obtained from native and HHP-treated buckwheat grains. 56

Table 4.1.5. Antioxidant properties and mineral content of flour samples obtained from native and HHP-treated buckwheat grains. 59

CHAPTER 2: **Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding tim**e.

Table 4.2.1. Particle size distribution and colorimetric parameters of flours samples obtained from native and HHP treated BW grains. 61

Table 4.2.2. Hydration, emulsion and foaming properties of flours samples obtained from native and HHP treated BW grains. 64

Table 4.2.3. Pasting properties of flours samples obtained from native and HHP treated BW grains. 67

Table 4.2.4. Thermal properties of flours samples obtained from native and HHP treated BW grains. 69

Table 4.2.5. Rheological properties of gel flours samples obtained from native and HHP treated BW grains. 71

Table 4.2.6. Phenol content (TP) and antioxidant capacity (TAC) of flours samples resulting from HHP-treatments and native BW grains. 73

Table 4.2.7. Rheological properties of gluten-free doughs as a function of BW flour replacement level and type of BW flour (native/modified) 75

Table 4.2.9. Bread quality properties of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified) 78

Table 4.2.10. Crumb grain features and crumb and crust colour parameters of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified). 80

Table 4.2.11. Total phenol content (TPc) and antioxidant capacity (TAC) of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified). 83

Table 4.2.12. Sensory properties of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified). 85

CHAPTER 3: **Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours. Study of pre-soaking treatment and holding time as processing facto**rs

Table 4.3.1. Particle size distribution and colorimetric parameters of flour samples obtained from native and HHP treated paddy rice grains. 88

Table 4.3.2. Functional properties (hydration, emulsion and foaming) of flour samples obtained from native and HHP treated paddy rice grains. 92

Table 4.3.3. Pasting properties of flour samples obtained from native and HHP treated paddy rice grains. 95

Table 4.3.4. Rheology properties of gel flour samples obtained from native and HHP treated paddy rice grains. 96

Table 4.3.5. Vitamin and mineral content of flour samples obtained from native and HHP treated paddy rice grains. 99

Table 4.3.6. Total phenol content (TPC) and total antioxidant capacity (TAC) of flour samples obtained from native and HHP treated paddy rice grains. 101

Table 4.3.7. Rheological properties of the gluten-free flour dough samples as a function of WR flour replacement level and type of WR flour (native/modified). 104

Table 4.3.8. Bread quality properties of gluten-free breads as a function of WR flour replacement level and type of WR flour (native/modified) 107

Table 4.3.9. Crumb grain characteristics and colorimetric parameters of the crust and crumb gluten-free bread samples as a function of WR flour replacement level and type of WR flour (native/modified). 110

Table 4.3.10. Sensory evaluation of gluten-free breads as function of WR flour (native/modified) replacement level (15% - 50%). 114

CHAPTER 4: **Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread**

Table 4.4.1. Rheological properties of gluten-free doughs. 116

Table 4.4.2. Proximal composition of gluten-free breads. 118

Table 4.4.3. Total phenol content and total antioxidant capacity of gluten-free breads. 119

Table 4.4.4. Bread quality properties of gluten-free breads. 122

Table 4.4.5. Crumb grain characteristics of gluten-free breads. 124

Table 4.4.6. Crumb and crust colour parameters of gluten-free breads. 125

Table 4.4.7. Sensory properties of gluten-free breads 127

LIST OF FIGURES

CHAPTER 1: **Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles**

Figure 4.1.1. SEM images of flour samples obtained from native and HHP-treated buckwheat grains: A: Native; B: 1C+S40; C: 2C+S40, at different magnifications: 6000x (1), 12000x (2) and 20000x (3). 47

Figure 4.1.2. Pasting profiles of flour samples obtained from native and HHP-treated buckwheat grains: 1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. 50

Figure 4.1.3. Effect of HHP treatment on viscoelastic moduli evolution vs frequency (Hz). Empty symbols represent the elastic modulus (G′); filled symbols represent the viscous modulus (G′′). 1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. 52

CHAPTER 2: **Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time.**

Figure 4.2.1. Pasting profiles of flour samples obtained from native and HHP treated BW grains. 0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min (A). 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min (B). 66

Figure 4.2.2. Photographs of bread loaves (top view) and slices of gluten-free breads made from 100% rice flour (R) and by replacing rice flour with native (N) or modified (M) BW flour at different replacement levels (1: 15%; 2: 30%; 3: 50%; and 4:: 70%) 81

Figure 4.2.3. Sensory properties of a 100% rice-based GF bread (100% RF) and different GF breads obtained by replacing rice flour with BW flour (native –N; modified -M) at different replacement levels (15%, 50%). 84

CHAPTER 3: **Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours. Study of pre-soaking treatment and holding time as processing factors**

Figure 4.3.1. Scanning electron microscopy (SEM) photomicrographs of whole rice flour samples obtained from native and HHP-treated grains at different magnifications: 3000x (1), 5000x (2) and 10000x (3). A: native flour; B, C: flours obtained from HHP treatment at 15 min of unsoaked and pre-soaked grains, respectively; D, E: flours obtained from HHP treatment at 30 min of unsoaked and pre-soaked grains, respectively. 90

Figure 4.3.2. Pasting profiles of flour samples A: Native and samples obtained from unsoaked HHP-treated paddy rice grains at 600MPa for 5, 15 and 15 min (5U, 15U and 30U). B: Native and samples obtained from pre-soaked and HHP-treated paddy rice grains at 600MPa for 5, 15 and 15 min (5S, 15S and 30S). 94

Figure 4.3.3. Photographs of bread loaves (top view) and slices of gluten-free breads made from 100% rice flour (100%RF) and by replacing rice flour with native (WRF Native) or modified (WRF HHP) WR flour at different replacement levels (15, 30, 50 and 70%). 112

Figure 4.3.4. Sensory properties of gluten-free breads according to the replacement level (15/50%) of the rice flour of the bread formula with whole rice flour (N-native; M-modified by HHP). A bread made with 100% rice flour (control) was used as reference. 113

CHAPTER 4: **Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread**

Figure 4.4.1. Scanning electron microscope images of the bread crumbs. (1a,2a): control bread sample; (1b,2b): bread containing coarse buckwheat hull particles at an addition level of 3%; (1c, 2c and 2d): breads containing coarse buckwheat hull particles at an addition level of 6%. 121

Figure 4.4.2. Images of the internal crumb structure of gluten-free breads. C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. 124

Figure 4.4.3. Effect of the addition of buckwheat hulls on the sensory properties of gluten-free breads. C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. 126

I. INTRODUCTION

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1.1. Background

Baking is one of the world's most popular processing methods for starchy staples because it imparts specific sensory characteristics to the final product, which are widely accepted by consumers. Among these, flavour, aroma and texture are the most important and characteristic. Wheat flour-based bakery products are obtained from doughs, which, due to their unique mechanical properties in terms of viscoelasticity, cohesiveness and extensibility, offer particular machinability and gas retention capacity during fermentation, which is key to the development of products such as leavened bread. The functionality of wheat dough is mainly dependent on the proportion of the gluten-forming proteins glutenins and gliadins and their interactions with other flour components (Taylor et al., 2016; Y. Zhou et al., 2021). The use of refined or white wheat flour in breadmaking is common because it results in breads that are more appreciated by consumers for their sensory properties (Kurek et al., 2017). Similarly, commercial gluten-free bakery products are also based on starches and flours, mainly maize and white rice, respectively (Roman et al., 2019).

Refined flours, on the other hand, are nutritionally poorer than their whole counterparts because the milling process removes the germ and the outer seed coat or bran, which contain valuable nutritional elements such as proteins, dietary fibre, fat, micronutrients and bioactive compounds (Khalid et al., 2017; J. Xu et al., 2019). Fortified gluten and gluten-free bakery products that meet health-conscious consumers' preferences are gaining a prominent place in the bakery market. It is widely recognised that fortified baked goods with nutrient-dense whole flours could be an effective strategy to meet the dietary requirements for fibre and other micronutrients generally limited in Westernised and celiac diets (Khalid et al., 2017; Kurek et al., 2017; Ktenioudaki & Gallagher, 2012; Torres et al., 2016). Sources of fortification can also be derived from minor cereals (Q. Wang et al., 2021), pseudocereals (Dega & Barbhai, 2023), legumes (Monnet et al., 2019) (Wandersleben et al., 2018) or other plant sources such as hemp (Mikulec et al., 2019). Furthermore, the use of uncommon crops for this purpose could be an interesting alternative for farmers due to the potential added value of these crops. Increasing agricultural diversity could be a step towards a healthier ecosystem, reduced agricultural economic volatility (Nelson & Burchfield, 2023) and more sustainable food chains (Monnet et al., 2019), thus contributing to improving system productivity and sustainability (Dwivedi et al., 2017).

However, this nutrient-enriching formulation strategy often results in lower sensory quality of the resulting breads (Kurek et al., 2017). Components such as phytic acid (Kurek et al., 2017), phenolic compounds (J. Xu et al., 2019) or dietary fibres (Y. Zhou et al., 2021) impair proper flour functionality and dough yield. Fibres are particularly detrimental, as they can act as water competitors, hampering the necessary dough hydration. This effect produces a negative effect on dough rheology, reducing the elastic properties and inducing a more viscous behaviour, which leads to weaker doughs (Khalid et al., 2017). It also alters the water availability for starch gelatinisation, limiting the granule starch swelling and amylose leaching, which affects the formation of a proper crumb structure (Ronda et al., 2017). In addition, the presence of phenolic acids in the fibres, such as ferulic acid, could alter the functionality of the gluten network by increasing the extensibility of the dough. (Khalid et al., 2017). The final product often shows undesirable sensory properties such as reduced brightness, lower specific volume and increased crumb hardness (Y. Zhou et al., 2021). Dosage level, particle size and botanical origin are conditions to be taken into account for the addition of fibre in baked product formulations (Gómez et al., 2003). In gluten-free (GF) bakery formulations, some adverse effects of the interaction of fibre with the gluten substitute used, usually hydrocolloids, should also be taken into account (Martínez et al., 2014).

Studies aimed at improving the quality of fortified bread have investigated various alternatives. One of them is the addition of improvers, such as vital gluten, in combination with surfactants with or without shortening (Shogren et al., 1981), a strategy that was not completely effective in counteracting loaf volume reduction and crumb grain impairment. The inclusion of natural materials, such as rosehip and cephalaria, has also been studied, with positive results in improving the rheological properties of whole wheat dough (Boz et al., 2010). Some positive results were also observed with the addition of soluble dietary fibres (SDF) compared to insoluble ones (IDF), such as longer dough development time (Gómez et al., 2003) or higher volumes in gluten (Dalgetty & Baik, 2006) and gluten-free bread (Martínez et al., 2014).

In recent years, an increasing number of studies on the application of non-thermal emerging technologies (e.g., ultrasound, non-thermal plasma, ozonation, ultraviolet light, pulsed light or high hydrostatic pressure) to improve the quality of food products of plant origin have been reported (Z. H. Zhang et al., 2019; Barbhuiya et al., 2021; Ding et al., 2021). In cereal-based matrices, these technologies can alter the main components of flour, protein and starch, enabling the production of physically modified ingredients (Han et al., 2020; Venkateswara Rao et al., 2021) . The functionality of the plant material resulting from these processing methods is drawing attention to the production of baked goods. These ingredients could be a promising alternative to chemical additives and thus help reduce or even eliminate the use of preservatives and other synthetic additives, facilitating the industry's goal of offering "clean label" products that are in high demand by conscious consumers looking for healthier diets and lifestyles (Barros et al., 2021).

High hydrostatic pressure (HHP), also known as cold pasteurisation, has been reported to exert significant effects on starch and protein polymers (Castro et al., 2020; Barros et al., 2021). This technology offers interesting benefits in the food industry, not only in terms of shelf life extension but also in terms of preserving the natural flavour and nutrient profile of the original food material (Amsasekar et al., 2022). In addition, compared to thermal treatments, this technology uses less energy, which means less environmental impact (Huang et al., 2016). HHP technology has also been proposed to improve textural properties, increase the bioavailability and bioaccessibility of bioactive compounds and minerals and reduce the risk of allergies in some food products (Barba et al., 2015).

There is now a growing body of research on HHP technology applied to cereal-derived matrices such as starch, flour or grains to physically modify their components and improve their native functionality (J. Zhang et al., 2022; Cappa, Lucisano, et al., 2016; Ahmed, Mulla, & Arfat, 2017; Gharibzahedi & Smith, 2021; Gutiérrez et al., 2022). Throughout the introduction of this doctoral thesis, an initial description of the HHP technology is given, followed by a detailed analysis of the effect of HHP application on the main flour constituents, starch and protein. The aim of this literature review was also to address changes reported in the literature on the techno-functional properties of HHP-modified biopolymers, as well as on more complex matrices that could be used as bakery ingredients of high nutritional interest. The impact of using HHP-modified ingredients on the dough and the resulting breads was also deeply analysed. Finally, some strategies to improve the value of the nutritional profile in terms of the content of bioactive compounds of starch-rich food ingredients through HHP technology and the use of by-products from crop waste of nutritional interest were also addressed.

1.2. HHP Technology: Principles, Fundamentals and Processing

The industrial HHP treatment process is generally carried out by placing the food to be treated in a hermetically sealed and flexible container and then introducing it into the pressure chamber. Once the processing conditions of pressure level (100–600 MPa) and holding time have been established, the pressure is built up by means of a pump and pressure intensifier and then transmitted to the food via a liquid transfer medium, usually water, that can be recycled after processing. Although it is considered a non-thermal treatment, adiabatic heating must be taken into account (Knoerzer, Buckow, et al., 2010), which is approximately 3 °C per 100 MPa for water (Patazca et al., 2007). In addition, combined pressure and temperature treatments can be carried out using temperature control devices and insulated vessels (Knoerzer, Smith, et al., 2010; Knoerzer, 2017; Barros et al., 2021).

The principles on which this technology is based are the isostatic principle, which assumes that the pressure is applied uniformly, instantaneously and homogeneously to the food, and Le Chatelier's principle, which refers to the application of pressure with an effect on volume leads to a change in the equilibrium of the system (Castro et al., 2020). As a consequence of the HHP treatment, the pressurised material may undergo phase transitions, changes in molecular configuration and chemical reactions (V. M. B. Balasubramaniam et al., 2015). On this basis, and depending on the processing conditions, food biomolecules are affected. The impact of pressure on proteins can cause unfolding, partial denaturation or changes in the electronic configuration of some amino acid side chains (W. Wang et al., 2022). In turn, HHP treatment on starch under certain conditions of pressure level, starch:water ratio and holding time can affect non-covalent interactions, leading to changes at the supramolecular level and, hence, on their techno-functional properties (Dominguez-Ayala et al., 2022). The following section describes the effect of HHP treatments on starch and protein, the two main biopolymers present in flours and cereal derivatives, in more detail.

1.3. Impact of HHP Treatments on the Main Biopolymers of Starchy Raw Materials

1.3.1. Effect of HHP Treatments on Starch

The emerging interest in the physical modification of native starches is based on the need to improve their functionality in baked goods with reduced chemical additive content (M. Liu et al., 2018; Perez-Rea & Antezana-Gomez, 2018). Non-thermal technologies, such as HHP, can meet this purpose for their ability to disrupt the granule crystallinity in the presence of water, enabling new functionalities together with the generation of new label-friendly ingredients (BeMiller, 2018). Depending on the botanical origin of the starch and its amylose content, the presence of water and the HHP processing conditions (pressure, holding time and temperature), the starch modification effect or the degree of gelatinisation achieved may be variable (V. M. Balasubramaniam et al., 2017). In order to understand the mechanisms underlying the impact of HHP on this biomolecule, a number of recent investigations have focused on this area.

At the atomic level, an investigation made using molecular dynamics simulation explored the changes induced in the starch molecule conformation at different levels of applied pressure (Zhi-guang et al., 2020). In that study, an increase in molecular stability was found as the fluctuation range (root mean square fluctuation) of the molecules decreased due to pressure. The authors also observed changes in the conformation of amylopectin and amylose with increasing pressure in terms of a reduction in the distance between the amylopectin chains and the two double amylose chains. They explained that this effect could be related to deformations (holes and cavities) on the starch granule surface promoted by the HHP treatment. With increasing pressure, they also reported antagonistic changes of non-covalent bonding forces at the level of supramolecular structure, resulting in the alteration of the native crystalline starch structure. This could be associated with changes in X-ray diffraction patterns (M. Liu et al., 2018) as well as with the disappearance of birefringence patterns (Castro et al., 2020). The influence of HHP treatment on the ordered state of crystallinity with different amylose/amylopectin ratios in maize starches has been investigated (Yang et al., 2016). A significant reduction in the SAXS (small-angle X-ray scattering) peak area of the waxy and normal maize starches compared to those with high amylose content (B-type) was observed. This higher resistance to compression of B-type starches has been attributed to the shorter amylose linkages, which leave less space for compression through the lamellar structure and limit the flexibility to absorb internal stresses. The more open helices arrangement of B-type starches allows larger amounts of water molecules accommodation (36 instead of 8 for A-type), resulting in stronger hydrogen bond networks to stabilise the helix structure against pressure forces. On the other hand, A-type starches have scattered branching points within the crystalline region, establishing "weak points" in the granular structure and making it more vulnerable. Therefore, the A-type structure typically presented on cereals (such as rice, corn and wheat) and pseudocereals (buckwheat) is more sensitive to being gelatinised by HHP treatment (Pei-Ling et al., 2012).

The process of water molecules entering and binding to starch molecules, together with the weakening of starch intramolecular hydrogen bonds, is driven by compressive forces once they exceed a certain threshold, allowing the existing structure to be disrupted and starch gelatinisation to begin. The effects of pressure on starch at a micron-size granule level have been extensively studied and are generally represented by a wide range of changes in the morphological and functional properties of starch granules, particularly in their swelling and solubilisation properties (Dominguez-Ayala et al., 2022; Yang et al., 2017). Similar to heat-driven gelatinisation, in which hydration, swelling of the amorphous region and loss of birefringence processes occur, in the pressure-driven gelatinisation process, the crystalline regions are prevented from melting because amylose helps to stabilise amylopectin, thus interrupting starch gelatinisation of the crystalline region and maintaining the granular conformation (Knorr et al., 2006). However, at high enough pressure, total gelatinisation can occur (W. Li et al., 2012), even for B-type starches (Yang et al., 2016). The extent of gelatinisation can be modulated by processing conditions (starch:water ratio, pressure level, holding time, temperature) (Yang et al., 2016), allowing intermediate levels of crystalline degradation or partial gelatinisation to be obtained, with different changes in starch functionality.

1.3.2. Effect of HHP Treatments on Protein

Studies on the effects of HHP treatments on biomolecules started in the 1960s and were focused on the impact on proteins, nucleoproteins and membranes of pressure-sensitive microorganisms (Gross & Jaenicke, 1994). The stability of biosystems under high pressure could be predicted by Le Chatelier's principle, as the application of pressure will shift the biosystem to a new equilibrium state occupying a smaller volume through molecular interactions (Mozhaev et al., 1994). The structural thermodynamic equilibrium of proteins depends mainly on three types of interactions: ionic, hydrophobic and hydrogen bonding. Ion pairs are strongly influenced by the pressure in an aqueous solution, resulting in the arrangement of water molecules in their vicinity due to the electrostriction compression effect. Hydrophobic groups can be similarly affected when the pressure level causes protein unfolding, exposing hydrophobic residues and facilitating interactions between them through van der Waals forces. In addition, the application of HHP has been related to the formation of hydrogen bonds with small changes in the activation volume (Gross & Jaenicke, 1994).

The effect of pressure on protein unfolding is different from that driven by temperature. The thermal process may completely and irreversibly unfold the protein, breaking covalent bonds and displacing non-polar hydrocarbons towards the solvent medium. On the other hand, pressure rarely alters covalent bonds but mainly affects the tertiary and quaternary structures of proteins. The pressure-unfolding mechanism begins when pressure forces induce water molecules to enter the interior of the protein, destabilising non-polar groups. The pressure sensitivity of proteins, therefore, depends on the conformational flexibility of their structure, which is maintained despite the loss of some non-polar domains due to the inclusion of water molecules (Knorr et al., 2006). It has been reported that at pressures above 200 MPa, changes occur in the protein structure of globulins, leading to aggregation as a result of protein–protein interactions. In contrast, below 200 MPa, only some tertiary and quaternary conformational changes occur, as these pressure levels affect weak bonds, such as van der Waals' forces, hydrophobic interactions and electrostatic and hydrogen bonds. Depending on the protein, pressure application above 300 or 400 MPa generally leads to irreversible denaturation. Therefore, according to the most abundant protein fraction, structural pressure-induced changes could have a major impact on protein functionality, with changes in solubility and hydration behaviour, interfacial activity and rheological properties (Barbhuiya et al., 2021).

1.4. Impact of HHP Treatments on Techno-Functional Properties of Starch and Protein Biopolymers

1.4.1. Effect of HHP Treatments on Techno-Functional Properties of Starch

Starch is a polymeric carbohydrate consisting of numerous glucose units linked by glycosidic bonds and is the most abundant and important carbohydrate in flours. Starchy foods are the primary source of carbohydrates for most people, and starch provides basic functionality for the development of common bakery products. However, native starches do not always offer the functionalities currently required for the food product development industry, especially in complex formulations where the use of chemical additives is reduced, or even clean-label food products are desired (BeMiller, 2018). In this context, there is growing interest in enhancing the functionality of starches through physical modification. Improvements in swelling, solubility or gelatinisation are the main areas of interest in starch modification. HHP technology has the ability to modify the structure of the starch molecule, facilitating water entry into crystalline regions due to the effect of pressure weakening the double helix (Ding et al., 2021). Pressure increases the water diffusion into the starch amorphous region, leading to crystal disruptions. However, pressure gelatinisation depends on extrinsic conditions such as starch type and hydration (W. Li et al., 2012) and intrinsic processing conditions such as pressure level, temperature and holding time (BeMiller, 2018). This pressure gelatinisation differs from heat-induced gelatinisation, in which the amylose and amylopectin molecules and residual granules are solubilised to form a starch paste (Angioloni & Collar, 2013). In contrast, in pressure-induced gelatinisation, the starch granules are deformed but retain their granular shape (Balakrishna et al., 2020). Table 1 shows the available results on the changes induced by HHP treatment on the techno-functional properties of plant starches according to the treatment conditions applied.

The degree of gelatinisation achieved by pressure treatment correlates with water-binding capacity (WBC), as reported by Rumpold and Knorr (2005), who observed that wheat, tapioca and starch suspensions (5%) increased their WBC with increasing pressure. When comparing fully gelatinised samples, the highest WBC was observed for tapioca starch, followed by potato, but at 450 MPa, the wheat starch sample showed the highest WBC because, unlike the other treated samples at that pressure level, it was completely gelatinised (Rumpold & Knorr, 2005). Increased water retention capacity with the pressure has also been reported for other starch sources, such as corn (Cappa, Lucisano, et al., 2016) and quinoa (Ahmed, Thomas, et al., 2018). The latter authors related this increase to the observed increase in damaged starch. They also stated that pressure-damaged starch was more easily swollen. However, the hydration behaviour of HHP-modified starch granules could be different depending on the test temperature. Li et al. (W. Li et al., 2012) reported higher swelling power and water solubility at 50–60 °C of rice starch treated with HHP (600 MPa for 30 min at room temperature) compared to native rice starch. However, at test temperatures above 70 °C, the HHP-treated rice starch showed an opposite effect with a decrease in swelling and solubility (W. Li et al., 2012). This behaviour could be caused by an aggregation of amylose molecules due to the effect of pressure, which favoured the promotion of lipid–starch associations at 50–60 °C, leading to an increase in water retention capacity and solubility. However, at higher temperatures, the ordered state of the rearranged amylose molecules may have prevented them from melting, limiting amylopectin swelling and amylose solubilisation. This behaviour was consistent with the findings for HHP-treated common buckwheat starch (H. Liu et al., 2016). However, this trend was not followed for quinoa and pea starch, where higher solubility was found at higher temperatures (Ahmed, Thomas, et al., 2018; M. Liu et al., 2018).

Variations in the pasting profiles of HHP-treated starches have also been reported in the literature, depending on the starch source and the treatment conditions. B-type diffraction pattern starches, such as those found in potatoes, were more resistant to pressure treatment (Colussi et al., 2018). Conversely, starches with A-type diffraction patterns, such as those of cereals, were more sensitive to pressure and showed significant changes in the pasting profile (W. Li et al., 2012). Li et al. (W. Li et al., 2012) reported different pasting profiles of HHP-treated rice starch depending on the pressure level. At pressures below 480 MPa, the peak, trough and final viscosities were higher than those observed for native rice starch. These authors associated the increase in these viscosities with the increase in the swelling power of these granules, which had a fragmented crystalline structure. However, a significant drop in maximum, minimum and final viscosity was also observed for starch samples treated with HHP at 600 MPa (W. Li et al., 2012). They explained that at this level of pressure, the amylose and lipid developed a helical complex that intertwined with the amylopectin molecules, limiting their ability to swell and preventing them from melting, improving their paste stability (W. Li et al., 2012). A similar pasting behaviour at 600 MPa was also observed for pea starch (M. Liu et al., 2018), corn and quinoa starch (G. Li & Zhu, 2018), as well as for buckwheat starch (H. Liu et al., 2016).

Table 1.1. Technological changes in starch promoted by high-hydrostatic-pressure treatments.

Botanical Starch Source	HHP Treatment Conditions	Effect on Techno-Functional Properties	References
Cereals			
Wheat	P: 0.1–500 MPa Time: 15 min T: 25–66 °C S/W: 5% (*w/w*)	Increased water-binding capacity.	Rumpold & Knorr, 2005
Barley	P: 400–550 MPa Time 0–75 min T: 30 °C S/W: 10% and 25% (*w/w*)	Increased gel consistency with the pressure and holding time (10%). G′ increased with the pressure (25%). G′ increased with longer holding times (25% *w/w*, P = 400–450 MPa).	Stolt et al., 2000
Corn	P: 400–600 MPa Time: 5–10 min T: 20–40 °C S/W: 40% (*w/w*)	Increased water and sodium carbonate retention capacity. Increased sucrose retention capacity. Decreased breakdown viscosity (P = 600 MPa). Increased setback viscosity (P= 600 MPa, 40 °C, time: 10 min).	Cappa, Lucisano, et al., 2016
Rice	P: 120–600 MPa Time: 30 min T: room temperature S/W: 20% (*w/w*)	Increased swelling power and solubility (P = 600 MPa, 50–60 °C). Decreased swelling power and solubility (P = 600 MPa, 70–90 °C). Increased peak, trough and final viscosities (P = 120–480 MPa). Increased pasting temperature and decreased peak, breakdown and setback viscosities (P = 600 MPa).	W. Li et al., 2012
Sorghum	P: 300–600 MPa Time: 10 min T: 20 °C S/W: 25% (*w/w*)	Increased complex viscosity values at the beginning of gelatinisation. Decreased breakdown viscosity (P > 300 MPa).	Vallons & Arendt, 2009b
Tubers			
Potato	P: 400–600 MPa Time10 min T: 21 °C S/W: 1:3 (*w/w*)	Increased peak and breakdown viscosities (P = 400 MPa). Increased final viscosity and peak time (retrograded samples for 7 days at 4 °C).	Colussi et al., 2018
Roots			
Tapioca	P: 400–600 MPa Time: 15–30 min T: 22–25 °C S/W: 1:3, 1:4 (*w/w*)	Increased G′ values with increasing the pressure level and concentration. Increased gel firmness with increasing the holding time. Increased mechanical strength with increasing the concentration.	Ahmed et al., 2014
Pseudocereals			

Quinoa	P: 300–600 MPa Time: 15 min T: 26 °C S/W: 1:3; 1:4 (*w*/*w*)	Increased water-holding capacity (P = 600 MPa). Increased water solubility index (from 1.99 to 3.45%). Increased G′ with increasing the pressure and starch concentration.	Ahmed, Thomas, et al., 2018
	P: 100–600 MPa Time: 5 min T: room temperature S/W: 10% (*w*/*v*)	Decreased water solubility index at 55–95 °C (P ≥ 500 MPa). Decreased swelling power at 75–85 °C (P ≥ 500 MPa). Increased swelling power at 55–65 °C (P ≥ 500 MPa). Decreased consistency coefficient (K) (P ≥ 500 MPa). Increased G′ and decreased G″ (P ≥ 500 MPa). Decreased pasting temperature (P = 600 MPa). Decreased peak viscosity (P ≥ 500 MPa). Increased peak viscosity (P = 500 MPa).	G. Li & Zhu, 2018
Buckwheat	P: 120–600 MPa Time: 20 min T: room temperature S/W: 20% (*w*/*v*)	Increased swelling power and solubility at 50–60 °C (P ≥ 360 MPa). Decreased swelling power at 70–90 °C (P ≥ 120 MPa). Decreased hardness, adhesiveness, gumminess and chewiness of starch gels. Increased pasting temperature and peak time. Decreased peak, breakdown and setback viscosities.	H. Liu et al., 2016
Legumes			
Pea	P: 150–600 MPa, Time: 25 min T: 30 °C S/W: 15% (*w*/*w*)	Increased water absorption and solubility index and swelling power. Increased peak, breakdown and setback viscosities (P = 150–450 MPa). Decreased peak, breakdown and setback viscosities (P = 600 MPa).	M. Liu et al., 2018

P: pressure; T: temperature; S/W: starch-to-water ratio.

1.4.2. Effect of HHP Treatments on Techno-Functional Properties of Proteins

As was previously explained, pressure forces can alter the protein functional groups by affecting their quaternary, tertiary and even secondary structure (Ahmed, Al-Ruwaih, et al., 2018). These modifications can lead to changes in water retention capacity, emulsifying and foaming properties and viscoelastic behaviour, which could be used to improve breadmaking performance in a similar way to chemical additives. Table 2 shows the findings reported in the literature on the techno-functional changes in proteins promoted by HHP treatments.

Different studies have reported an improvement in the hydration properties of HHP-treated proteins (Zhu et al., 2017; Ahmed, Al-Ruwaih, et al., 2018; Cao et al., 2018). An increase in the water-holding capacity of pine nut protein fractions (Cao et al., 2018) and kidney bean protein isolates (Ahmed, Al-Ruwaih, et al., 2018), as well as in the water absorption capacity of rice bran proteins (S. M. Zhu et al., 2017) with increasing pressure levels, has been observed, which has been attributed to protein unfolding. The loss of structure favoured the increase of exposed functional hydrophilic groups, providing more water-binding sites. In addition, this unfolding of proteins may also expose inaccessible hydrophobic groups from the protein core. This structural change may also improve the oil absorption capacity of proteins, as has been observed in HHP-treated rice bran proteins (S. M. Zhu et al., 2017) and pine nut proteins (Cao et al., 2018).

Protein solubility is one of the most important properties from a techno-functional point of view and plays an important role in other properties such as emulsion, foaming and gelling capacity (Yada, 2004). It has been reported that pressure, together with enzymatic hydrolysis, can reduce the size of peptides by breaking peptide bonds and thus increase solubility (Queirós et al., 2018). S. M. Zhu et al. (2017) observed significant changes in the solubility of isolated rice bran proteins associated with the pressure level applied, with a significant increase in solubility observed in samples pressurised between 100 and 200 MPa and a decrease at pressures above 200 MPa. It was suggested that the increasing result observed was due to the partial opening of protein structures at low pressure levels (S. M. Zhu et al., 2017). Similarly, an increase in the solubility of pine nut protein fractions was also observed with HHP treatment, particularly at 200 MPa. This effect was attributed to pressure-induced changes in the spatial structure of the proteins (Cao et al., 2018). However, higher pressures resulted in the formation of protein structures that hindered solubility (S. M. Zhu et al., 2017). Qin et al. (2013) and H. Li et al. (2012) attributed a decrease in solubility in walnut and soybean protein isolates for the generation of agglomerates produced at pressures above 400 MPa and 300 MPa, respectively.

Contradictory results have been reported on the effect of protein unfolding in relation to the foaming capacity of proteins after HHP treatment. S. M. Zhu et al. (2017) observed improvements in the foaming capacity of rice bran protein treated with HHP. The authors related this effect to an increase in surface hydrophobicity caused by the unfolding of protein. However, contrary results were observed for the foaming capacity after HHP treatments in kidney bean protein isolate (Ahmed, Al-Ruwaih, et al., 2018), pea protein isolate (Chao et al., 2018) and soybean protein isolate ($P > 300$ MPa) (H. Li et al., 2012). Chao et al. (2018) suggested that the decrease in foaming capacity is induced by pressure-mediated protein unfolding, which led to a reduction in protein flexibility and an aggregation between them, hindering their ability to encapsulate air bubbles.

Different results have also been reported for the emulsifying capacity of pressure-treated proteins, depending on the level of pressure applied. While at moderate pressures (100 MPa for rice bran protein and 200–400 MPa for bean protein isolate), the emulsion capacity increased; at higher pressures, there was no increase, or there was even a decrease in this property reported (Ahmed, Al-Ruwaih, et al., 2018; Zhu et al., 2017). Similar results were obtained with walnut (Qin et al., 2013) and soy protein isolates (H. Li et al., 2012), as a decrease in the emulsion activity index was observed with increasing pressure levels. These authors attributed the improvement in emulsifying at moderate pressure levels to an increase in the degree of protein unfolding, which could lead to a larger surface area for the oil/water interface. The observed decreases in emulsion stability were attributed to a decrease in the molecular flexibility of the proteins due to the formation of aggregates induced by disulphide bonds (Queirós et al., 2018; H. Li et al., 2012).

Regarding rheological properties, viscoelastic moduli of the HHP-treated wheat proteins showed different results. After HHP treatment at 500 MPa (60 °C), glutenin showed a two-fold increase in the elastic modulus (G'), whereas the viscoelastic moduli of gliadin decreased by approximately 50% (Kieffer et al., 2007). These results were attributed to the higher pressure sensitivity of glutenin compared to gliadin due to its higher thiol group content, which could increase disulphide cross-linking. This is in line with the findings of Cao et al., (2018) for pine nut proteins, where a higher viscoelastic modulus was observed with a pressure treatment of 400 MPa. These authors also associated the pressure-induced cross-linking with improvements in protein gel strength. In the study of (Kieffer et al. (2007), the pressure treatment at 400 MPa (40 °C) led to an increase in the resistance to extension and a decrease in extensibility of gluten at longer holding times.

Table 1.2. Technological changes in proteins promoted by high-hydrostatic-pressure treatments.

Protein Source	HHP Treatment Conditions	Effect on Techno-Functional Properties	References
Cereals			
Gluten, gliadin and glutenin	P: 0.1–800 MPa Time: 5–30 min T: 30–80 °C P/W: Hydrated in excess of water	Increased gluten resistance to extension and decreased extensibility (P = 400 MPa, 40 °C). Decreased gluten extensibility, cohesiveness and strength (P ≥ 600 MPa). Decreased G′ and G″ (gliadin) (P = 500 MPa). Increased G′ and G″ (glutenin) (P = 500 MPa).	Kieffer et al., 2007
Rice bran protein	P: 100–500 MPa Time: 10 min T: 20 °C P/W: 1% with phosphate buffer (50 mM, pH 7) (*w*/*v*)	Increased protein solubility (P ≤ 200 MPa/pH 2–3; pH 6–10). Decreased protein solubility (P > 200 MPa). Increased water absorption capacity (P = 500 MPa). Increased oil absorption capacity (P = 200 MPa). Increased foam capacity (P = 500 MPa). Increased foam stability. Increased emulsifying activity (P = 100 MPa). Increased emulsifying stability (P ≤ 400 MPa). Decreased least gelation concentration (P = 200 MPa).	S. M. Zhu et al., 2017
Tubers			
Potato protein concentrate (PPC) and isolate (PPI)	P: 200–600 MPa Time: 10 min T: 20–40 °C P/W: 1% (*w*/*w*) pH 6/7 with 0.1 M hydrochloric acid/sodium hydroxide	Decreased PPC (P > 400 MPa, 40 °C and pH 7) and PPI solubility (P ≥ 400 MPa, pH 6). No change in the time required for foam formation. Increased foam instability.	Baier & Knorr, 2015
Sweet potato protein	P: 400 MPa Time: 30 min T: 25 °C P/W: 4% with Tris-HCL buffer (50 mmol·L^{-1}, pH 7) (*w*/*w*) With or without addition of salts (NaCl, $MgCl_2$, $CaCl_2$)	Increased G′ with the addition of salts. Increased water-holding capacity (WHC) with NaCl. Decreased WHC with $MgCl_2$ and $CaCl_2$.	Zhao et al., 2018
Legumes			
Kidney bean protein isolate	P: 200–600 MPa Time: 15 min T: 23 °C P/W: 1:4; 1:5 (*w*/*w*)	Increased water-holding capacity. Decreased foaming capacity (from 76.7 to 42.1%). Increased emulsifying activity and stability. Increased elastic-like rheological behaviour with increasing protein concentration.	Ahmed, Al-Ruwaih, et al., 2018
Pea protein isolate (PPI)	P: 200–600 MPa Time: 5 min	Decreased oil droplet size (better emulsion quality) (P = 600 MPa, pH 3).	Chao et al., 2018

	T: 23 °C P/W: 1%, PPI: phosphate buffer (pH 7) (*w*/*v*)	Increased emulsion stability (P = 600 MPa, pH 3 and pH 7). Increased foaming capacity.	
Soy protein isolate (SPI)	P: 200–500 MPa Time: 15 min Time: 5–20 min (300 MPa) T: 20 °C P/W: 1% (*w*/*v*) (pH 6.8)	Increased solubility, water-holding capacity, emulsion activity index and foam capacity (P = 200–300 MPa and 5–15 min). Decreased solubility, water-holding capacity, emulsion activity index and foam capacity (P > 300 MPa and 20 min at P = 300 MPa. Decreased emulsion stability index and foam stability.	H. Li et al., 2012
Nuts			
Pine nut protein isolate	P: 100–400 MPa Time: 10 min T: 20 °C P/W: 10% (*w*/*v*)	Increased solubility (P = 200 MPa). Increased water- and oil-holding capacity (P = 400 MPa). Increased gel consistency (G*) (P = 100/400 MPa). Increased gel resistance to the strain (P = 400 MPa).	Cao et al., 2018
Walnut protein isolate	P: 300–600 MPa Time: 20 min T: room temperature P/W: 1% with phosphate buffer (0.2 mol·L^{-1}, pH 8) (*w*/*v*)	Decreased solubility with increasing pressure. Increased foaming capacity and foaming stability. Increased emulsion activity index (P ≤ 400 MPa). Decreased emulsion activity index (P > 400 MPa). Decreased emulsion stability index.	Quin et al., 2013

P: pressure; T: temperature; P/W: protein-to-water ratio.

1.5. Impact of HHP Treatment in Complex Matrices

As shown in the previous section, depending on the processing conditions, the HHP technology could produce functionalities in starch and proteins that could be of interest for improving baking performance. As a complement to the HHP treatments carried out on these biopolymers, it is also interesting to develop treatments for more complex matrices such as flours, where starch and protein are complemented by other constituents such as fibre (Y. Zhou et al., 2021). In these systems, the effects of HHP treatments on their functional properties are determined by their complex composition and differ from those achieved by treatments on isolated polymers. Ahmed et al. (2007) found that the protein-free rice starch suspension was completely gelatinised at 550 MPa, whereas its rice flour counterpart required 650 MPa for the same holding time. N. Sharma et al. (2018) reported that the presence of protein could decrease the degree of starch gelatinisation. This could be due to the effect of water competition between starch and protein, leaving less water available for starch gelatinisation by HHP (Balakrishna et al., 2020). In addition, this effect could be even more prominent if the HHP-modified protein had increased its water-binding capacity (Zhu et al., 2017; Ahmed, Al-Ruwaih, et al., 2018). Similarly, the presence of fibre has been shown to reduce starch gelatinisation (Santos et al., 2008). Therefore, since complex formulations, such as flours, exhibit changes in their functional behaviour due to possible interactions between their components (Collar et al., 2006; Champenois et al., 1998; N. Xu et al., 2021), several studies have been carried out to elucidate the techno-functional response of HHP application in these matrices. Table 3 shows the HHP impact on the techno-functional properties of the resulting flours.

For pressure-treated whole wheat flour and jasmine rice flour, a linear increase in water-holding capacity was found with increasing pressure level and flour-to-water ratio (from 1:1 to 1:4; *w/w*) as was shown by Ahmed, Mulla, & Arfat (2017) and Ahmed, Mulla, Arfat, et al. (2017) respectively. In addition, an increase in the water-holding capacity of non-hydrated wheat flour (14.6% moisture content) was also observed with increasing pressure and holding time (Jakobi et al., 2018). In these reports, changes in hydration behaviour were attributed to alterations in particle size due to HHP treatment, with an increase in the surface area as a reduction in particle size was observed. Ahmed, Mulla, & Arfat (2017) also suggested that pressure favoured damaged starch granules, thus facilitating their swelling. In other studies in which a pressure treatment was applied to pre-soaked grains of whole grain suspensions of brown rice and buckwheat, an increased water absorption capacity of the resulting flours was also found (Zhu et al., 2016; Gutiérrez et al., 2022). In those studies, it was suggested that the HHP treatment might have increased the hydrogen bonds between water and starch molecules, thus increasing their water absorption capacity. However, in contrast to the results reported by Gutiérrez et al. (2022), S. M. Zhu et al. (2016) observed a decrease in the swelling power of brown rice flour samples measured at 50 °C. This decrease was attributed to the presence of dispersed fibres partially destroyed by the pressure treatment which inhibited the swelling of the brown rice flour.

Unlike other reports (Zhu et al., 2017; Ahmed, Al-Ruwaih, et al., 2018; Chao et al., 2018), a decrease in the foaming and emulsifying properties of the resulting buckwheat flours was observed after HHP treatments on whole grains. As a result, it was proposed that the application of pressure promoted changes, leading to a loss of surfactant properties. The authors suggested that it could be related to changes in the distribution patterns of hydrophilic/hydrophobic groups of proteins (Gutiérrez et al., 2022). It has been reported that HHP may alter the balance of non-covalent bonds, increasing the exposure of functional groups such as disulphide groups. This could lead to the stretching of the protein molecules (Cao et al., 2018), reducing their flexibility with the cross-linking of disulphide bonds and losing efficacy in emulsion formation, as stated by Cabra et al. (Cabra et al., 2008).

Numerous reports have shown that the impact of HHP treatments led to an overall modification in the pasting viscosity profiles of the flours. A reduction in peak, breakdown and setback viscosities in HHP-treated flours has been reported for wheat (McCann et al., 2013; Ahmed, Mulla, & Arfat, 2017; Angioloni & Collar, 2012b), rice (Ahmed, Mulla, Arfat, et al., 2017), waxy rice (Cappa, Lucisano, et al., 2016), sorghum (Angioloni & Collar, 2012b) and buckwheat (Gutiérrez et al., 2022). In pressure-treated legume flour, a decrease in pasting temperature has also been reported for green pea and chickpea samples, but it was not always possible to obtain an RVA profile as it depends on the starch content of the sample (Angioloni & Collar, 2013). Hence, HHP treatments may lead to changes in the starch molecules that would be detected in RVA tests. The extent of these changes would be associated with the mechanisms that facilitate pressure gelatinisation and the HHP treatment conditions. Thus, if a pressure level threshold is not reached, the gelatinisation process will not occur (Vallons et al., 2011). In addition, the presence of water is also required (Balakrishna et al., 2020). Therefore, the higher the pressure and water availability, the higher the degree of gelatinisation that can be achieved (McCann et al., 2013), as this allows the infiltration of water into the starch molecule, leading to a partial gelatinisation of the inner regions of the starch granule (Angioloni & Collar, 2012b). The degree of gelatinisation achieved could be measured by a decrease in enthalpy. However, in composite matrices, it has been observed that the pressures required in flour are higher than in starch to induce enthalpy changes (McCann et al., 2013). S. M. Zhu et al. (2016) have suggested that the decrease in enthalpy should be attributed to a combination of starch gelatinisation and protein unfolding. The decrease in peak viscosity observed in flour samples could also be a characteristic of the presence of pre-gelatinised starch (McCann et al., 2013). Furthermore, it has been suggested that pressure gelatinisation induces changes in the helical structure of the amylose and amylopectin branches, which could lead to a restriction in amylose leaching due to a reinforcement of the granular structure (McCann et al., 2013). This might explain the decrease in breakdown viscosity values mentioned above, as the destabilisation effect on the crystallite and subsequent melting in the amorphous region would be reduced (Angioloni & Collar, 2012b). Complementary, Cappa, Lucisano, et al. (2016) observed that HHP treatments seemed to have a greater effect on those samples with high amylose content, as the higher pasting temperature was attributed to the more compact starch structure generated by the HHP treatment. Similarly, in the study by Gutiérrez et al. (2022), the reduction in breakdown viscosity values was attributed to a reinforced crystalline structure due to possible starch protein/fibre entanglement. Consequently, these authors ascribed the reduction in setback viscosity to a decrease in amylose leaching through this reinforced structure, allowing a lower content of free amylose molecules to be further retrograded.

Although a wide variability of results has been observed when analysing the rheological properties of gels made from HHP-treated flours by oscillatory measurements, an overall increase in the elastic modulus (G′) has been observed in wheat (Ahmed, Mulla, & Arfat, 2017), oat (Hüttner et al., 2009a), rice (Ahmed et al., 2007), buckwheat and tef (Vallons et al., 2011) and chickpea flours (Alvarez et al., 2014a; Alvarez et al., 2015). The increase in G′ with pressure has been attributed to the partial gelatinisation effect combined with protein aggregation (Ahmed, Mulla, & Arfat, 2017; Deora et al., 2014). Some authors have also pointed out the importance of the flour-to-water ratio (F/W) in increasing the mechanical strength of the gel. Ahmed, Mulla, & Arfat (2017) suggested that the higher complex viscosity (η*) values observed at higher concentrations of whole wheat flour were caused by higher molecular interactions and a strengthened structure with increasing pressure. Similar conclusions were reached by Ahmed et al. (2007) and Alvarez et al. (2014) when they observed the same trend in basmati rice flour and chickpea slurries, respectively. However, Hüttner et al. (2009a) attributed the increase in G′ observed in the oat dough treated at pressures above 350 MPa mainly to the swelling of the starch granules. On the other hand, a decrease in viscoelasticity was observed in a Thai jasmine rice flour dispersion at 600 MPa with increasing F/W ratio from 1:1 to 1:4. The plasticising effect of the rice flour was attributed to an increased shear-thinning behaviour with increasing pressure (Ahmed, Mulla, Arfat, et al., 2017).

Table 1.3. Technological changes on diverse, complex matrices (flours and grains) promoted by HHP treatments.

HHP-Treated Complex Matrix	HHP Treatment Conditions	Effect on Techno-Functional Properties	References
Cereals			
Wheat flour	P: 200–600 MPa Time: 5 min T: 25 °C F/W: 33–56% of moisture content	Decreased pasting profile viscosities (56%).	McCann et al., 2013
	P: 0.1–600 MPa Time: 10 min T: room temperature F/W: 14.6% of moisture content	Increased water retention capacity (from 65.68 to 73.77%).	Jakobi et al., 2018
Wheat flour (whole)	P: 300–600 MPa Time: 10 min T: 26–38 °C F/W: 1:1; 1:2; 1:3, 1:4 (*w/w*)	Increased water-holding capacity. Increased water solubility index. Increased texture hardness and decreased stickiness. Decreased peak, breakdown and final viscosities (1:2, *w/w*). Increased G′.	Ahmed, Mulla, & Arfat, 2017
Wheat/oat/millet and sorghum flours	P: 0.1–500 MPa Time: 10 min T: 20 °C F/W: 1:0.6 and 1:1 (*w/w*)	Decreased peak viscosity values (wheat, oat, sorghum). Decreased breakdown and setback viscosities (wheat and sorghum). Increased breakdown and setback viscosities (oat).	Angioloni & Collar, 2012b
Oat flour	P: 200–500 MPa Time: 10 min T: 20°C F/W: 1:0.95 (*w/w*)	Increased extension of the linear viscoelastic region. Yield stress increased. Decreased loss tangent (P ≥ 350 MPa).	Hüttner et al., 2009a
Rice and waxy rice flours	P: 400–600 MPa Time: 5–10 min T: 20–40 °C F/W: 40% of moisture	Decreased peak viscosity (rice flour). Increased pasting temperature (P = 600 MPa.) Decreased peak viscosity (rice flour). Decreased breakdown viscosity.	Cappa, Lucisano, et al., 2016
Basmati rice flour	350–650 MPa 7.5–15 min T: 22–26 °C F/W: 1:2, 1:3, 1:5 (*w/w*)	Increased G′ (with pressure and holding time). Increased gelatinisation degree.	Ahmed et al., 2007
Thai jasmine rice flour	P: 300–600 MPa Time: 10 min T: 25 °C F/W: 1:1, 1:3, 1:4 (*w/w*)	Increased water absorption capacity and solubility index (with increasing the flour-water ratio and the pressure level). Decreased peak viscosity (F/W = 1:2). Decreased pasting temperature (P ≥ 400 MPa). Decreased trough and breakdown viscosities. Decreased setback viscosity. Increased G′ (F/W = 1:3, *w/w*). Increased complex viscosity (η*) values.	Ahmed, Mulla, Arfat, et al., 2017

Brown rice grain	Pre-soaking (30 °C, 3 h) P: 200–500 MPa Time: 5–15 min T: room temperature G/W: 1:1.6 (*w/v*)	Increased water absorption (from 6.2 to 21.3%). Decreased swelling power (50 °C). Increased swelling power (70 °C). Increased solubility values (70–90 °C).	S. M. Zhu et al., 2016
Sorghum flour	P: 200–600 MPa Time: 10 min T: 20 °C F/W: 40% (*w/w*)	Increased complex viscosity (P > 300MPa). Decreased complex viscosity (P < 300 MPa). Increased loss tangent (P = 200–300 MPa). Decreased loss tangent (600 MPa).	Vallons et al., 2010
Pseudocereals			
Buckwheat and tef flour	P: 200–600 MPa Time: 10 min T: 20 °C F/W: 40% (*w/w*)	Increased pasting temperature (tef batters at 400 MPa). Decreased breakdown and setback. Increased complex modulus and decreased loss tangent (tan δ) (buckwheat). Decreased complex modulus and increased tan δ (tef batters up to 200 MPa). Increased complex modulus and decreased tan δ (tef batters at >200 MPa).	Vallons et al., 2011
Unhulled buckwheat grains	Pre-soaking of the grains (40 °C, 4 h) P: 600 MPa Time: 30 min (1 cycle), 15 min (2 cycles) T: room temperature G/W: 1:4; (*w:w*)	Increased water absorption capacity (12%) and swelling power (pre-soaking). Decreased emulsifying and foaming capacities and stabilities. Decreased peak (18%), breakdown (93%) and setback (29%) viscosities (with pre-soaking and 1 cycle). Decreased complex modulus (1 cycle and pre-soaking).	Gutiérrez et al., 2022
Legumes			
Chickpea, green pea and soybean flours *.	P: 200–450 MPa Time: 10 min T: 20 °C F/W: 1:0.6, 1:1 (*w:w*)	Decreased breakdown viscosity (chickpea and green pea; F/W = 1:1). Increased peak viscosity and holding strength (chickpea and green pea). Decreased pasting temperature (chickpea and green pea). * The soybean flour samples did not allow a regular RVA profile to be obtained due to the low starch content.	Angioloni & Collar, 2013
Chickpea flour	P: 150–600 MPa, Time: 15 min T: 25 °C F/W: 1:2, 1:3, 1:4, 1:5 (*w:w*)	Increased G″ (P = 600 MPa). Increased G′ values (1:2 and 1:3, P = 600 MPa). Decreased loss tangent (1:2, P = 600 MPa).	Alvarez et al., 2014
	P: 200–600 MPa Time: 5–25 min T: 10–50 °C F/W: 1:5 (*w:w*)	*For heat-induced gels at 75 °C:* Increased G′ and G″ at 50 °C, P = 600 MPa, 5 min Increased G′ at 10 °C, P = 200 MPa, 25 min Decreased G′ and G″ for the rest of HHP treatment conditions *For heat-induced gels after storage 1 week (4 °C):*	Alvarez, Fuentes, et al., 2015

	Increased G' and G'' at 10 °C, P = 200 MPa, 5–15 min; ii. P = 400 MPa, 5–25 min. Increased G' and G'' at 25 °C, P = 200 MPa, 25 min. Increased G' and G'' at 50 °C, P = 200–400 MPa, 25 min. Increased G' at 25 °C, i. P = 200 MPa, 15 min; ii. P = 400 MPa, 5 and 25 min. Increased G' and G'' at 50 °C, 200 MPa, 15 min Decreased G' and G'' for the rest of HHP treatment conditions

P: pressure; T: temperature; F/W: flour-to-water ratio; G/W: grain-to-water ratio.

1.6. Impact of HHP Treatments on Dough Properties and Bread Quality

A number of investigations have been carried out in order to determine the functionality of the HHP-modified ingredients as structure-promoting agents. These include empirical and fundamental rheological tests in doughs that were performed to collect measures such as dough consistency, extensibility, stickiness and/or cohesion, as these properties are closely related to bread quality, particularly in gluten-free formulas (Ronda et al., 2017). Furthermore, the impact of this physically modified ingredient on leavened bread quality parameters such as specific bread volume, crumb texture or bread staling has also been assessed. The main effects of HHP-treated ingredients on dough rheology and bread quality are summarised in Table 4.

It has been reported that HHP can modify the strength of gluten (Kieffer et al., 2007; Bárcenas et al., 2010). Therefore, HHP treatments could improve the functionality of wheat flours with poor breadmaking properties (McCann et al., 2013). This technology has been proposed to improve bread quality in wheat-based formulations with high-fibre ingredients that are prone to promote detrimental effects (Barros et al., 2021). Insoluble fibres lead to physical disruption of the gluten network (Y. Zhou et al., 2021) or create break points where gas can more easily escape during proofing (Martínez et al., 2014).

The importance of applying appropriate HHP conditions to induce higher functionality is of great relevance as numerous studies have found opposite effects. It has been reported that increasing the pressure level and/or holding time increased the viscoelastic modulus of HHP-treated wheat-based cake batter (Barcenilla et al., 2016). Similarly, Angioloni & Collar (2012b) observed significant increases in the storage and loss modulus of doughs containing wheat flours treated with HHP at 350 MPa and above (50% of replacement level). Similar increases were also reported using GF flour (oats, millet and sorghum) treated at 500 MPa for replacement wheat flour between 40 and 60%. In large deformation mechanical tests, wheat-based doughs containing HHP-treated flours resulted in increasing values in hardness and adhesiveness at 150 MPa (Bárcenas et al., 2010) or at 500 MPa (Angioloni & Collar, 2012b). These authors also reported a loss in dough cohesiveness, an increase in resistance to extension and a decrease in the dough extensibility. Rheological changes could be a consequence of HHP-induced structural changes in starch and protein, such as starch pre-gelatinisation and gluten strengthening through disulphide bond formation (Zannini et al., 2012; Angioloni & Collar, 2012b). Therefore, HHP conditions could lead to an overstructuring effect of the combined action of both structural changes. Kieffer et al. (2007) reported higher resistance to extension in gluten samples at high pressures (800 MPa) and temperature conditions (60 °C). This increased resistance could hinder the machinability of the doughs (Angioloni & Collar, 2012b). In this regard, other authors have reported that wheat-based breads containing HHP-modified wheat flour or other cereals such as oats, millet and sorghum showed some detrimental characteristics such as a reduction in specific volume (Bárcenas et al., 2010), increase in crumb hardness and also a loss in cohesiveness (Angioloni & Collar, 2012a).

Angioloni and Collar explored the effect of HHP treatments on legume flours for the possibility of favouring the formation of a protein network through new bonds (e.g., disulphide bonds) despite the generally low methionine, cysteine and tryptophan content of these flours (Angioloni & Collar, 2013; Collar & Angioloni, 2017). They observed promising structuring effects in HHP treatments (≥350 MPa) on more hydrated legume flour batters (1:1, compared to 1:0.6; *w*/*w*). These effects were related to the formation of structure-promoting disulphide bonds and the formation of urea-insoluble aggregates, in agreement with the observations of Hüttner et al. (2009a). The resulting wheat-based breads containing HHP-treated legume batters also showed a decrease in specific volume and a noticeable increase in crumb hardness and staling rate. However, with the addition of hydrocolloid (3% of CMC), not only was the hardening of the breads reduced but also the firming kinetics and overall acceptability were closer to those legume breads used as controls (without HHP treatment), which were highly acceptable (Collar & Angioloni, 2017).

Matsushita et al. (2020) reported a significant ($p < 0.05$) increase in the specific volume of strong wheat-based breads obtained by a combined action of HHP treatment (43 MPa) and enzyme supplementation (0.2%) on doughs. Those results were attributed to the action of the enzymatic bakery mixture of α-amylase and hemicellulose, which degraded damaged and gelatinised starch and pentosane, improving gas retention during proofing. In addition, the catalytic activity of the enzymes was improved by HHP treatment at 43 MPa. These breads also had a softer crumb texture compared to a control bread at each storage time measured (1–3 days). Therefore, the combined action of low-pressure treatment and enzymes could be an effective tool to overcome the increase in staling reported in other studies where breads were made with HHP-treated wheat flours (Rumpold & Knorr, 2005).

It has been suggested that structure strengthening could be a valid tool to improve the baking performance of gluten-free flour matrices (Angioloni & Collar, 2012b). In these terms, starch pre-gelatinised by HHP treatments has been proposed as a structuring agent (Zannini et al., 2012). Some promising investigations have been carried out for developing GF breads using HHP-modified flours. Studies have reported improved bread quality properties using GF flours HHP-treated at low pressure levels. Hüttner et al. (2010) observed significant increases in bread-specific volume and softer crumb hardness compared to the control and those obtained at higher pressures of breads made with replaced oat flour (10%) with an HHP-treated one at 200 MPa. Similarly, the use of HHP-treated sorghum flour (200 MPa) at the same replacement level had no adverse effect on bread properties (Vallons et al., 2010). In both investigations, significant increases in the elastic solid behaviour of the doughs were observed with HHP treatments above 350 MPa (Hüttner et al., 2010) and 400 MPa (Vallons et al., 2010). The increase in dough consistency at high pressure levels, which could impair proper bread development, was attributed by Hüttner et al. (2010) to the combined action of protein network formation and starch gelatinisation. However, Vallons et al. (2010)

attributed the strengthening effect mainly to the starch gelatinisation since the rheological test carried out on batters with the addition of NEM (N-ethylmaleimide solution) as a thiol exchange inhibitor had little effect on the rheological properties of the doughs. Conversely, a weaker batter structure was found at 200 MPa. To explain the opposite results found in the batter consistency at 200 MPa of the HHP treatment, both investigations attributed the structural changes occurring in the proteins at this pressure level either to a depolymerisation of the protein (Vallons et al., 2010) or to the weakening of electrostatic and hydrophobic bonds (Hüttner et al., 2010). Hüttner et al. (2010) also suggested that the weakened protein structure did not alter the uniform starch gel network developed during baking. In addition, the modified protein might have improved its foaming properties, as observed by Chao et al. (2018), leading to better textural properties of the crumb. Contrary to these studies, the application of HHP treatments at 600 MPa to maize starch or rice flour in a GF bread formulation (with the addition of structuring agents such as HPMC and psyllium) did not impair the bread quality characteristics, as a low specific volume loss (5–7%) was found, and a softer crumb texture was observed. A significant delay in staling was also found (Cappa, Barbosa-Cánovas, et al., 2016a).

Table 1.4. Impact of HHP-treated ingredients on dough properties and bread quality.

HHP-treated Ingredient	HHP Treatment Conditions	Effect on Dough Properties	Effect on Bread Quality	References
Wheat-based formula				
Wheat starch	P: 600 MPa Time: 15 min T: room temperature S/W: 5% (*w/w*)		Slightly increased firmness and decreased elasticity after storage (5 days).	Rumpold & Knorr, 2005
Wheat flour	P: 50–250 MPa Time: 1–4 min T: room temperature F/W: wheat flour mixed with water up to 500 BU	Increased dough hardness and adhesiveness (P ≥ 100 MPa).	Bigger gas cells with an uneven distribution. Decreased specific bread volume. Increased crumb hardness. Increased moisture content. Reduced luminosity, a* and b* of crust and crumb.	Bárcenas et al., 2010
Wheat flour	P: 200–600 MPa Time: 5 min T: 25 °C F/W: 1:1, 1:2 (*w/w*) (33% and 56% of moisture content)	*At 33% of moisture content:* Increased dough strength. Higher development time and stability (P ≥ 400 MPa).		McCann et al., 2013
Wheat flour	P: 0–100 MPa Time: 10 min T: room temperature F/W: wheat flour mixed with water up to 500 BU	*Doughs containing 0.2% bakery enzyme and HHP-treated flour at 43 MPa:* Increased gas retention.	*Doughs containing 0.2% of bakery enzyme and HHP-treated flour at 43 MPa:* Increased specific bread volume.	Matsushita et al., 2020

			Decreased bread crust luminosity and changed the colourimetric parameters. Improved breadcrumb structure. Reduced hardening kinetics of bread crumbs from dough containing enzymes and HHP-treated flour. Weakened gluten network in breads made from HHP-treated flour without the bakery enzyme.	
Oat, millet, sorghum and wheat flour	P: 200–500 MPa Time: 10 min T: 20 °C F/W: 1:1 (*w/w*)	*At 500 MPa:* Increased dough hardness and resistance to extension. Decreased dough cohesiveness (wheat and millet). Decreased extensibility (wheat). Increased viscoelastic moduli (G′ and G″).	*At 350 MPa:* Decreased specific bread volume and crumb cohesiveness. Increased crumb hardness (sorghum flour). Increased overall acceptability (wheat, millet and sorghum flours). Decreased bread staling (wheat and oat flours). Increased bread staling (millet and sorghum flours).	Angioloni & Collar, 2012b; Angioloni & Collar, 2012a
Chickpea, greenpea and soybean flours	P: 200–450 MPa Time: 10 min T: 20 °C F/W: 1:0.6 (*w/w*) F/W: 1:1 (*w/w*)	*At P ≥ 350 MPa (1:1, w/w):* Increased retardation time (chickpea and soybean flours). Decreased instantaneous compliance and increased zero shear viscosity. Reduced stickiness (chickpea and green pea flours).	*At 350 MPa (1:1, w/w):* Increased the initial crumb hardness. Decreased specific bread volume. Increased crumb firming kinetics.	Angioloni & Collar, 2013; Collar & Angioloni, 2017
Gluten-free formula				
Oat flour	P: 200–500 MPa Time: 10 min T: 20 °C F/W: 1:0.95 (*w/w*)	*At 10% of replacement level and at P ≥ 350 MPa:* Decreased loss tangent.	*At 10% of replacement level and at 200 MPa:* Increased specific bread volume. *At 40% of replacement level and at 500 MPa:* Decreased specific volume. *At 10, 20 and 40% of replacement level and at 200 MPa:*	Hüttner et al., 2010

		Decreased crumb hardness at day 5.	
Corn starch, rice flour	P: 600 MPa Time: 5 min T: 40 °C F/W: 1:0.5 (*w*/*w*)	Lower crumb water activity. Decreased specific bread volume. Changes in a* Decreased breadcrumb hardness. Increased moisture retention. Decreased crumb hardness at 24 and 72 h.	Cappa, Barbosa-Cánovas, et al., 2016a
Sorghum flour	P: 200–600 MPa Time: 10 min T: 20 °C F/W: (40% *w*/*w*)	*At 10% and at 600 MPa*: Decreased specific bread volume. *At 2% and 600 MPa:* Decreased crumb hardness after 72 h.	Vallons et al., 2010

P: pressure; T: temperature; S-F/W: starch/flour-to-ater ratio.

1.7. High Hydrostatic Pressure (HHP) as A Strategy to Enhance the Nutritional Value of Food Matrices

The use of staple foods as vehicles for dietary micronutrient fortification is widely used as a public health strategy to meet some nutritional needs of the population. Examples include the fortification of white rice, wheat and maize flours. In some countries, fortification of white rice flour with minerals and vitamins is mandatory and is associated with nutritional deficiencies in the population, as the micronutrient-rich bran layer is discarded during rice processing and milling (WHO, 2018). Some populations, such as those with celiac disease, often show deficiencies in micronutrients and bioactive compounds due to their gluten-free diet, so new approaches are being developed that focus on fortifying gluten-free bakery products (Torres et al., 2016). Different methods can be applied to obtain a fortified product, from spray drying, coatings or even direct mixing formulas. Innovative food processing techniques involving minimal or no thermal treatment have gained interest as alternatives for their benefits in the preservation of sensory characteristics and thermolabile bioactive compounds. As a non-thermal pasteurisation technology, HHP is considered to be a processing technique with minimal loss of nutritional and sensory properties (Amsasekar et al., 2022) and can therefore be considered a suitable technology for micronutrient fortification. Pressure-mediated inward diffusion of nutrients from an enriched medium is the most direct way to enrich the target food, a process known as high-pressure impregnation (HPI) (Balakrishna et al., 2020). In this process, nutrients are incorporated into the food product by pressure forces that may impart micro-fractures or affect the permeability of the food material surface, which would facilitate a process of mass transfer by osmotic pressure. Based on this mechanism of action, the concentration of the nutrient in the medium is an important factor to benefit from osmotic phenomena, as well as the state of the food matrix, with a porous or permeable one being highly desirable to increase the diffusion rate (Karwe et al., 2016). HPI treatments have been used effectively to promote quercetin enrichment in frozen–thawed cranberries (Mahadevan et al., 2015), calcium in mango cubes (Lamilla et al.,

2021) and baby carrots (Gosavi et al., 2018), curcuminoids in pineapple slices (George & Rastogi, 2017) or anthocyanins in apple slices (George et al., 2016). Since pressure forces can damage cell structures, HHP treatments in stiffer food matrices, such as cereal grains, can also affect their constituent tissues, reducing their natural resistance to mass transfer (Khan et al., 2019). In the study of Balakrishna et al. (2021), an HPI treatment (600 MPa, 50/70 °C, 5–20 min) was carried out to fortify white rice with thiamine, calcium and zinc. They observed significant increases in the concentration of these nutrients, particularly with increasing temperature and holding time.

The effect of pressure-mediated cell wall damage can also be exploited to improve the bioactive profile of the pressurised food product. Pressures of 30 MPa have been reported to be sufficient to promote cell structural damage in germinated brown rice. Therefore, enzymatic hydrolysis could therefore be accelerated in the denatured substrate, resulting in increased biosynthesis of compounds such as antioxidants (γ-Oryzanol), tricin 40-O-(threo-b-guaiacylglyceryl) ether (TTGE), arabinoxylans, γ-aminobutyric acid (GABA) and vitamins such as E and B (M. Y. Kim et al., 2015). Although it has been shown that enzyme activity can be inhibited at pressures above 100 MPa (M. Y. Kim et al., 2015), an increase in antioxidant capacity has been observed in germinated brown rice treated with HHP at 100–500 MPa (Xia, Wang, et al., 2017). These authors attributed this effect to the release of antioxidant compounds bound to cell walls and organelles due to the turbulence and shear effects promoted by the HHP treatment. Similarly, other authors have also reported increases in antioxidant capacity after HHP treatments in alternative food products such as *Prosopis chilensis* seeds (Briones-Labarca et al., 2011), sweet potato flour (Cui & Zhu, 2019) and buckwheat flour (Z. Zhou et al., 2015). Recent research has also shown positive results with the HHP treatment of whole seeds as an effective and simple method of fortifying flour from the natural compounds found in the outer layers of the seeds. Gutiérrez et al. (2022) observed an increase in the phenolic content of buckwheat flour after HHP treatments of whole grains. Furthermore, Balakrishna & Farid (2020) showed an increase in the thiamine content of white rice flour after HHP treatment (450/600 MPa, 15/30 min, 50/70 °C) of paddy rice. These authors attributed this improvement to pressure-induced inward diffusion of thiamine naturally present in the outer parts of the seed (natural coat), similar to the infusion provided by the pressure-driven mass transport phenomenon of the HPI treatment. The results of this study concluded that treating whole rice grains with HHP could be an interesting alternative to parboiling for industrial applications.

Few studies are available on the use of HHP-treated flours for the nutritional enrichment of bread in terms of bioactive properties. Positive results in this respect were obtained by Angioloni & Collar (2012a), who observed a significantly ($p < 0.05$) higher antiradical activity in breads made with HHP-treated wheat, oat, millet and sorghum flours (350 MPa, 10 min, 20 °C) than in those made with native flours.

1.8. Valorization of by-products as heath promoting ingredients in gluten-free baking products

Dietary fibre (DF) is currently attracting particular interest from consumers and researchers. Many claims have been associated with its intake, from the reduced glycaemic response, increased faecal volume, maintenance of normal blood cholesterol levels to the reduction of certain types of cancer (Dahl & Stewart, 2015). Institutions such as the European Food Safety Authority (EFSA) recommend a fibre intake of at least of 25 g/day for normal laxation in adults and an intake above this amount for a reduced risk of coronary heart disease, type 2 diabetes and improved weight maintenance (Stephen et al., 2017). There are many fibre sources in foods, including legumes, fruits, vegetables and whole grains, in which the fibre is found especially in the outer parts of the seeds.

Biochemical properties of DF such as viscosity, fermentability and gel-forming ability, have been associated with their potential health benefits (Dahl & Stewart, 2015). While soluble, high viscous and gel-forming fibres such as β-glucans or psyllium may have health benefits in controlling glycaemia and cholesterol, and fibres with low fermentability may have a constipation or laxative effect. In turn, the physiological effects of insoluble fibres depend on their particle size; whereas fine particles can lead to a constipating effect, coarse particles can have a laxative effect (McRorie & McKeown, 2017).

Despite the evidence for the health benefits of DF intake, there is still a lack of DF in the Western diet (Barber et al., 2020). Among special needs populations, celiac disease patients (approximately 1% of the Western population) are particularly susceptible to DF deficiency, due to a general imbalance in the nutritional profile of specific gluten-free (GF) foods. This imbalance has been attributed to an increase in fat intake that displaced the consumption of fibre and complex carbohydrates (Cardo et al., 2021b). Different authors have also found that coeliac diets tend to be higher in fat and sugar and lower in fibre, proteins, micronutrients and bioactive compounds than non-coeliac diets (Cardo et al., 2021a; Babio et al., 2017; Torres et al., 2016).

Public institutions recommend increasing DF intake through dietary guidelines that emphasise the importance of choosing a variety of foods, including concentrated sources of fibre (Mobley et al., 2014). According to EU Regulation No 1924/2006 in EU, fibre-containing products can be specifically labelled for the use of the terms "source of fibre" and "high in fibre" as health claims if the product contains at least 3 and 6 g of fibre per 100 g, respectively, that demonstrate a beneficial physiological effect (Stephen et al., 2017). Strategies that can also help to meet DF consumption recommendations include adding small amounts of non-soluble fibre to foods (Barber et al., 2020) or the regular consumption of whole grains, as they are rich in fibre and other biologically active compounds, including vitamins, minerals and phenolics (R. H. Liu, 2007).

The innovation and development of acceptable-tasting fibre-enriched food products could be considered as a feasible strategy to encourage consumers to increase their intake of fibre. Bread can be considered as a suitable vehicle for fibre-enriched products, as it is a versatile and affordable staple food with balanced nutritional properties. The addition of DF to refined-flour-based breads can improve their nutritional profile, also providing micronutrients and bioactive compounds with antioxidant capacity such as flavonoids, carotenoids and polyphenols (R. H. Liu, 2007; Rodríguez et al., 2006). However, depending on the fibre type, the dosage and the particle size, the incorporation of DF in breadmaking has a greater or lesser effect on the rheological properties of the dough and the quality of the final product (Gómez et al., 2003; Anil, 2007). This is even more important in the production of GF breads, as the lack of gluten affects the viscoelastic properties of dough and hinders the gas retention of breads during proofing, resulting in poor quality breads (Pérez-Quirce et al., 2014).

The incorporation of unconventional sources of DF is attracting increasing interest (Chatterjee et al., 2016). Among these novel sources of DF, buckwheat (BW) is considered a raw material of great interest for the production of functional foods. In addition to its contribution to dietary fibre, this pseudocereal contains polyphenols in the form of flavonoids, which have a high antioxidant capacity (S. Q. Li & Howard Zhang, 2001) and other physiological effects such as the ability to lower blood pressure (Chatterjee et al., 2016). Among the parts of BW grains, dietary fibre is remarkably abundant in the outer layers of the seed, in the seed coat or the pericarp (hull). The latter plant material consists mainly of cellulose, lignin and non-cellulosic polysaccharides and contributes mainly to the insoluble fraction of DF (Steadman et al., 2001). Phenolic compounds are also mainly present in the BW hull (Heś et al., 2012), which is also a good dietary source of minerals (Steadman et al., 2001). The hulls are obtained during the processing of buckwheat kernels in roller or impact mills (Steadman et al., 2001). Although this raw material is generally considered as an industrial waste or a by-product (Dziedzic et al., 2012), several industrial uses for BW hulls have been proposed, such as pillow fillers, packaging containers for food cans and as a source of potash and natural dyes (M. Zhou et al., 2016). The potential of this by-product as a functional ingredient, for its dietary fibre content, could also contribute to waste reduction and indirect income generation (S. K. Sharma et al., 2016). Previous studies confirmed the possibility of obtaining extracts from these plant wastes, contributing to sustainability by promoting a 'circular economy' (Nobili et al., 2019). However, there are very few studies on the use of BW hull as an ingredient in food processing (L. Wang et al., 2023) and their use in GF bakery products, which are characterised with a starch-dominated matrix in which hydrocolloids play an important role in ensuring gas retention during the baking process, has not yet been proposed.

Similar to flours and other fibre sources, the degree of comminution of the BW hull particles is a key factor in their nutritional and functional value. It has been reported that BW hull micronization treatment could exert positive effects on bioactive and functional properties, enhancing its potential application in functional foods (F. Zhu et al., 2014). From a technological point of view, it would be of interest to know how the inclusion of buckwheat hull particles (BH) of different sizes in complex matrices such as GF dough and bread could affect their nutritional properties and organoleptic and textural quality.

II. OBJETIVOS

II. OBJETIVOS

El objetivo principal de la presente tesis doctoral fue el estudio de la aplicación de la tecnología de las altas presiones hidrostáticas (APH) sobre granos enteros de cereales y pseudocereales para la obtención de ingredientes de interés nutricional y tecnológico. La modificación física de estas matrices mediante la tecnología APH estuvo dirigida a permitir una mejor adecuación de las propiedades tecno-funcionales y nutricionales de las harinas obtenidas a partir de los granos tratados para la elaboración de productos horneados sin gluten.

Para lograr el cumplimiento de este objetivo, se plantearon los siguientes objetivos particulares, enmarcados en el plan de trabajo propuesto:

1. Evaluar el impacto de un pretratamiento de remojo de los granos seleccionados, previo a la aplicación del procesamiento APH.
2. Estudiar el potencial que presenta la aplicación de APH en diferentes ciclos de tratamiento para mejorar las propiedades tecnológicas y nutricionales de las harinas.
3. Analizar la respuesta tecno-funcional y nutricional de las harinas a través del manejo de los factores habituales del procesamiento con APH, como es el caso del nivel de presión y el tiempo de tratamiento.
4. Evaluar el efecto de la incorporación de harinas modificadas mediante APH provenientes de granos de especies sin gluten, como es el caso del arroz y el trigo sarraceno, en una formulación de productos horneados sin gluten.
5. Valorar el efecto de la incorporación de partículas de cascarilla de trigo sarraceno en los productos de panificación sin gluten, evaluando su impacto sobre diferentes parámetros de calidad y el perfil nutricional de los productos.

Para dar cumplimiento al primer objetivo, sobre las matrices vegetales seleccionadas, que fueron los granos enteros de trigo sarraceno, elegidos por su interés nutricional, y el grano de arroz, cuya harina se encuentra comúnmente presente en las formulaciones de productos sin gluten, se investigó el efecto de un pretratamiento de remojo previo aplicado durante 4 horas a 40° C en condiciones de exceso de agua. Empleando agua como medio transmisor de la presión, se evaluó el impacto del tratamiento APH sobre la matriz previamente hidratada, en comparación con la misma matriz sin el pretratamiento de remojo. Las harinas integrales resultantes fueron evaluadas desde un punto de vista tecno-funcional y nutricional. Este estudio es presentado en el capítulo 1 para la matriz de trigo sarraceno y en el capítulo 3 para la matriz de arroz.

Para la consecución del segundo objetivo, referido a la evaluación del efecto de la aplicación del tratamiento APH en diferentes ciclos de aplicación, se llevó a cabo un desarrollo experimental específico sobre una matriz de trigo sarraceno. El tiempo de tratamiento APH fue de 30 minutos, aplicado en un único ciclo de tratamiento, o en dos ciclos de 15 minutos/ciclo, mientras que el nivel de presión alcanzado fue de 600 MPa.

Este estudio fue abordado conjuntamente con el realizado para abordar el primero de los objetivos particulares de esta tesis, y se recoge en el capítulo 1 de este documento.

A fin de cumplir con el tercer objetivo, asociado a la capacidad de modulación de los tratamientos APH sobre la respuesta tecno-funcional y nutricional de las harinas integrales resultantes, se abordó un estudio experimental en el que se evaluaron diferentes factores de procesado ligados habitualmente a este tratamiento, como es el caso del tiempo de tratamiento o mantenimiento de la presión (se fijaron tiempos de 0/5/15 min para el trigo sarraceno y 5/15/30 min para el arroz) y el nivel de presión alcanzado (300/600 MPa para el trigo sarraceno y de 600 MPa para el arroz). El efecto de los tratamientos fue evaluado en términos de las propiedades de hidratación, emulsión, espumado, térmicas y de empastado de las harinas resultantes, de las propiedades reológicas a través de los geles obtenidos tras modificación hidrotérmica de las harinas, así como de sus propiedades bioactivas (capacidad antioxidante). Este estudio se recoge en el capítulo 2 para el trigo sarraceno, donde se empleó un tratamiento previo de remojo para los granos en las condiciones presentadas en el capítulo 1. Para la matriz de grano arroz, este estudio se presenta en el capítulo 3 y se realizó conjuntamente con el abordado para conseguir el primero de los objetivos particulares.

Para dar cumplimiento al cuarto de los objetivos se seleccionó una harina integral modificada físicamente mediante el tratamiento APH y cuyas propiedades pudieran presentar una ventaja tecnológica y nutricional para la elaboración de productos horneados sin gluten. En base a las anteriores experiencias, se seleccionaron unas condiciones de tratamiento APH de 600 MPa durante 15 min para ambas matrices (trigo sarraceno y arroz), aplicándose también un pretratamiento de remojo en las condiciones indicadas en el capítulo 1. Para la evaluación de la capacidad de panificación de estas harinas modificas, se realizó una sustitución parcial de la harina refinada de arroz, empleada como ingrediente base de una fórmula de pan sin gluten, con la correspondiente harina tratada en cuatro porcentajes de sustitución (15, 30, 50 y 70 %) comparándose con el efecto de incorporación de una harina de la misma naturaleza, pero en su forma nativa. El análisis comparativo se realizó en base a los resultados de los ensayos de reología dinámica de las masas de panificación, así como en función de las propiedades físicas y sensoriales de los panes resultantes. Estos estudios se encuentran recogidos en los capítulos 2 y 3 del presente documento.

El cumplimiento del quinto objetivo de la tesis se llevó a cabo mediante el empleo de dos fracciones de cascarilla de trigo sarraceno caracterizadas por su diferente tamaño de partícula (D_{50} = 62,7µm y 307µm). La cascarilla fue incluida en dos porcentajes de adición (3 y 6%, p/p) en una fórmula de pan sin gluten constituida a base de harina de arroz refinado y se valoró su incorporación a efectos de cumplir con los requisitos de las declaraciones nutricionales de "fuente de fibra" y "alto contenido en fibra" según el Reglamento (CE) Nº 1924/2006 de 20 de Diciembre, relativo a las declaraciones nutricionales y de propiedades saludables en los alimentos. El efecto de la inclusión de los dos tamaños de partícula y los porcentajes de adición fue evaluado en base al comportamiento reológico de las masas panarias, así como a las propiedades físicas y sensoriales de los panes resultantes. Este estudio se encuentra recogido en el capítulo 4 del documento.

II. OBJETIVES

II. OBJECTIVES

The main objective of this doctoral thesis was to study the application of high hydrostatic pressure (HHP) technology on whole cereal grains and pseudocereals to obtain ingredients of nutritional and technological interest. The physical modification of these matrices by HHP technology was aimed at achieving a better suitability of the techno-functional and nutritional properties of the flours obtained from the treated grains for the production of gluten-free baked goods.

In order to achieve this objective, the following specific objectives were established, under the proposed work plan:

1. To evaluate the impact of a soaking pre-treatment of selected grains prior to the application of APH processing.
2. To assess the potential of the application of APH in different treatment cycles to improve the technological and nutritional properties of the flours.
3. To analyse the techno-functional and nutritional response of the flours through the management of the usual factors of APH processing, such as pressure level and treatment time.
4. To evaluate the effect of incorporating APH-modified flours from gluten-free grain species, such as rice and buckwheat, in a gluten-free baked product formulation.
5. To study the effect of incorporating buckwheat hull particles in gluten-free bakery products, analysing their impact on different quality parameters and the nutritional profile of the products.

In order to meet the first objective, on the selected plant matrices, which were whole buckwheat grains, chosen for their nutritional interest, and rice grain, whose flour is commonly present in gluten-free product formulations, the effect of a pre-soaking pre-treatment applied for 4 hours at 40°C under conditions of excess water was investigated. Using water as the pressure transfer medium, the impact of the HHP treatment on the pre-hydrated matrix was evaluated in comparison to the same matrix without the soaking pre-treatment. The resulting whole flours were evaluated from a techno-functional and nutritional point of view. This study is presented in chapter 1 (section 4.1 of results and discussion) for the buckwheat matrix and in chapter 3 (section 4.3 of results and discussion) for the rice matrix.

In pursuit of the second objective, which referred to the evaluation of the effect of the APH treatment application in different application cycles, a specific experimental development was carried out on a buckwheat matrix. The HHP treatment time was 30 minutes, applied in a single treatment cycle or in two cycles of 15 minutes/cycle, while the pressure level reached was 600 MPa. This study was conducted in combination with the study carried out to address the first of the particular objectives of this thesis, and is presented in chapter 1 (section 4.1 of results and discussion) of this document.

In order to fulfil the third objective, associated with the modulation capacity of HHP treatments on the techno-functional and nutritional response of the resulting whole flours, an experimental study was undertaken in which different processing factors usually associated with this treatment were evaluated, such as the treatment or pressure maintenance time (0/5/15 min for buckwheat and 5/15/30 min for rice) and the pressure level reached (300/600 MPa for buckwheat and 600 MPa for rice). The effect of the treatments was evaluated in terms of the hydration, emulsification, foaming, thermal, and pasting properties of the resulting flours, the rheological properties of the gels obtained after hydrothermal modification of the flours, as well as their bioactive properties (antioxidant capacity). This study is reported in chapter 2 (section 4.2 of results and discussion) for buckwheat, where a pre-soaking treatment was used for the grains under the conditions presented in chapter 1 (section 4.1 of results and discussion). For the rice grain matrix, this study is presented in chapter 3 (section 4.3 of results and discussion) and was carried out in association with the study conducted to achieve the first particular objective.

In order to accomplish the fourth objective, a physically modified whole flour by HHP treatment was selected, whose properties could present a technological and nutritional advantage for the production of gluten-free baked goods. Based on the previous experiences, HHP treatment conditions of 600 MPa for 15 min were selected for both matrices (buckwheat and rice), and a soaking pre-treatment was also applied at the conditions indicated in chapter 1. For the evaluation of the baking ability of these modified flours, a partial replacement of the refined rice flour, used as a base ingredient in a gluten-free bread formula, with the corresponding modified flour was carried out at four replacement percentages (15, 30, 50 and 70 %) and compared with the effect of incorporating a whole flour of the same kind, but in its native state. The comparative analysis was carried out on the basis of the results of the dynamic rheology tests of the baking doughs, as well as on the basis of the physical and sensory properties of the resulting breads. These studies are presented in chapters 2 and 3 (section 4.2 and 4.3 of results and discussion, respectively) of this document.

The fifth objective of the thesis was achieved by using two buckwheat hull fractions characterised by different particle sizes (D50 = 62.7μm and 307μm). The husk was also included in two percentages of addition (3 and 6%, w/w) in a gluten-free bread formula based on refined rice flour. The incorporation of this ingredient was assessed in order to meet the requirements of the nutrition claims "source of fibre" and "high fibre" according to Regulation (EC) No 1924/2006 of 20 December 2006 on nutrition and health claims made on foods. The effect of the inclusion of the two particle sizes and addition percentages was evaluated on the rheological behaviour of the doughs, as well as on the physical and sensory properties of the resulting breads. This study is included in chapter 4 (section 4.4 of results and discussion) of the document.

III. MATERIALS AND METHODS

3.1 Materials

Buckwheat (BW) grains (Panda variety) kindly provided by Grupo BC Servicios 2011 (Palencia, Spain) were used. They showed the following proximal composition: 74.7% total carbohydrates (28.3% dietary fibre), 11.3% protein, 3.2% lipid, 2.1% ash and 8.7% moisture.

Split pieces of buckwheat (BW) hulls (BH) were obtained from two commercial Polish products (Sante, Warsaw, Poland): a coarse BW hull batch (CBH; D50: 307 μm) and a fine BW hull batch (FBH; D50: 62.7 μm). The proximate composition of both ingredients were as follows: 90.80% carbohydrates (87.34% dietary fibre), 6.22% protein, 2.23% ash and 0.75% fat for CBH as well as 93.78% carbohydrates (89.71% dietary fibre), 4.21% protein, 1.93% ash and 0.08% fat in dry weight basis (dw) for FBH.

Rough (paddy) rice (Japonica variety) were kindly provided by Herba Ricemills S.L.U. The proximate composition of the rice brown flour was 75.8% total carbohydrates (7.9% dietary fibre), 7.4% protein, 1.6% fat, 1.7% ash and 13.5% moisture.

The refined rice flour used in chapter 2 and 3 was supplied by Herba Ricemills S.L.U (Valencia, Spain) with a proximate composition of 79.9% total carbohydrates, 12.6% moisture, 6.5% protein, 0.1% lipid and <0.9% ash.

The refined rice flour used in chapter 4 was also provided by Herba Ricemills S.L.U (89.77% carbohydrates, 1.15% of dietary fibre, 9.04% protein, 0.39% ash and 0.81% fat in dry weight basis).

Sunflower oil, salt and sugar were purchased from a local supermarket as well as dried yeast (European, Istanbul, Turkey). Hydroxipropilmethylcellulose (HPMC, Methocel K4M Food Grade) was acquired from Dow Chemical (Midland, TX, USA).

The following chemicals obtained from Sigma-Aldrich, Co. (St. Louis, MO, USA) were used: gallic acid, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), Folin–Ciocalteu (FC) reagent, 2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+), 2,2′-diazobis-(2-aminodinopropane)-dihydrochloride (AAPH), fluorescein and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

3.2 Experimental designs

Section 4 of the results and discussion is subdivided into 4 chapters in order to respond to the objectives set out in section 2. The following particular designs of experiments have been proposed for each chapter:

Chapter 1. *Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles.* (section 4.1 of results and discussion).

The objective of this chapter was to investigate the effects of HHP treatment of buckwheat wholegrains on the techno-functional, nutritional and bioactive properties of the resulting flours. The effect of a soaking pre-treatment (4h at 40ºC) and the number of cycles (a single cycle of 30 min or a double cycle of 15 min each) in which the HHP treatment is applied were studied to assess the potential of this technology for whole grain HHP- treatment. This chapter was designed to respond to the specific objectives 1 and 2 proposed in section 2.

Chapter 2. *Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time.* (Section 4.2 of results and discussion).

This chapter investigated the influence of pressure level (300 – 600 MPa) and holding time (0 – 5 – 15 min) as factors in HHP treatment on buckwheat grain to modulate the techno-functional and bioactive response of the resulting flours. In addition, the impact of a modified flour resulting from selected HHP treatment conditions as an ingredient in a GF bread formulation on the quality of gluten-free bread was also investigated, in order to meet specific objective 3 and 4.

Chapter 3. *Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours*. Study of pre-soaking treatment and holding time as processing factors. (Section 4.3 of results and discussion).

This chapter evaluated the effect of HHP treatments applied to whole rice grains on the techno-functional and nutritional properties of the flours obtained. For this purpose, treatments at 600 MPa were carried out to evaluate the effect of different holding times (5 - 15 - 30 min). The convenience of carrying out a soaking pre-treatment prior to the HHP treatment was also evaluated. The evaluation of the impact of the treatments on the techno-functional and nutritional properties of the flours was carried out. Finally, a baking process was carried out in which the behaviour of the flours in real gluten-free systems was evaluated. This chapter has been conducted in order to meet the specific objectives (1, 3 and 4) proposed in section 2.

Chapter 4. *Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread.* (Section 4.4 of results and discussion).

The aim of this chapter was to evaluate the use of this by-product as a functional ingredient in a GF bread formula, assessing its impact on the rheological properties of the dough and the quality of the resulting GF bakery product. For this purpose, two batches of buckwheat hull (BH) particles (one corresponding to a fine BH particle size, D50: 62.7 μm and the other to a coarser one, D50: 307 μm) and two addition percentages (3 and 6 %) were studied in order to meet the requirements of the nutrition claims "source of fibre" and "high fibre" according to Regulation (EC) No 1924/2006 of 20 December 2006. This chapter was meant to respond to the specific objective 5 proposed in section 2.

3.3 High hydrostatic pressure treatment (HHP) and flour obtention

For each chapter, the flour obtention procedure and HHP-treatment was as follows:

Chapter 1. *Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles.* (section 4.1 of results and discussion)

First, a cleaning process was applied to ensure the safety of the grains. BW grains were packed with distilled water (grain to water ratio, 1:4) in co-extruded Polyethylene/Polyamyde pouch and sealed with minimum head space and separated in batches. Samples selected to soaking treatment were immersed in excess water for 4 hours at 40ºC. The above temperature was based on a preliminary study of soaking conditions (20ºC vs 40ºC) at 1 cycle of the HHP treatment (Gutiérrez et al., 2022).

In a second step, the effect of HHP was evaluated in soaked and un-soaked samples using a HHP unit (Wave 6000/135, NC Hyperbaric, Burgos, Spain) based in the Agrarian Technology Institute of Castilla y Leon (Spain) with a vessel of 135 L and 200 mm diameter. Samples were processed at 6.08 × 108 Pa (600 MPa) for a single cycle of 30 min, or a double cycle of 15 min each for a total holding time of 30 min. The pressure come-up speed was 120 MPa/min and after the holding time the pressure was instantaneously released.

After pressure treatment, grains were blotted, dehusked and ground using a Faribon mill (F6003 PH, Omas, Padova, Italia) and then milled with Fidibus Medium mill (Komo Grain Mills, Penninberg, Austria) making the final flour fraction went through a 250 µm mesh. All flours were stabilized to get a 12-13% of final moisture, and then packed and stored at 5ºC temperature until further used.

Chapter 2. *Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time.* (Section 4.2 of results and discussion).

The high hydrostatic pressure treatment was applied to BW whole grains using the following procedure. Briefly, grains were packed in co-extruded Polyethylene/Polyamide pouch with water (1:4, w/w) and allowed to soak for 4 h at 40 °C. Then samples were placed at a plastic vessel for the pressure treatment using a HHP Wave 6000/135 unit (NC Hyperbaric, Burgos, Spain). Six batches were prepared according to the processing conditions studied: 2 pressure levels (300 - 600MPa) and 3 levels of holding (treatment) time (0 - 5 - 15 min). Right after each pressure treatment, grains were blotted, dehusked and ground using firstly a CD1 mill (CHOPIN Technologies, Paris, France) and secondly a Fidibus Medium mill (Komo Grain Mills, Penninberg, Austria). Finally, the moisture content of the flours was reduced to stabilisation levels (12 – 13 %). The final particle size of the flour obtained was able to pass through a 250 µm mesh. All batches flours including a native one for control measures were stored at 4ºC.

Chapter 3. *Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours*. Study of pre-soaking treatment and holding time as processing factors. (Section 4.3 of results and discussion).

The pressure treatment procedure was as follows: separate batches of soaked (for 4h at 40 °C) or un-soaked rough rice grains were packed in polyethylene/polyamide bags coextruded with distilled water (1:4, w/w). The batches were then placed in plastic vessels for high hydrostatic pressure (HHP) treatment with an HHP Wave 6000/135 unit (NC Hyperbaric, Burgos, Spain). Each batch of pre-soaked (S) and un-soaked (U) grains was processed at 6.08 × 108 Pa (600 MPa) for the holding times of 5, 15 or 30 min. After the HHP treatment, grains were blotted at low temperature (35°C). Grain moisture was standardised at 16% to facilitate the dehulling process. A CD1 mill (CHOPIN Technologies, Paris, France) was used to dehull and grind the grains. The resulting whole flours (WR) characterised as unsoaked (U) and soaked (S) samples were equilibrated to 14 ± 1 % moisture and stored at 4ºC.

3.4 Methods

3.4.1 Particle size distribution of flours

The particle size distribution of the flours samples (native and HHP-modified buckwheat flour in chapters 1 and 2 and samples of native and HHP-modified brown rice flour in chapter 3) was obtained using a laser diffraction particle size analyser (Mastersizer 2000, Malvern Instruments Ltd, Malvern, UK). The flours were characterised by the median diameter (D_{50}) and the size dispersion [$(D_{90}-D_{10})/D_{50}$] as described by Abebe et al. (2015). Each sample was measured in triplicate.

3.4.2 Flour colour analysis

The colour characteristics of the flours (samples of native and HHP-modified buckwheat flour in chapters 1 and 2 and samples of native and HHP-modified brown rice flour in chapter 3) were described in the CIE L*a*b* and CIE L*C*h coordinates as was described by Abebe et al. (2015). The colour difference of each treated flour sample with respect to the native was also calculated following the equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. At least five measures were taken of each sample with the colorimeter PCE-CSM 5 (PCE Instruments, Meschede, Germany) and analysed with the software CQCS3 (Shenzhen ThreeNH Technology Co. Ltd., Shenzhen, China) 3.2 Version.

3.4.3 Scanning electron microscopy (SEM)

The structural morphology of the HHP modified buckwheat (chapter 1) and rice (chapter 3) flours was studied and compared with a native one by means of a scanning electron microscope (Quanta 200-F, FEI, Graz, Austria). In conditions of low-vacuum mode, the x-ray-equipped microscope performed micrographs at an accelerating voltage of 12 KeV with a secondary electron detector.

In chapter 4, the microstructure of ground bread samples was also analysed by scanning electron microscopy using the same device at high vacuum conditions using a secondary electron detector. Prior to analysis, each sample was coated with gold. Microphotographs of the metallised samples were acquired at an accelerating voltage of 15 keV at magnifications of 500×, 1000× and 3000×.

3.4.4 Techno-functional properties

Water holding capacity (WHC, g H2O absorbed/g sample), water absorption capacity (WAC, g H2O absorbed/g sample), water absorption and solubility index (WAI, g gel/g sample; WSI, g of soluble matter/100g sample, respectively) and swelling power (SP, g gel/g of insoluble matter) of flour samples (samples of native and HHP-modified buckwheat flour in chapters 1 and 2 and samples of native and HHP-modified brown rice flour in chapter 3) was obtained following the procedure described by Solaesa et al. (2020). The emulsion activity (EA) expressed as percentage of emulsion layer to total volume and emulsion stability (ES) expressed as percentage of emulsion layer after heating 30 min at 80ºC to total volume were determined. The foam capacity (FC, mL foam/g sample) and stability (FS, percentage of foam retained after 1 hour) were as well calculated using the procedure described by Vela, Villanueva, Grazielle, et al. (2023). All outcomes were referred on dry weight basis (dw). Measures of techno-functional properties were collected in triplicate.

3.4.5 Pasting properties

The flours pasting properties were determined following the AACC International Method 76-21.01 Standard 2 (AACC, 2017) and the procedure used by Vela et al. (2021) using a rheometer (Kinexus Pro +, Malvern Instruments Ltd, Malvern, UK). The viscosimetric profiles and the following pasting parameters: pasting temperature (PT), pasting time (Pt), peak viscosity (PV), trough viscosity (TV), breakdown (BV), final viscosity (FV) and setback (SV) were obtained for the flour samples (samples of native and HHP-modified buckwheat flour in chapters 1 and 2 and samples of native and HHP-modified brown rice flour in chapter 3) using Rspace 1.72 version software (Malvern Instruments Ltd, Malvern, UK).

3.4.6 Thermal properties

Data on starch Gelatinization and retrogradation transitions data were collected by differential scanning calorimetry (DSC3, STARe-System, Mettler Toledo, Greifensee, Switzerland). Following the methodology described by Vela, Villanueva, Grazielle, et al. (2023), the buckwheat flour samples used in chapter 1 and 2, were hydrated (30:70; w:w) and allowed to rest for 30 min before the analysis. Two temperature scans (0ºC-110ºC) 7 days apart, were carried out to obtain gelatinisation (first scan) and retrogradation (second scan) transitions. Data obtained were the enthalpy (ΔH, J/g flour, dw) and the transition temperatures: onset (TO), peak (TP) and endset (TE). Samples were analysed in duplicate.

3.4.7 Rheological properties of gels

A rheological characterisation using the sample gels produced from the pasting test was carried out. Gels from buckwheat (chapter 1) and whole rice flour samples (chapter 2 and 3) were placed between a serrated plate geometry (40 mm diameter) at a gap of 1 mm of Kinexus Pro + rheometer (Malvern Instruments Ltd, Malvern, UK) and allowed to rest for 5 min. Dynamic oscillatory tests at 25ºC was then carried out following the procedure used by Vela, Villanueva, Ozturk, et al. (2023). Frequency sweeps (from 10 Hz to 1 Hz) were performed at a constant strain of 1% within the linear viscoelastic region (LVR) previously stablish from strain sweeps (from 0.1% to 1000%) at a constant frequency of 1 Hz. The elastic (G_1') , viscous (G_1'') and the loss tangent ($(\tan \delta)_1$) coefficients were collected and fitted to the power law model as described by Abebe and Ronda (2014). The exponents a, b and c from the potential equations were taken to quantify the degree of dependence of the elastic, viscous and loss moduli respectively, with the frequency. The complex modulus (G^*_1) was also determined by the formula: $\{(G'_1)^2 + (G''_1)^2\}^{1/2}$. From the stress sweeps, τmax was taken as the maximum stress that the gel sample can withstand without altering its viscoelastic characterisation as well as the stress at the crosspoint (G` = G``).

3.4.8 Nutritional properties

3.4.8.1 Vitamin content

Following the procedure of Rico et al. (2020), the determination of the content of group B vitamins (thiamine, niacin and pyridoxine) was obtained for each whole rice flour sample used in chapter 3. The results were expressed as mg vitamin/100 g dw.

3.4.8.2 Mineral content

Flour samples were incinerated and the dry-way mineralized and ashes were diluted with 5 ml of HNO3 (6,5%). After reaching and keep the boiling temperature for two minutes, chill and filtered using mili-Q water into 100 ml flask pouring HNO3 (65 %), samples were set in a graphite furnace tube atomizer (Varian GTA 120Z, Agilent Technologies, California, USA) and the SpectrAA 240 FS atomic absorption espectrophotmeter (Varian, Agilent Technologies, California, USA) to analyze the content in B, Ca, Fe, Mn, Zn for buckwheat flour samples in chapter 1 and to analyse the content in P, Ca, Fe, Mn, K, Zn in whole rice flour sample in chapter 3. Results were expressed in mg/100g (dw).

3.4.8.3 Total phenol content (TPC) and total antioxidant capacity (TAC)

Based on the procedure of Rico et al. (2021), extracts of flour samples (from buckwheat flour samples from chapters 1, 2 and brown rice flour samples from chapter 3) and extracts of bread samples (from breads made in chapter 2 and 4) were obtained as follows: 1 g of each sample (pieces of the bread samples were broken into small pieces and then ground) was mixed with 10 ml of a methanol:water solution (1:1, v:v) previously acidified to pH 2 (HCL 0.1M). After shaking in an orbital shaker (250 rpm) for 30 min at 25ºC and centrifuged (4000 rpm, 10 min) the supernatant was filtered (Whatman paper nº1) and collected. Over the residue, 10 ml of the same solution was poured and the same procedure was repeated. A third re-extraction of the residue with 5 ml of the solvent was carried out (only for whole rice flour samples extraction). The collected supernatants were combined and adjusted to 25 ml with the solvent. Aliquots of each extract were stored at -80ºC for further analysis.

The total phenol content (TP) of the extracts was obtained with the method described in Rico et al. (2021) using a microplate reader (Fluostar Omega, BMG, Ortenberg, Germany) at 765 nm. A calibration curve made with Gallic acid as standard (98-699 µM) was used and results were expressed as mg gallic acid equivalents (GAE)/100 g of sample (dw).

The total antioxidant capacity (TAC) of the samples extracts was determined following the procedure described in Rico et al. (2021) by DPPH•, ABTS•+, ferric reducing ability potential (FRAP) and oxygen radical absorbance capacity (ORAC) assays. The antioxidant activity on solid samples (quencher, Q-) was also determined by Q-DPPH• and Q-ABTS•+ assays. Data were collected with a microplate reader (Fluostar Omega, BMG). The results were expressed in dw as mg Trolox equivalent (TE) per 100 g of sample (DPPH, Q-DPPH, ABTS, Q-ABTS and ORAC) and as mmol Fe^{+2}/100 g (FRAP). At least duplicates of each sample were evaluated.

3.4.9 Dough formulation and breadmaking procedure

The ingredients and breadmaking procedure used in chapter 2 for gluten-free bread samples obtention were the following: a refined rice flour, a HHP-modified whole buckwheat flour (63.4 % carbohydrates, 4.8% dietary fibre, 13.2 % protein, 3.8 % fat, 1.9 % ash and 12.9 % moisture) from HHP treatment (600 MPa, 15 min) of pre-soaked buckwheat grains and a native buckwheat flour which were also used for comparative purposes.

Doughs samples were prepared using a GF bread formula on the basis of 100 g of rice flour (13% moisture content): 95 g water, 6 g sunflower oil, 5 g sucrose, 3 g dry yeast and 2 g HPMC. The resulting BW flour after HHP treatment (600 MPa for 15 min) or a native BW flour was used at four replacement levels (15, 30, 50, 70 %) of the rice flour. A control dough using 100 % of rice flour was also prepared. The GF dough were obtained following the methodology described by Pérez-Quirce et al. (2014). Finally, time, temperature and relative humidity for the proofing and baking processes is detailed in the work by Villanueva et al. (2022).

The procedure used for breadmaking in chapter 3 was the following: Doughs were prepared on a 100 g of refined rice flour basis (13% moisture) with the following formula: 100 g water, 6 g sunflower oil, 5 g sucrose, 3 g dry yeast and 2 g HPMC. A japonica whole rice (WR) flour (native) or a WR modified one was incorporated at levels of 15, 30, 50 and 70% replacing the refined rice flour. The modified WR flour used came from an HHP treatment of pre-soaked japonica rough rice at 600 MPa for 15 min of holding time. This flour presented the following proximate composition: 75.8% total carbohydrates (7.5 % dietary fibre), 7.7 % protein, 1.8% fat, 1.7% ash and 13% moisture. Doughs and breads were obtained following the methodology described by Villanueva et al. (2022) with some modification. 180 g of each sample dough were placed in aluminum pans for 40 min at 32ºC and 85% relative humidity in a HPP260eco Memmert chamber (Memmert GmbH, Buechenbachm, Germany). Baking was performed using a S400 Sveba Dahlen oven (Sveba Dahlen AB, Fristad, Sweden) at 180 °C for 20 min. The resulting breads were allowed to cool down prior performing the bread quality analysis for 1 h. Duplicates of each bread formulation were prepared. After baking bread was stored at 4ºC for 7 days to analyse the staling effect.

The dough formulation and baking procedure used to produce the breads in chapter 4 were as follows:
Dough samples were using the following gluten-free (GF) formula on the basis of 100 g of rice flour (13% moisture content): water (110%), sunflower oil (6 %), sugar (5 %), HPMC (2 %) and salt (1.5 %). Given the dietary fibre content of each BH sample and based on 100 g of rice flour, the BH added was the required amount to obtain at least 3% and 6% of fibre content coming from BH in order to comply with EU Regulation No. 1924/2006 on the health claims "source of fibre" and "high fibre", respectively. The amounts of BH on a wet basis added to the formulation were 3.61 % and 3.83 % of CBH and FBH, respectively, for the dough and bread samples CB3 and FB3, respectively, and 7.23% and 7.66 % of CBH and FBH, respectively, for the samples CB6 and FB6. The

dough preparation and the breadmaking process were performed following the procedure of Villanueva et al. (Villanueva et al., 2019a). Briefly, after mixing the solid and liquid ingredients for 8 min with a professional mixer (Model 5KPM50, KitchenAid, St. Joseph, MI, USA) at speed 4, the dough samples were placed into aluminium pans for proofing (28 °C and 85% relative humidity) for 50 min. After that, the pans were distributed for baking (40 min at 190 °C) in an oven (EMD-Salva, Salva, Lezo, España). Finally, bread samples were allowed to cool down for 1 h before carrying out analysis. One bread of each formulation was stored at 4 ± 2 °C in polyethylene bags in order to study the effect on staling at seven days.

3.4.10 Rheological properties of doughs

The rheology of the doughs without yeast was carried out with the same dynamic oscillatory tests indicated for gel rheology characterization, following the procedures described in section 3.10 with minor modifications. The frequency sweeps were conducted from 10 Hz to 1 Hz (at 0.05% of constant strain), while the strain sweeps were performed from 0.01% to 100% (in chapter 2) and from 0.01 % to 1000 % (in chapter 3) (at 1 Hz of constant frequency). The same procedure was used to determine the rheological properties of the doughs produced in chapter 4, but in this case a stress sweep was carried out from 0.1 to 500 Pa prior to the frequency sweep from 10 to 0.1 Hz in the linear viscoelastic region (LVR). The stress value selected to perform the frequency sweeps was fixed at 0.5 Pa.

3.4.11 Evaluation of bread quality

The bread samples, once cooled for 1 h and removed from the moulds, were weighed to calculate the baking loss. The specific volume of bread samples containing buckwheat flour (chapter 2) was collected by seed displacement (AACC Method 10-05.01, 2010) using the bread volume tester JMTY (Hangzhou, China). The bread volume of samples containing whole rice flour samples (chapter 3) and buckwheat hull particles (chapter 4) was obtained using the Volscan Profiler 300 (Stable Microsystems, Surrey, UK) and the specific volume was calculated by dividing the volume by the weight of the loaf. Bread crumb texture properties were assessed by a TPA in a double compression cycle of fresh and storage (at 4 °C for 7 days) bread samples using the procedure described by Villanueva et al. (2019). The crumb grain properties were evaluated using image software (ImageJ 1.51 j8, Wayne Rasband, USA) of a digitized slice bread image (30 x 50 mm at the centre, 600 dots per inch) with an HP Scanjet scanner (G3110, Hewlett Packard Enterprise, Palo Alto, CA, USA). The parameters evaluated were the following: mean cell area (mm^2), cell density ($cells/cm^2$), crumb grain uniformity (ratio of the number of small to large cells) and cell wall thickness (mm). Images of the breads were taken using the PowerShot SX410 IS camera (Canon, Japan). Crust and crumb colour was determined using a Minolta colorimeter (CN-508i, Minolta, Co. LTD, Tokyo, Japan), following the procedure used by Villanueva et al. (Villanueva et al., 2019). Colour differences with respect to the control were obtained using the equation: $\Delta E = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$.

3.4.12 Proximal Composition of Bread Samples

A proximate analysis was performed in the breads samples produced for chapter 4 by measuring carbohydrate, protein, fat, moisture and ash of the bread samples. The nitrogen content was determined with the Dumas method (AOAC 2005, method 990.03, 2005) in an elemental analyser (Leco Corp., St. Joseph, MI, USA). A conversion factor of 6.25 was used to obtain the protein content. Total fat was assessed using a fat extracting unit (Soxtec System 2055 Tecator, Foss, Hillerød, Denmark) using dried extracts obtained from bread samples with petroleum ether (BP 40–60 °C) (AOAC 2005, method 2003.05) (Principle & Apparatus, 2005). Ash content was determined by incineration of the samples in a muffle furnace at 550 °C during 24 h (AOAC 2005, method 923.03) (Principle & Apparatus, 2005). Finally, carbohydrates were calculated with difference. The total dietary fibre (TDF) concentration was obtained using a kit (Sigma, St. Louis, MO, USA) and the manufacturer´s instructions based on AOAC method 985.29 (Principle & Apparatus, 2005). Results were expressed in dry weight basis (dw). All measurements were performed in duplicate.

3.4.13 Sensory evaluation

A multiple comparison test was conducted to assess the differences in the intensity of sensory attributes between bread samples made with the flour obtained from the HHP treatment (600 MPa, 15 min; with a pre-soaking treatment of the grain) and the native flour at two different substitution levels (15 % and 50 %). 11 semi-trained panellist rated the intensity on a structure scale, ranging from 1 (lowest attribute intensity) to 9 (highest attribute intensity) of the following six attributes: crust uniformity, taste and odour intensity, aftertaste, crumb hardness and chewiness) for the sensory evaluation carried out in chapter 2 and crust uniformity, flavour and taste intensity, aftertaste, crumb humidity, crumb hardness and cohesiveness for the sensory evaluation tested for the sensory test performed in chapter 3. A GF 100% rice-based bread was given as a reference bread and placed in the middle of the scale (5).

A descriptive sensory test was conducted on control and fibre-enriched breads in chapter 4. A total of 54 panellists (aged 25–55) rated several attributes for each sample using a nine-point hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like). The breads were assessed on the basis of crumb and crust colour, flavour, crumb texture, taste, aftertaste and overall acceptability.

3.4.14 Statistical analysis

A multifactorial analysis of variance was carried out to evaluate the effect of individual factors and their interaction. One-way ANOVA was also performed, using Fisher´s Least Significant Difference test (LSD), to assess significant ($p<0.05$) differences between the flours as well as the breads samples. Statistical software (Statgraphics Centurion 18-X64, Bitstream, Cambridge MN, USA) was used for the analysis

IV. RESULTS AND DISCUSSION

CHAPTER 1

Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles.

4.1 Evaluation of the impact of high hydrostatic pressure (HHP) treatments on whole buckwheat grains. Analysis of grain soaking pre-treatment and number of HHP treatment application cycles.

4.1.1 Particle size distribution of flours

Flour granulometry data can provide useful information about relevant functional and nutritional properties as those results could affect the processing performance of flours Vela et al. (2021). In table 1 is presented the particle size distribution of flours. The soaking pre-treatment had a significant ($p<0.01$) effect on D_{50}. Native flours exhibited the lowest values, being significantly $p<0.05$) different from all treated samples. Un-soaked HHP-treated flours (1C and 2C) showed a significant ($p<0.05$) average increase of 22,3% in this parameter, whereas pre-soaked HHP-treated samples (1C+S40 and 2C+S40) had an increase of 56,3% regarding the native flour. Size dispersion was significantly ($p<0.01$) affected by the number of HHP cycles and the soaking pre-treatment. Treated samples with single cycle showed significantly ($p<0.05$) lower values than samples with double cycles either with or without pre-soaking treatment. Moreover, all pre-soaked samples had significantly ($p<0.05$) lower values than those un-soaked. All flour samples including the control were obtained with the same milling procedure hence, HHP treatments could exert some structural changes to the buckwheat grain, promoting a greater shear milling resistance and modifying the grain fracturability. Structural modifications after HHP treatments have been previously observed by Ravichandran et al. (2018) in rice grains. Those authors observed a negative activation volume of high-pressured paddy grains by a compaction phenomenon.

Table 4.1.1. Granulometry, colour difference, hydration, emulsion and foaming properties of flour samples obtained from native and HHP-treated buckwheat grains.

SAMPLE	D_{50} (μm)	$(D_{90}-D_{10})/D_{50}$	WHC (g/g)	WAC (g/g)	OAC (g/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)	FC (ml/g)	FS (%)	EA (%)	ES (%)
Native	102.8 a	2.05 c	2.77 a	1.17 a	1.1 a	5.68 b	5.09 a	5.99 b	0.71 d	44.4 b	56 e	20.8 d
1C	125.8 b	2.04 c	2.98 b	1.23 b	1.2 b	5.54 ab	5.08 a	5.84 ab	0.26 b	0.0 a	44 d	11.1 c
2C	125.2 b	2.08 d	2.80 a	1.14 a	1.2 b	5.47 a	4.92 a	5.76 a	0.39 c	0.0 a	33 c	5.6 b
1C+S40	161.6 c	1.88 a	3.55 c	1.22 b	1.2 b	5.93 c	5.23 ab	6.25 c	0.00 a	0.0 a	6.0 a	3.8 a
2C+S40	160.8 c	1.93 b	3.46 c	1.31 c	1.3 b	6.18 d	6.14 b	6.58 d	0.19 b	0.0 a	18 b	3.1 a
SE	0.7	0.01	0.05	0.01	0.0	0.05	0.31	0.06	0.04	2.4	1.0	0.4
Number of HHP Cycles	ns	**	*	ns	ns	ns	ns	ns	**	-	ns	**
Soaking pre-treatment	**	**	**	**	ns	**	ns	**	**	-	**	**
Cycles x Soaking	ns	ns	ns	**	ns	*	ns	*	ns	-	**	**

1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. D_{50}: median diameter; $(D_{90}-D_{10})/D_{50}$: Size dispersion; ΔE: Difference of colour between each treated sample and the control; WHC: Water holding capacity, WAC: Water absorption capacity, OAC: Oil absorption capacity, WAI: Water absorption index, WSI: Water solubility index, SP: Swelling power, FC: Foam capacity, FS: Foam stability, EA: Emulsion activity, ES: Emulsion stability. Samples with different small letters show significant differences between treatments ($p<0.05$). SE: Pooled standard error from ANOVA. ** $p<0.01$, *$p<0.05$, ns: not significant.

4.1.2 Scanning electron microscopy (SEM)

Effects of the HHP and combined treatments on the microstructure of the flour samples were investigated. Figure 1 shows the micrographs of starch granules of BW flour samples. Starch granules of native flours (A images) exhibited the typical spherical-polygonal appearance, ranged between 2-14 µm of diameter, with a smooth surface and small indentations (Zhu, 2016). Images of treated samples (B and C corresponding to 1C+S40 and 2C+S40, respectively) revealed noticeable changes in the starch granular structure such as some deformations and increased surface roughness of the granules. Furthermore, the well-defined boundaries observed in control granules seemed to be dimmer, although the integrity of most granules was retained. These effects were in agreement with the findings of previous studies on the effect of HHP on buckwheat starch structure (Vallons and Arendt, 2009). The appearance of visible effects on starch after HHP treatment is reasonable as buckwheat starch is associated with A-type crystalline polymorph which is more vulnerable than other types to HHP treatments (Balasubramaniam et al., 2016). The loss of the shape and surface integrity in the starch granule was increased in the case of pre-soaked samples treated with double cycle. In those samples extragranular material, was observed probably of a proteinic nature, entwining and connecting the starch granules. Accordingly with Cao et al. (2018) HHP treatment could exert some modifications on protein structure to produce higher protein particle specific surface area, protein aggregation and more surface roughness. These structural modifications would be more pronounced in pre-soaked HHP-treated flours as can be observed in Figure 1.

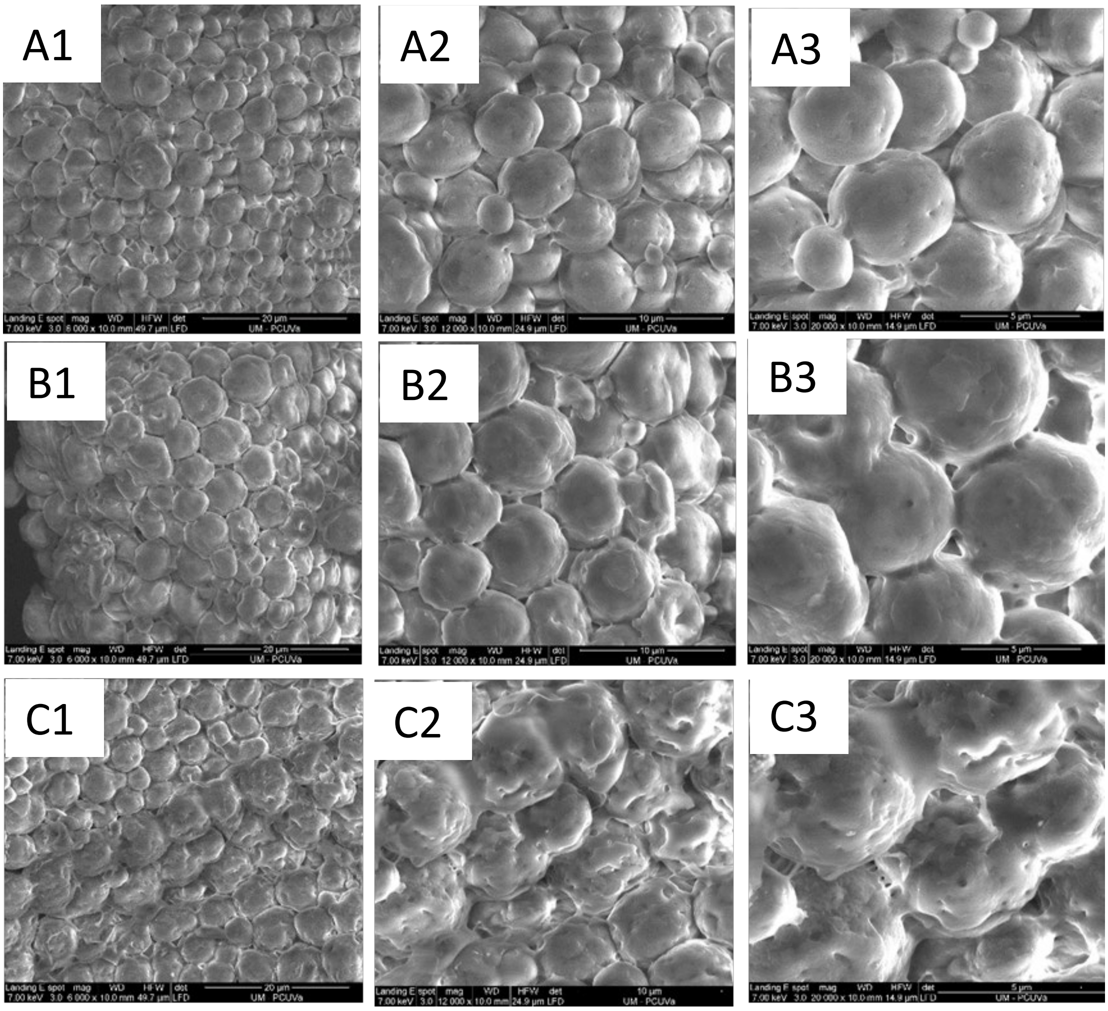

Figure 4.1.1. SEM images of flour samples obtained from native and HHP-treated buckwheat grains: A: Native; B: 1C+S40; C: 2C+S40, at different magnifications: 6000x (1), 12000x (2) and 20000x (3)

4.1.3 Flour colour analysis

Sample colour parameters are summarized in Table 4.1.2. Soaking pre-treatment significantly ($p<0.05$) affected the luminosity (L*) of the samples. All treated samples showed a significant decrease ($p<0.05$) of the luminosity as compared to the control. Pre-soaked and HHP double-cycle treated samples (2C+S40) exhibited the lowest values. Colour differences between native and treated flours (ΔE) were lower to 5, so the changes in BW flour colour were not sensory noticeable. Only a slight but significant ($p<0.05$) variation of the hue (h) was found for the un-soaked and single-cycle treated sample (1C) regarding the native flour. In contrast, significantly ($p<0.05$) bigger Chroma values (C*) for all treated samples in comparison with the native were found. Among the treated samples, significantly ($p<0.05$) increased values were observed for the double-cycled samples compared to the single-cycled ones irrespective of the soaking pre-treatment. Similar results have previously been reported by Yu et al. (2017), who evaluated the effect of HHP treatment with and without soaking on brown rice grains. Those authors stated the soaking treatment could help to diffuse pigments present in the outer layers of the grain to the water, so that the depressurization process could promote the migration of these components present in the pressure transmission water to the inner parts of the grains trough fissures. This phenomenon could be intensified when double pressurisation cycle is used and could explain the differences found in Chroma for HHP double-cycle treated samples compared to HHP single-cycle ones.

Table 4.1.2. Flour chromatic parameters and size dispersion of flour samples obtained from native and HHP-treated buckwheat grains.

SAMPLE	L*	a*	b*	C*	h	ΔE
Native	79.0 c	2.35 a	8.07 a	8.4 a	73.7 b	-
1C	77.1 b	2.75 b	8.89 b	9.3 b	72.8 a	2.1a
2C	75.5 a	2.82 bc	9.32 c	9.7 c	73.1 ab	3.7b
1C+S40	74.9 a	2.94 c	9.69 d	10.0 c	73.2 ab	4.6b
2C+S40	75.5 a	2.96 c	9.89 d	10.3 d	73.4 ab	4.0b
SE	0.4	0.06	0.1	0.1	0.2	0.4
Number of HHP Cycles	ns	ns	*	**	ns	ns
Soaking pre-treatment	*	*	**	**	ns	**
Cycles x Soaking	*	ns	ns	ns	ns	**

1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. Samples with different small letters show significant differences between treatments ($p<0.05$). SE: Pooled standard error from ANOVA. ** $p<0.01$, *$p<0.05$, ns: not significant.

4.1.4 Functional properties.

Results of the flour hydration properties (WHC and WAC), oil absorption capacity (OAC), gel hydration properties (WAI, WSI and SP) and surfactant activity properties (emulsifying activity and stability –EA, ES-, foaming capacity and stability –FC, FS-) are displayed in Table 1.

The impact of the soaking pre-treatment in the water holding and absorption capacity was significant ($p<0.01$) as pre-soaked samples exhibited a substantial increase, especially in WHC (between 25-28%) regarding the native one. Significant ($p<0.01$) effect of the soaking pre-treatment was also found in WAC. In this case, the pre-soaked with double-cycle samples showed a significant increase ($p<0.05$) of 12% compared to the control. Being carbohydrates and proteins mainly responsible for a flour water binding capacity (Vela et al., 2021), modification of these components would explain variations in the BW flour hydration properties observed after treatments. It was reported HHP treatment led to cold gelatinization of starches and enhanced their ability to bind water (Balakrishna et al., 2020). In addition, Yu et al. (2017) reported an increase in hydrogen bonding between water and starch after HHP processing which could promote water absorption.

Results showed a significant ($p<0.05$) increase in OAC (9%-14%) of treated samples regarding the native flour. However, none of the studied factors significantly affected this parameter. Changes in the mechanism of oil entrapment by proteins as consequence of HHP treatment have been reported by Cao et al. (2018) to explain this behaviour.

The effect of soaking pre-treatment on gel hydration properties was significant as HHP pre-soaked samples showed significantly ($p<0.05$) higher values in WAI and SP than un-soaked ones. As reported by Ravichandran et al (2018), a higher degree of gelatinization in paddy grains was promoted not only by HHP but also by a soaking pre-treatment. This effect could lead to an increase in the swelling volume, as well as an increase in the water absorption as the hydrogen bonding between starch molecules could be replaced by hydrogen bonding with water (Herlina, 2017).

Meanwhile, in WSI only the pre-soaked and HHP double-cycle treated sample had a significant ($p<0,05$) difference with the native one The protein agglomeration and increased solubility after HHP treatment of grains have been previously reported by Błaszczak et al. (2007).

Both cycle and soaking pre-treatment exerted a significant ($p<0.01$) effect on the FC of BW flours. A significant ($p<0.05$) and remarkable reduction in the foaming capacity of all studied samples regarding the native flour was observed. Pre-soaked samples showed the lowest FC values and these samples did not show any ability to retain the foam generated during the test. A similar trend was found in the emulsion activity and stability of tested samples, as a significant ($p<0.05$) and important drop was observed in these parameters, especially for the pre-soaked ones. Surface activity properties are mainly driven by the protein fraction of the BW flour. Balasubramaniam et al. (2016) reported that high-pressure promotes complex protein modifications which could impair the tertiary structure of proteins, disfavouring hydrophobic interactions. This phenomenon might have led to changes in the surfactant properties of HHP treated BW flours hindering their capacity to generate stable emulsions and foams.

4.1.5 Pasting properties

Viscosity profiles and pasting properties of samples are shown in Figure 2 and in Table 4.1.3, respectively. Pasting viscosity profile is mainly determined by starch behaviour but in whole-flours, other components such as fibre, proteins and fats may affect their pasting properties (Vela et al., 2021). The impact of soaking pre-treatment was remarkable as viscosity profiles of pre-soaked samples showed an overall decrease. The multifactor analysis of variance also revealed a statistically significant effect for soaking pre-treatment factor in Peak (PV), Breakdown (BV) and Final (FV) viscosities ($p<0.01$), and in Trough (TV) and Setback (SV) viscosities ($p<0.05$). Meanwhile, the "Cycle" factor affected significantly to FV ($p<0.05$). No significant changes regarding pasting temperature (PT) were observed, as around 65ºC was the temperature at which starch granules started to swell in all tested samples. Opposite results were found by Liu et al. (2016), who reported an increase in PT of a BW starch:water mixture subjected to a HHP treatment. Those authors attributed this effect to changes in the starch structure induced by HHP, enhancing the granule thermostability. This effect might be limited in the present study due to the presence of hull and the complex nature of the BW grain matrix that could mitigate the effects of the pressure on starch granules. Conversely, combined treatments had significant effect on pasting properties as both pre-soaked and HHP single and double cycle treated samples showed lower PV values than native flour (18% for 1C+S40 and 16% for 2C+S40) and the un-soaked ones. This decrease was consistent with previous findings of Liu et al. (2016). Furthermore, all HHP treated samples showed a significant ($p<0.05$) decrease in BV, ranging up from 88% for 2C+S40 to 93% for 1C+S40. Higher stability of the swollen granules under continuous heating and shearing was also been noted by Liu et al. (2016). Authors linked the reduction in BV with a limited granular swelling, as HHP promoted the granule's ability to withstand the continued heating and shearing, improving their stability. Similar results were obtained by Lin and Fernández-Fraguas, (2020) who stated this enhanced stability versus shearing and heating as a consequence of a reinforced crystalline structure and an entanglement between starch-protein/fibre caused by the high-pressure treatment. This increased stability in the starch granule might explained the significant ($p<0.05$) decrease in the SV results obtained in the HHP treated samples regarding the control (29% for 1C+S40 and 23% for 2C+S40). The increased rigidity of the starch granules might reduce amylose leaching during heat-paste cycle and hence, there would be less amylose free molecules able to retrogradate in the cool-paste cycle. Liu et al. (2016) also suggested the formation of stable amylose-lipid complexes could decrease starch retrogradation and SV parameter.

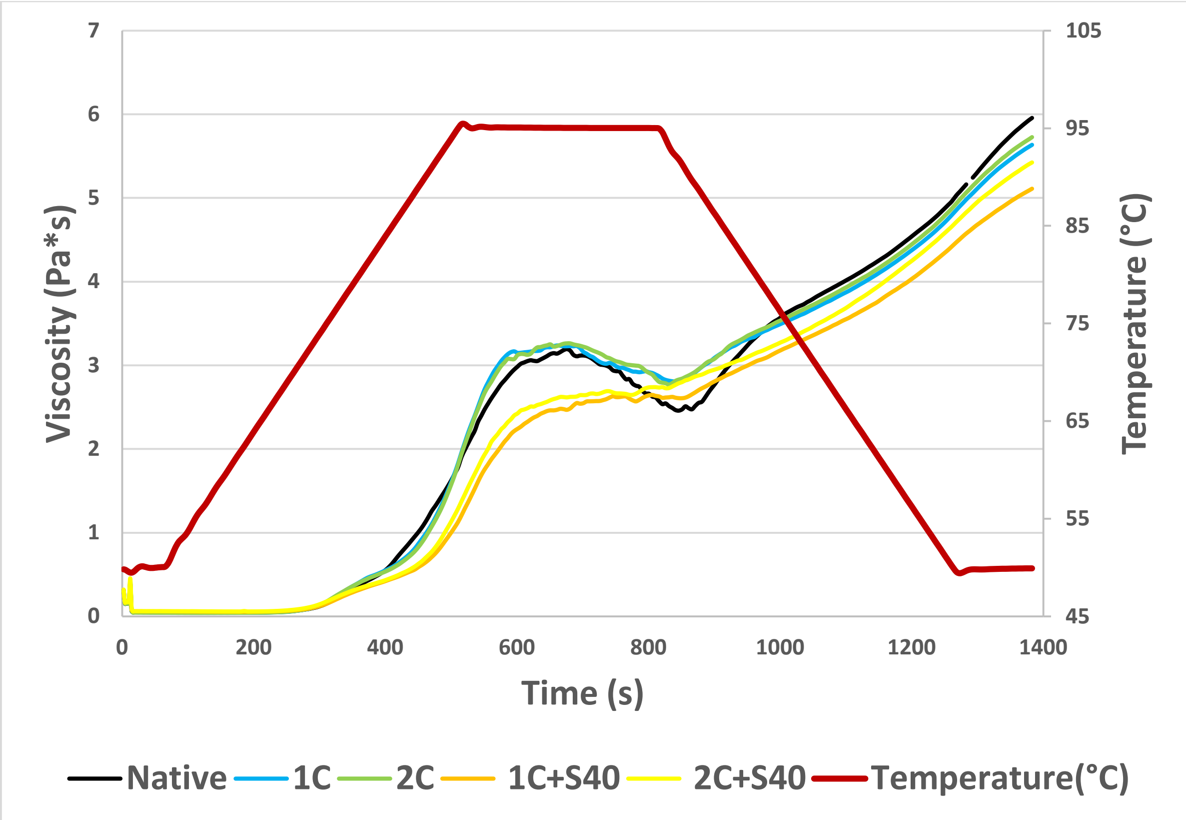

Figure 4.1.2. Pasting profiles of flour samples obtained from native and HHP-treated buckwheat grains: 1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample.

4.1.6 Rheological properties of gels

The mechanical spectra of control and treated gel samples obtained in the dynamic oscillatory test is showed in Figure 3, while the rheology parameters are summarized in Table 4.1.3. All treated samples showed a significant decrease ($p<0.05$) regarding the elastic and viscous moduli in comparison with the native gel sample, except the un-soaked HHP double-cycle sample in the elastic modulus. The G1` values of those treated samples decreased from 24 to 28% for 1C and 1C+S40 samples, respectively. The decreasing values for the viscous modulus compared to the control ranged from 30 to 36%. Therefore, the complex modulus values had significantly ($p<0.05$) lower outcomes for pre-soaked samples and 1C. On the basis of these results it can be stated that treatments given in this work led to a weakened gel structure. The loss tangent values of treated samples also showed significantly ($p<0.05$) lower values than the control except for 1C+S40. Although all samples exhibited a solid-like behaviour as Tan (δ)1 values were less than 1, results denoted a tendency to increase the weight of the viscous component of the gels after HHP treatment. This might be due to treatment enhanced granule structure resistance, so less amylose was available for gelling as suggested by Li and Zhu (2018).

All treated samples showed a significant ($p<0.05$) decrease in exponent value "a" denoting a reduction in the frequency dependence of G' comparing with the control sample. This agrees with the findings obtained by Li and Zhu (2018) after studying frequency sweeps of HHP treated starch gels of maize and quinoa. This lower value of the exponent "a" was associated by those researches with an enhance of the long-term stability of quinoa starch gel.

The soaking pre-treatment factor and its interaction with "Cycle" factor were found significant ($p<0.01$ and <0.05, respectively) in the maximum stress which gels could withstand before disrupting their structure (τmax). Significant ($p<0.05$) increased τmax values was shown for almost all treated samples, although it was less pronounced for pre-soaked samples. It was possible HHP treatment could promote some molecular interactions and/or starch structure modifications which raised the gel stability.

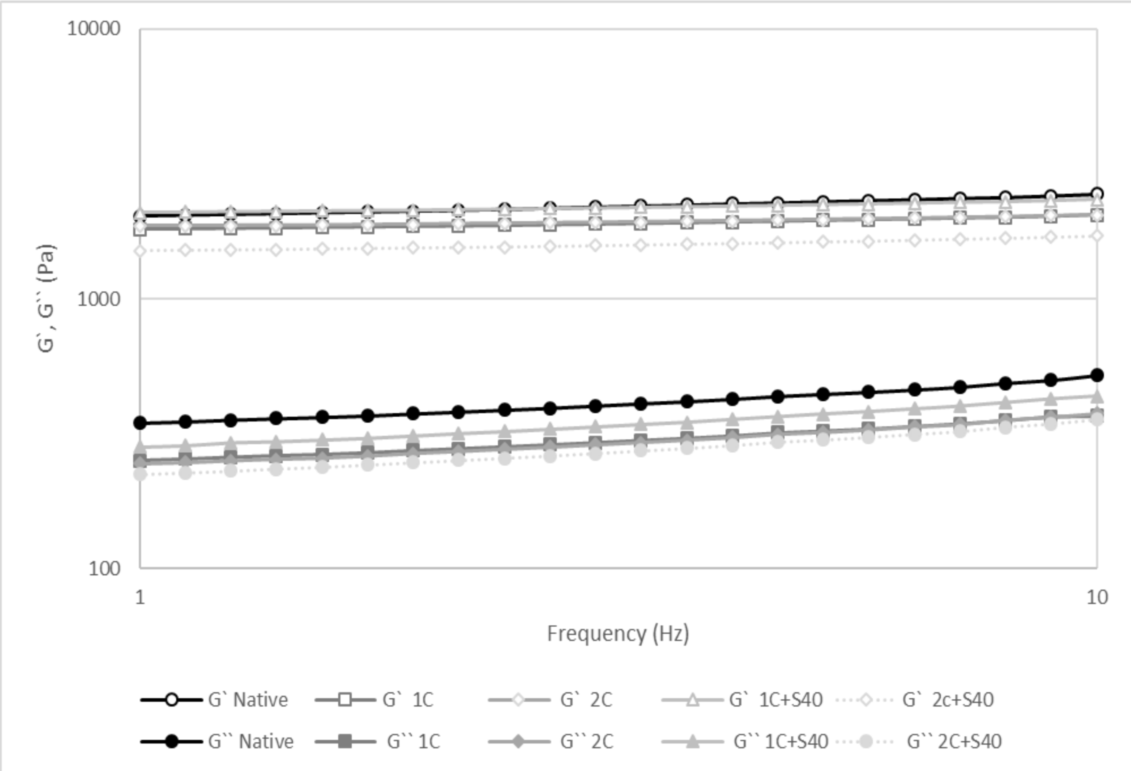

Figure 4.1.3. Effect of HHP treatment on viscoelastic moduli evolution vs frequency (Hz). Empty symbols represent the elastic modulus (G′); filled symbols represent the viscous modulus (G′′). 1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample.

Table 4.1.3. Pasting and rheology parameters of flour samples obtained from native and HHP-treated buckwheat grains.

SAMPLE	PT (ºC)	PV (Pa · s)	TV (Pa · s)	BV (Pa · s)	FV (Pa · s)	SV (Pa · s)	G'_1 (Pa)	a	G''_1 (Pa)	b	Tan $(\delta)_1$	c	G* (Pa)	Crosspoint (Pa)	$\tau_{máx}$ (Pa)
Native	64.4^{a}	3.20^{b}	2.40^{a}	0.79^{d}	6.03^{d}	3.63^{c}	2015^{b}	0.08^{b}	338^{c}	0.170^{a}	0.168^{c}	0.09^{a}	2043^{b}	494^{a}	352^{a}
1C	65.9^{a}	3.25^{b}	2.80^{c}	0.44^{b}	5.64^{bc}	2.83^{b}	1540^{a}	0.052^{a}	222^{ab}	0.189^{ab}	0.144^{ab}	0.14^{b}	1554^{a}	583^{a}	530^{c}
2C	65.5^{a}	3.28^{b}	2.77^{c}	0.51^{c}	5.73^{c}	2.96^{b}	1845^{b}	0.041^{a}	238^{b}	0.186^{ab}	0.129^{a}	0.15^{b}	1860^{b}	794^{b}	684^{d}
1C+S40	65.7^{a}	2.63^{a}	2.54^{ab}	0.09^{a}	5.11^{a}	2.57^{a}	1455^{a}	0.051^{a}	226^{ab}	0.205^{b}	0.156^{bc}	0.15^{b}	1471^{a}	528^{a}	451^{bc}
2C+S40	65.7^{a}	2.69^{a}	2.63^{bc}	0.06^{a}	5.43^{b}	2.79^{b}	1495^{a}	0.054^{a}	218^{a}	0.199^{b}	0.146^{ab}	0.15^{b}	1511^{a}	519^{a}	396^{ab}
SE	0.7	0.06	0.06	0.01	0.07	0.06	76	0.006	5	0,008	0.005	0.01	76	47	22
Number of HHP Cycles	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soaking pre-treatment	ns	**	*	**	**	*	ns	ns	ns	ns	ns	ns	ns	*	**
Cycles x Soaking	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. PT: Pasting Temperature, PV: Peak Viscosity, FV: Final Viscosity, TV: Trough Viscosity, BV: Breakdown Viscosity, SV: Setback Viscosity. G'_1 and G''_1 represent elastic and viscous moduli at a frequency of 1 Hz. τ_{max} represent the maximum stress in viscoelastic lineal region (LVR) obtained from the strain sweep. The crosspoint value represents the stress at G\` = G\`\`.SE: Pooled standard error from ANOVA. Samples with different small letters show significant differences between treatments ($p<0.05$). ** $p<0.01$, *$p<0.05$, ns: not significant.

4.1.7 Thermal properties.

The thermal properties obtained from DSC tests and used to analyse the gelatinization (first scan) and retrogradation (second scan) transitions of the samples are shown in Table 4.1.4. Endothermic thermal transition data showed two peaks, the first has been related to starch gelatinization and the other has been associated with protein denaturation and amylose-lipid complex dissociation (Lin and Fernández-Fraguas, 2020). The gelatinization thermal parameters values of the native flour were found to be in the range of results reported by Zhu (2016). Soaking pre-treatment had a significant ($p<0.05$) effect in gelatinization enthalpy (ΔHgel). Pre-soaked samples exhibited a significant ($p<0.05$) reduction in ΔHgel compared to the native one (20% for 1C+S40 and 25% for 2C+S40) and to the un-soaked samples. This result suggests that starch in pre-soaked samples could be partially gelatinized regardless of the number of cycles of HHP-treatment. Gelatinization could happen as buckwheat starch has an A-type crystalline structure, which has the highest sensitivity to cold-gelatinize in a high-pressure treatment (Balasubramaniam et al., 2016). Another key factor to trigger cold-gelatinization is the presence of water (Balakrishna et al., 2020). Kim et al. also reported that at least 50% of moisture content is necessary to partially gelatinize a starch-water suspension. Considering that the pressure was applied over whole buckwheat grains, probably the presence of the hull could provide a physical barrier limiting moisture diffusivity, mainly in not pre-treated by soaking samples. However, the soaking pre-treatment might provide enough moisture to the inner parts of buckwheat grains to produce some degree of gelatinization. Ravichandran et al. (2018) also confirmed that gelatinization degree for pre-soaked and HHP treated paddy grains was higher than that of un-soaked ones.

All pre-soaked samples also exhibited a significant ($p<0.05$) reduction in the gelatinization temperature range (ΔT), caused by an increase in the onset temperature (To-gel). The ΔT reduction of BW starch samples treated with HHP has been previously reported by Liu (2016). Lower values of the gelatinization peak width indicate higher starch crystallites homogeneity and a better organized granular structure, requiring a shorter temperature range to fully hydrate.

The amylose-lipid complex dissociation peak had an enthalpy of 0.9 J/g in the native sample, which is consistent with the data reported by Zhu (2016). All treated samples showed lower values, although not significantly different, than the native flour. Small differences were also found by Lin and Fernández-Fraguas (2020) in the peak temperature of the amylose-lipid complex dissociation.

No significant differences were observed in the retrogradation enthalpy (ΔHret) caused by amylopectin recrystallization after one week of storage at 4ºC. Li and Zhu (2018) also reported minor variations in the retrogradation temperatures studying the thermal effect of HHP-treatment on quinoa and maize starch. In contrast, in this second scan, a significant ($p<0.05$) reduction in the amylose-lipid enthalpy (ΔHam-lip) was found for the treated samples compared to the native one. In particular, pre-soaked samples exhibited a significant ($p<0.05$) decrease in ΔHam-lip compared to un-soaked ones. ΔHam-lip in the second scan also showed increased values compared to the first scan. Vela et al. (2021) suggested that these higher values appeared because better complex formation conditions occurred as consequence of amylose leakage above the gelatinisation temperature.

Table 4.1.4. Thermal properties of flour samples obtained from native and HHP-treated buckwheat grains.

	First scan							Second scan						
SAMPLE	**ΔH_{gel} (J/g)**	**$T_{o\text{-}gel}$ (ºC)**	**$T_{p\text{-}gel}$ (ºC)**	**$T_{e\text{-}gel}$ (ºC)**	**ΔT (ºC)**	**$\Delta H_{am\text{-}lip}$ (J/g)**	**$T_{p\text{-}am\text{-}lip}$ (ºC)**	**ΔH_{ret} (J/g)**	**$T_{o\text{-}ret}$ (ºC)**	**$T_{p\text{-}ret}$ (ºC)**	**$T_{e\text{-}ret}$ (ºC)**	**ΔT (ºC)**	**$\Delta H_{am\text{-}lip}$ (J/g)**	**$T_{p\text{-}am\text{-}lip}$ (ºC)**
Native	8.9 ^{b}	56.9 ^{a}	66.4 ^{a}	75.0 ^{a}	18.1 ^{c}	0.9 ^{a}	96.9 ^{a}	2.4 ^{a}	33.6 ^{a}	48.7 ^{a}	59.9 ^{a}	26.3 ^{a}	2.15 ^{c}	95.6 ^{a}
1C	8.3 ^{b}	56.8 ^{a}	66.5 ^{a}	74.6 ^{a}	17.8 ^{bc}	0.8 ^{a}	96.1 ^{a}	2.6 ^{a}	25.5 ^{a}	48.5 ^{a}	60.3 ^{a}	34.9 ^{a}	1.48 ^{b}	95.5 ^{a}
2C	8.8 ^{b}	56.9 ^{a}	66.4 ^{a}	74.2 ^{a}	17.3 ^{bc}	0.4 ^{a}	96.3 ^{a}	2.9 ^{a}	37.9 ^{a}	47.2 ^{a}	58.9 ^{a}	21.0 ^{a}	1.54 ^{b}	94.0 ^{a}
1C+S40	7.1 ^{a}	58.5 ^{b}	66.6 ^{a}	74.3 ^{a}	15.8 ^{a}	0.5 ^{a}	96.9 ^{a}	2.3 ^{a}	38.2 ^{a}	47.8 ^{a}	58.4 ^{a}	20.2 ^{a}	1.13 ^{a}	93.9 ^{a}
2C+S40	6.7 ^{a}	58.2 ^{b}	66.4 ^{a}	74.0 ^{a}	15.8 ^{a}	0.3 ^{a}	97.7 ^{a}	3.1 ^{a}	28.4 ^{a}	48.4 ^{a}	67.8 ^{a}	39.4 ^{a}	1.02 ^{a}	93.0 ^{a}
SE	0.3	0.2	0.2	0.3	0.2	0.2	0.9	0.5	4.8	0.1	3.0	6.0	0.07	1.0
Number of HHP Cycles	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soaking pre-treatment	*	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns
Cycles x Soaking	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	*	ns	ns

1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. ΔH_{gel}: Enthalpy of gelatinization, T_o, T_p, $T_{e\text{-}gel}$: Onset, peak and endset temperature of gelatinization, ΔT: $(T_e\text{-}T_o)_{gel/ret}$, $\Delta H_{am\text{-}lip}$: Enthalpy of amylose-lipid complex dissociation, $T_{p\text{-}am\text{-}lip}$: Peak temperature of the amylose-lipid complex dissociation, ΔH_{ret}: Enthalpy of retrograded amylopectin, T_o, T_p,$T_{e\text{-}ret}$: Onset, peak and endset temperatures of melted retrograded amylopectin. Samples with different small letters show significant differences between treatments ($p<0.05$). SE: Pooled standard error from ANOVA. ** $p<0.01$, *$p<0.05$, ns: not significant.

4.1.8 Total phenol and total antioxidant capacity

The total phenol content (TP) and total antioxidant capacity (TAC) of buckwheat flours were determined in extracts using DPPH and in the flour samples using direct DPPH method (Table 4.1.5).

Control samples showed a TP value of 311 mg GAE/100g, similar to those reported by Quettier-Deleu et al. (2000), although variations of TP in buckwheat flour have been reported in the literature associated with variety, location and environmental conditions (Zhang et al., 2017). TP was significantly affected by the soaking pre-treatment ($p<0.01$) and the number of HHP cycles applied ($p<0.05$). A significant ($p<0.05$) increase was observed in TP in un-soaked and pressurized samples using one cycle (1C) meanwhile not significant differences with native flour were observed when a double cycle was applied to the sample (2C). Other authors have already reported an increase in phenol content in other vegetal matrixes after HHP treatment (Balasubramaniam et al., 2016). This effect was associated with the improvement in the extractability of bioactive compounds due to the physical effect of the pressure. The cell permeabilization and the pressure-driven mass transport triggering a mass-transfer phenomenon (osmosis) could be also taken as plausible explanation (Balakrishna et al., 2020). The use of soaking pre-treatment significantly ($p<0.05$) improved TP content over the native flour regardless of the number of HHP cycles. This pre-treatment combined with HHP probably promoted a better extractability of phenol content increasing the solubility of the phenols.

The evaluation of total antioxidant capacity showed that native sample showed higher DPPH values (373 mg TE/100g) than un-soaked and pressurized samples regardless of the use of single or double HHP treatment. However, the use of soaking pre-treatment produced an enhancement of the antioxidant properties of pressurized with one cycle samples reaching values not significantly different to the native flour. A similar behaviour was observed in the study when direct method (Q-DPPH) was used. The multifactor analysis of variance showed a significant effect of the soaking pre-treatment ($p<0.01$). The application of HHP reduced the DPPH value (41%) although the use of soaking enhanced the antioxidant activity of both single or double HHP treated samples. The overall increase in the antioxidant markers of the pre-soaked samples respecting the un-soaked ones could be attributed to the increased extractability of phenolic compounds, already reported to be potent radical scavengers (Bautista-expósito et al., 2020). Q-DPPH values were higher compared to those observed in extracts DPPH. This difference has been associated to a higher proportion of non-extractable cell-wall bound phenols and hence, non-measurable by classical methods (Rico et al. 2020).

Few information is available on the impact of HHP treatment of whole grains on the antioxidant capacity of resulting flours. Zhou et al. (2015) observed a reduction in the percentage of DPPH inhibition of HHP treated buckwheat flour (600 MPa, 2 cycles, 15 min/cycle, room temperature). In addition, high retaining of the antioxidant capacity (DPPH) of hydrophilic extracts after high-pressure treatment (200 MPa during 4 and 9 min) of BW raw groats was previously reported by Błaszczak (2013). No significant changes ($p>0.05$) in the antioxidant capacity of the extracts against the DPPH radical were also observed by Xia and Li, (2018) when HHP-treated matrices of wholegrain brown rice were evaluated (150-450 MPa, 10 min). Balasubramaniam et al. (2016) have stated HHP affects only non-covalent bonds so bioactive compounds should be preserved after HHP treatment.

4.1.9 Minerals content

Table 3 shows the minerals content of control and HHP treated flours. The data obtained in the present study for the control sample were similar to those reported by Steadman et al. (2001) for whole buckwheat groats fraction. The minerals content was significantly affected by the soaking pre-treatment, except for calcium, while the number of HHP cycles significantly affected the calcium and iron content. In general, un-soaked flour samples had a lower minerals content than native sample regardless of the number of cycles used and the mineral analysed, showing that the application of HHP treatment without soaking produced a negative effect on the mineral content. However, the soaking pre-treatment enhanced the concentration of B, Fe, Mn and Zn in the flours compared to un-soaked ones, probably due to the increase in their extractability. As it was observed in the case of TP, contradictory information has been published about the influence of HHP treatment on the minerals content of flours. Briones-Labarca et al. (2011) reported a loss of minerals content after HHP treatment while Xia and Li (2018) observed a retention of Zn and Fe after a mild HHP treatment (30-90 MPa, 5 min). Although Xia and Li (2018) reported hydrothermal treatments could release minerals to the medium, those probably could interact with other component such as phytic acid or protein, reducing the diffusion to the pressure transmission water. Therefore, variations in the minerals content could be related to changes in phytate content or protein hydrolysis during HHP treatment. In addition, those authors suggested other factors as enzyme activity could be involved in fluctuations of minerals content in the grains.

Table 4.1.5. Antioxidant properties and mineral content of flour samples obtained from native and HHP-treated buckwheat grains.

SAMPLE	TP (mg Eq. AG/ 100g)	DPPH (mg Eq. Trolox /100g)	Q-DPPH (mg Eq. Trolox /100g)	B (mg/100g)	Ca (mg/100g)	Fe (mg/100g)	Mn (mg/100g)	Zn (mg/100g)
Native	311 a	373 b	401 b	0.445 bc	35 b	7.00 b	0.50 b	1.93 c
1C	338 b	252 a	245 a	0.404 ab	30 ab	3.85 a	0.40 a	1.69 ab
2C	297 a	219 a	230 a	0.363 a	20 a	3.15 a	0.40 a	1.57 a
1C+S40	361 b	366 b	391 b	0.487 c	30 ab	7.10 b	0.50 b	1.84 bc
2C+S40	347 b	238 a	374 b	0.564 d	25 ab	3.45 a	0.45 ab	1.89 c
SE	7.4	24.0	18.0	0.016	3,0	0.37	0.02	0.05
Number of HHP Cycles	*	*	ns	ns	*	**	ns	ns
Soaking pre-treatment	**	ns	**	**	ns	**	*	**
Cycles x Soaking	ns	ns	ns	*	ns	*	ns	ns

1C: HHP single-cycle treated sample; 2C: HHP double-cycle treated sample; 1C+S40: Pre-soaked and HHP single-cycle treated sample; 2C+S40: Pre-soaked and HHP double-cycle treated sample. TP: Total phenols; DPPH: antioxidant capacity against the DPPH radical in sample extracts; Q-DPPH: antioxidant capacity against the DPPH radical in solid samples. Samples with different small letters show significant differences between treatments ($p<0.05$). SE: Pooled standard error from ANOVA. ** $p<0.01$, *$p<0.05$, ns: not significant.

CHAPTER 2

Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time.

4.2 Characterisation of whole buckwheat flours obtained from HHP treatments of whole grains for the production of improved gluten-free functional ingredients: effect of pressure and holding time.

4.2.1 Particle size distribution of the flours

Flour granulometry data are presented in table 4.2.1. The factors HHP pressure level and holding time had a significant ($p<0.01$) effect on the median diameter (D_{50}). The flours resulting from applying the higher pressure level of the HHP treatments at holding times of 5 and 15 min., showed significantly ($p<0.05$) higher D_{50} values than the native flour. However, an opposite effect was found for those samples treated at 300 MPa. For the size dispersion, the effect of holding time was found to be significant ($p<0.01$). In this case, all treated samples showed significantly ($p<0.05$) lower values than the native flour. Moreover, the samples obtained from a treatment time of 0 min had the closest value to the native flour. Longer treatment times significantly decreased the size dispersion of t the flour samples.

HHP could induce volume compaction of the treated matrix (Ravichandran et al., 2018a), thus modifying grain fracturability and increasing median diameter values, as shown in previous studies (Gutiérrez et al., 2022). This effect was more pronounced in the samples treated at 600 MPa and with the longest holding times (5, 15 min). The increase in flour size particle could also be related to starch modification at a pressure level of 600 MPa, which could lead to aggregation of the modified starch granules (Ahmed et al., 2016).

Table 4.2.1. Particle size distribution and colorimetric parameters of flours samples obtained from native and HHP treated BW grains.

SAMPLE	D_{50} (µm)	Size Dispersion	L*	a*	b*	ΔE	C*	h
Native	109.1 ^{b}	2.42 ^{d}	84.4 ^{c}	2.91 ^{bc}	7.8 ^{b}	-	8.3 ^{b}	69.5 ^{b}
0-300	101.9 ^{a}	2.31 ^{c}	80.9 ^{b}	2.59 ^{a}	7.1 ^{a}	3.6 ^{c}	7.6 ^{a}	69.9 ^{bc}
5-300	101.6 ^{a}	2.06 ^{a}	86.2 ^{d}	3.04 ^{c}	8.7 ^{c}	2.1 ^{b}	9.2 ^{c}	70.8 ^{d}
15-300	101.7 ^{a}	2.14 ^{b}	84.8 ^{c}	3.05 ^{c}	8.6 ^{c}	1.0 ^{a}	9.1 ^{c}	70.4 ^{cd}
0-600	107.7 ^{b}	2.28 ^{c}	85.8 ^{d}	2.79 ^{b}	8.6 ^{c}	1.7 ^{ab}	9.1 ^{c}	72.1 ^{e}
5-600	125.8 ^{c}	2.06 ^{a}	84.2 ^{c}	3.37 ^{d}	9.1 ^{d}	1.5 ^{ab}	9.7 ^{d}	69.8 ^{bc}
15-600	127.0 ^{c}	2.08 ^{a}	78.8 ^{a}	3.36 ^{d}	8.5 ^{c}	5.6 ^{d}	9.1 ^{c}	68.5 ^{a}
SE	0.7	0.02	0.3	0.06	0.1	0.3	0.1	0.2
				Multifactor ANOVA				
Holding time	**	**	**	**	**	**	**	**
Pressure level	**	ns	**	**	**	**	**	ns
Holding time x pressure level	**	ns	**	ns	**	**	**	**

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. D_{50}: Median diameter; where 50% of particles had smaller particle size; Size dispersion: $(D_{90}-D_{10})/D_{50}$; L*: luminosity; a* and b*: chromatic colour coordinates; ΔE: Difference of colour between each treated sample and the flour obtained from native grains; C*: Chroma; h: hue. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.2.2 Colour of the flours

The colour parameters of the flour samples are also shown in Table 4.2.1. Pressure level and holding time factors had a significant ($p<0.01$) effect in those parameters. Slight but also significant differences ($p<0.05$) were found between the samples according to the HHP treatment conditions. The sample from the most intense treatment conditions (600 MPa, 15 min) showed the most notable changes, with a significant decrease ($p<0.05$) in luminosity and an increase in a* and b* values. This sample also showed the greatest colour variation (ΔE) with respect to the colour of the native flour. Minor but statistically significant ($p<0.05$) changes, were also observed for hue (h) in the samples from HHP treatments compared to the native one. A lower but significant ($p<0.05$) chroma (C*) value was found in the 0-300 sample, while significantly ($p<0.05$) higher values for this parameter were observed in the other samples compared to the native flour. The increases in chroma values are related to increases in the intensity of the red and yellow colours of these samples. Previously, increases in the chroma values of BW flours resulting from HHP (Gutiérrez et al., 2022) and ultrasound (Harasym et al., 2020) grain treatments have been reported. These authors attributed these increases to a diffusion effect of the soluble pigments present (carotenoids and xanthophyll) from the bran to the endosperm.

4.2.3 Functional properties

The functional properties of the native and HHP treated BW flours are presented in Table 4.2.2. The pressure level and its interaction with the holding time factors significantly ($p<0.05$) influenced the water absorption capacity (WAC) of the resulting flours. The samples treated at 300 MPa showed a significant ($p<0.05$) decrease (5 – 7 %) in the WAC values with respect to the native flour sample. On the contrary, the flours obtained from the highest pressure level treatments showed a significant ($p<0.05$) increase in their water-binding capacity (7- 10 %). It has been reported that biochemical changes in proteins and carbohydrates, the main components of flour, can lead to changes in their water absorption capacity. Błaszczak et al. (2007) stated that HHP treatments above 150-200 MPa could promote the disruption of the protein tertiary structure, resulting in the exposure of non-polar amino acids to the surrounding water. It has also been observed that non-polar amino acid groups can hold much less water than polar ones (Kuntz et al., 1971). This phenomenon could explain the decrease in WAC observed for the samples treated at 300 MPa. Conversely, it has also been suggested that pressures above 400 MPa could lead to cold gelatinisation of starches (Balakrishna et al., 2020) which could counteract the hydrophobicity of the HHP-modified proteins in flours, as HHP modified starch has been shown to have an increased water-binding capacity (Ahmed, Thomas, et al., 2018).

A significant effect ($p<0.01$) of the holding time and pressure level factors was found for WAI and SP parameters. Pressure treatments above 300 MPa or holding times longer than 0 min at 300 MPa significantly ($p<0.05$) increased the WAI and SP values of the samples. Meanwhile, a significant ($p<0.05$) decrease in WSI was observed for samples treated for 0 min (with a decrease of almost 18% for the 0-300 sample) or 5 min at 300 MPa (8 %). An increase in the swelling power of common buckwheat starch after HHP treatment (50 °C, 20 min) has already been reported by increasing the pressure level from 360 to 600 MPa (H. Liu et al., 2016). The authors suggested the appearance of amylose aggregates which induced greater water retention. That work also showed a reduction in the WSI of starch samples with HHP treatments at 70°C from 120 MPa to 600 MPa. The result was attributed to the formation of amylose-lipid complexes with HHP treatment, which reduced the mobility of soluble amylose molecules.

Holding time and pressure level also significantly ($p<0.01$) affected the emulsion and foaming properties of the resulting flours. A significant ($p<0.05$) loss of emulsion activity (EA) was observed for samples obtained from HHP treatments of 300 MPa for 15 min or 600 MPa for 5 and 15 min. There was also a significant loss ($p<0.05$) in emulsion stability (ES) (46% - 73%) for samples from 600 MPa or 300 MPa for 5 min treatments. The loss of foaming properties of the samples was also significant ($p<0.05$) for those treated at 600 MPa or 300MPa for 5 and 15 min. This behaviour was consistent with results previously reported by Gutiérrez et al. (2022). Depending on the HHP treatment conditions, some authors have suggested that high pressures could hinder emulsion activity of flours by changing the distribution pattern of the unfolded protein, favouring a higher proportion of hydrophilic groups on the surface (Lin & Fernández-Fraguas, 2020a). Similarly, other authors have reported a decrease in foaming capacity (FC) after HHP treatment of pea protein isolates. They attributed this to the formation of unfolded proteins and subsequent aggregation between them. These aggregates could have reduced the flexibility of the protein and its ability to encapsulate air bubbles (Chao et al., 2018).

Table 4.2.2. Hydration, emulsion and foaming properties of flours samples obtained from native and HHP treated BW grains.

SAMPLE	WAC (g/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)	FC (mL/g)	FS (%)	EA (%)	ES (%)
Native	1.21 c	6.12 a	5.68 c	6.49 a	0.70 c	28.6 b	54.4 cd	10.3 c
0-300	1.12 a	6.20 a	4.67 a	6.51 a	0.65 c	15.5 a	52.8 bcd	10.3 c
5-300	1.15 ab	6.55 bc	5.24 b	6.91 bc	0.50 b	-	51.4 bc	5.6 b
15-300	1.15 b	6.73 d	5.52 c	7.12 d	0.50 b	-	50.0 b	2.8 a
0-600	1.33 e	6.44 b	4.92 a	6.77 b	0.50 b	-	55.6 d	5.6 b
5-600	1.29 d	6.70 cd	5.52 c	7.09 cd	0.40 a	-	38.9 a	2.8 a
15-600	1.29 d	6.83 d	5.42 bc	7.22 d	0.40 a	-	38.6 a	2.8 a
SE	0.01	0.05	0.09	0.06	0.00	0.4	1.1	0.1
				Multifactor ANOVA				
Holding time	ns	**	**	**	**	**	**	**
Pressure level	**	**	ns	**	**	**	**	**
Holding time x Pressure level	*	ns	ns	ns	ns	**	**	**

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. WAC: Water absorption capacity, OAC: Oil absorption capacity, WAI: Water absorption index, WSI: Water solubility index, SP: Swelling power, FC: Foaming capacity, FS: Foaming stability, EA: Emulsion activity. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.2.4 Pasting properties

The pasting parameters and viscosity profiles are shown in Figure 1 and in Table 4.2.3, respectively. The HHP processing factors (holding time, pressure and their interaction) significantly influenced the results for peak, trough, breakdown ($p<0.01$) and setback viscosity ($p<0.05$). No significant changes were found in the pasting temperature (PT) of the samples, except for those obtained from a treatment at 600 MPa for 5 and 15 min, which showed a significant ($p<0.05$) decrease in this parameter compared to the native sample. In contrast, a significant increase ($p<0.05$) in the peak viscosity (PV) was observed, for those samples resulting from holding times of 0 min or those at 300 MPa regarding the native PV value. An overall and significant ($p<0.05$) increase in trough viscosity (TV) of the flour samples compared to the native one was observed. This resulted in a general decrease in the breakdown viscosity (BV) values of the samples. The results observed for final viscosity (FV) followed a similar trend to that observed for PV, except for the samples treated at 600 MPa for5 and 15 min, with an overall significant ($p<0.05$) increase in viscosity in the treated flours compared to the native flour. With the exception of the samples resulting from the treatments at 300 MPa for at 0 and 15 min, the setback viscosity values of the flours showed a significant ($p<0.05$) decrease with respect to the native flour.

The results obtained in the present study for the viscosity profiles and particularly for PV were different from those previously reported by Gutiérrez et al. (2022) for flour from BW grains treated at 600 MPa, probably due to the different holding time used in the previous study (30 min). Literature have also shown a decrease in PV with increasing pressure (120-600 MPa) after 20 min treatment of common BW starch slurry at 20 % (w:v) (H. Liu et al., 2016). However, other authors have observed an increase in the PV of HHP-treated lentil starch dispersions (1:4, w/w) (Ahmed et al., 2016) or in a lotus starch (15%, w/w) (Guo et al., 2015). This previous study attributed the increase in PV to swollen starch granules which had a higher resistance. It is known that the extent of gelatinization is given by the HHP processing conditions (H. S. Kim et al., 2012). For a given starch concentration and temperature, the pressure level and holding time determine the gelatinisation degree (H. S. Kim et al., 2012). As the presence of water is also a prerequisite for triggering pressure gelatinization (Balakrishna et al., 2020), it can be assumed that the degree of hydration improves with increasing pressure and holding time (H. S. Kim et al., 2012). Therefore, further functional changes are expected under more intense HHP treatment conditions, such as the higher thermal stability observed for the samples treated at 600 MPa during 5 and 15 min. These results were similar to those previously reported by Gutiérrez et al. (2022) and Liu et al. (2016). These authors attributed this improved thermostability to changes in the crystalline structure, the formation of amylose-lipid complexes or entangled amylopectin molecules, which increased the rigidity of the starch granule and thus limited the leaching of amylose. This effect could explain the lower SV values, as the lower the amylose content in the medium, the lower the amount of retrograded amylose could occur. On the other hand, under mild treatment conditions, such as the treatments applied at 300 MPa, the structural changes induced by HHP would be more limited, although sufficient to promote rapid swelling of the starch granules.

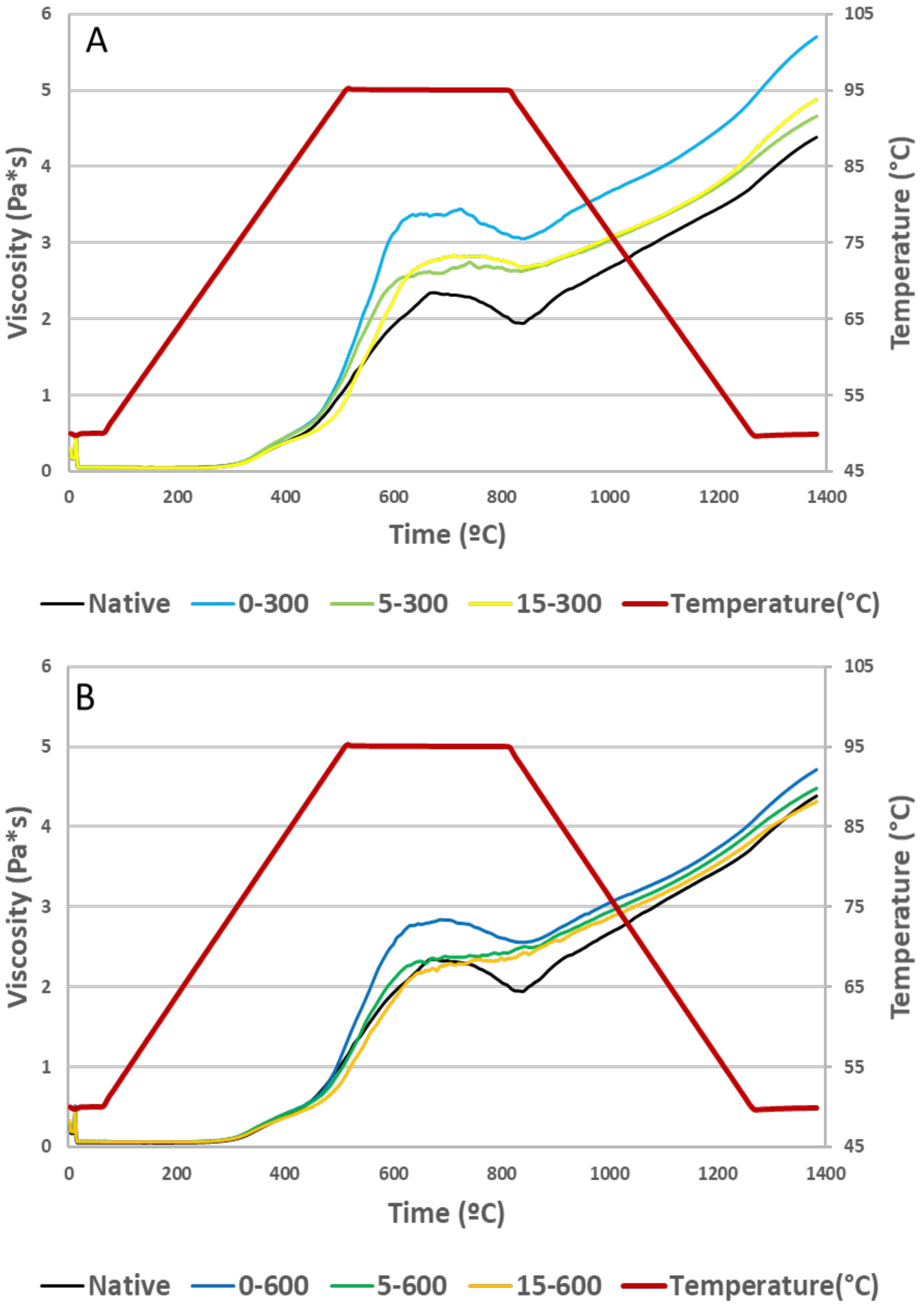

Figure 4.2.1. Pasting profiles of flour samples obtained from native and HHP treated BW grains. 0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min (A). 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min (B).

Table 4.2.3. Pasting properties of flours samples obtained from native and HHP treated BW grains.

SAMPLE	PT (ºC)	PV (Pa · s)	TV (Pa · s)	BV (Pa · s)	FV (Pa · s)	SV (Pa · s)
Native	67.71 b	2.35 a	1.93 a	0.42 cd	4.38 ab	2.45 c
0-300	67.96 b	3.54 d	3.30 d	0.24 b	5.77 e	2.47 c
5-300	67.17 ab	2.75 b	2.61 c	0.14 ab	4.65 cd	2.04 a
15-300	67.67 ab	2.74 b	2.24 b	0.51 d	4.56 bcd	2.33 bc
0-600	67.68 ab	2.85 c	2.56 c	0.3 bc	4.72 d	2.16 ab
5-600	66.68 a	2.39 a	2.35 b	0.04 a	4.48 abc	2.12 ab
15-600	66.68 a	2.33 a	2.27 b	0.06 a	4.32 a	2.05 a
SE	0.3	0.02	0.05	0.05	0.06	0.07
Multifactor ANOVA						
Holding time	ns	**	**	**	**	*
Pressure level	ns	**	**	**	**	*
Holding time x Pressure level	ns	**	**	**	**	*

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. PT: Pasting Temperature, PV: Peak Viscosity, FV: Final Viscosity, TV: Trough Viscosity, BV: Breakdown Viscosity, SV: Setback Viscosity. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.2.5 Thermal properties

The effect of HHP treatment on the thermal properties of the resulting BW flours are displayed in Table 4.2.4. Thermal scanning showed two endothermic peaks related to starch gelatinisation and amylose-lipid dissociation (Vicente et al., 2023a). The former is associated with the melting of A-type crystals, typical of BW starch (Z. Zhou et al., 2015). The thermal properties observed for the native flour were in the range of those previously reported (Perez-Rea & Antezana-Gomez, 2018; Zhu, 2016). The gelatinisation enthalpy results were significantly ($p<0.05$) influenced by pressure and holding time. A significantly ($p<0.05$) higher enthalpy was found for the 0-300 sample, while a significantly ($p<0.05$) lower value of this parameter was found for the samples resulting from a treatment at 600 MPa for 15 min compared to the native value. A decrease in the gelatinisation enthalpy has previously been reported for BW flours resulting from HHP treatment of grains (Gutiérrez et al., 2022) and for those resulting from HHP treatment of starch suspensions (Vallons & Arendt, 2009a). Lower gelatinisation enthalpy values could be associated with partially gelatinised samples (Fu et al., 2012). In addition, increasing the intensity of the pressure treatment could increase the extent of gelatinisation, as less energy is required to disrupt the hydrogen intra-helixes bonds of the crystalline regions as the pressure level increases (Yang et al., 2017). Increased gelatinisation extent with increasing holding time was also observed by Zhang et al.,

(2022) after HHP applying HHP to oat starch slurries at 500 MPa and increasing the pressure-treatment time from 10 min to 15 min.

Slight, but also significant ($p<0.05$) changes were observed in the temperature thermal parameters (To, Tp and Te). Decreased values of onset (To) and peak (Tp) temperatures were observed for the flour resulting from HHP treatment at 300 MPa for 0 min compared to the native one, but with increasing treatment intensity (5/15 min – 600 MPa samples) a trend of increasing To and decreasing endset temperature (Te) was shown. Thus, a significant ($p<0.05$) reduction in the gelatinisation temperature range (ΔT) values of these samples was observed with respect to the native flour. A similar reduction was previously reported by Vallons & Arendt (2009) and Liu et al. (2016) for BW starch suspensions (25% and 20% w/w, respectively) at the highest pressure levels studied (600 MPa). This behaviour was also observed in pressure treated BW whole grains (Gutiérrez et al., 2022) and was attributed to a higher organisation of the crystalline structure.

A second melting transition peak related to the formation of V-type starch-lipid complexes was detected. The melting enthalpy of the native sample was 1.2 J/g, which is in the range of those values previously reported by (F. Zhu, 2016) for BW starch. Such inclusion complexes are usually formed with amylose due to its flexible chain structure (Lu & Baik, 2015). The flours resulting from the treatments at 600 MPa for 5 and 15 min had a significantly lower value ($p<0.05$) compared to the native flours, showing similar values (0.5 J/g) to those previously reported by Gutiérrez et al. (2022) after HHP treatment of pre-soaked whole BW grains at 600 MPa for 30 min. Since the melting enthalpy of starch-lipid inclusion complexes could be influenced by the amount of amylose (Chumsri et al., 2022; Fernández-Martín et al., 2008), these decreasing values could be the result of a lower availability of amylose chains to interact with the endogenous lipids present in BW starch (F. Zhu, 2016) due to a possible disruption of the amorphous region with the increased intensity of the HHP treatments. In addition, it is known that the starch-lipid complex could hinder the swelling capacity of starch (Lu & Baik, 2015). Therefore, the decrease in the amylose-lipid enthalpy in the samples resulting from the most intense HHP treatment (15 min at 600 MPa) could be related to the increase in the SP values observed in this study.

The flour samples showed no significant changes in the retrogradation enthalpy at the second scan performed after 7 days. These results were consistent with those reported by Fernández-Martín et al. (2008). These authors found that retrogradation had very little effect on pressure-induced starch gels. However, the enthalpy of the amylose-lipid complex showed higher values compared to those observed in the first scan. This increased enthalpy was previously observed by Gutiérrez et al. (2022) and Vicente et al. (2023) in retrograded BW flours. Authors such as Solaesa et al. (2022) have attributed this effect to a better condition for the formation of amylose-lipid complexes due to amylose leakage with the heating of the first scan. There was only a significant difference ($p<0.05$) in the enthalpy of the amylose-lipid complex between the native sample (3.5 J/g) and the sample resulting from the most intense treatment (1.61 J/g). A significant ($p<0.05$) decrease in this thermal property, was also observed by Gutiérrez et al. (2022) for the resulting BW flours after treatment of whole grains at 600 MPa for 30 min. This effect could be related to the reduced availability of amylose after high intensity HHP treatment of grains , which affected the formation of V-type complexes in the first scan.

Table 4.2.4. Thermal properties of flours samples obtained from native and HHP treated BW grains.

	First scan							Second Scan		
Sample	ΔH gel (J/g)	To-gel (◦C)	Tp-gel (◦C)	Te-gel (◦C)	ΔT (◦C)	ΔH am-lip (J/g)	Tp am-lip (◦C)	ΔH ret (J/g)	ΔT (◦C)	ΔH am-lip (J/g)
Native	7.9 b	57.4 cd	66.7 c	75.0 bc	17.7 b	1.2 b	97.4 c	1.82 a	20.8 a	3.50 b
0-300	8.9 c	56.2 a	66.3 a	74.5 abc	18.3 b	1.1 ab	93.2 a	2.18 a	20.6 a	2.63 ab
5-300	8.1 bc	56.7 ab	66.6 bc	74.8 abc	18.2 b	0.8 ab	95.4 b	1.81 a	21.1 a	2.40 ab
15-300	8.3 bc	57.1 bc	66.7 c	75.1 c	18.0 b	1.3 b	95.7 b	1.67 a	19.6 a	3.44 b
0-600	8.5 bc	56.9 abc	66.4 ab	74.3 ab	17.4 ab	0.7 ab	93.6 a	1.85 a	19.9 a	3.10 ab
5-600	7.9 b	57.8 de	66.7 c	74.2 a	16.4 a	0.4 a	92.5 a	1.74 a	19.8 a	1.92 ab
15-600	6.8 a	58.0 e	66.7 c	74.2 a	16.2 a	0.5 a	92.5 a	1.93 a	20.1 a	1.61 a
SE	0.2	0.2	0.1	0.2	1.8	0.2	0.1	0.18	0.9	0.35
Multifactor ANOVA										
Holding time	*	ns	*	ns	ns	ns	ns	ns	ns	ns
Pressure level	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Holding time x Pressure level	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. ΔH gel: Enthalpy of gelatinization, To, Tp, Te-gel: Onset, peak and endset temperature of gelatinization, ΔT: (Te-To)gel/ret, ΔHam-lip: Enthalpy of amylose-lipid complex dissociation, Tp-am-lip: Peak temperature of the amylose-lipid complex dissociation, ΔH ret: Enthalpy of retrograded amylopectin. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0,05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95% confidence level (p<0.05), ns: not significant.

4.2.6 Rheological properties of gels

The rheological properties of the gels obtained from the native and HHP modified flours are shown in Table 4.2.5 The storage modulus predominated over the viscous for all gel samples resulting in a solid-like gel rheological behaviour. Gel samples obtained from HHP treated flours had significantly ($p<0.05$) lower $G`_1$ values than those from native flour. The same decreasing trend was observed for the $G``_1$ values of the gels. The samples resulting from treatments at 300 MPa showed a significant ($p<0.05$) decrease in G^*_1 values with increasing holding time. The HHP treatments also resulted in a significantly ($p<0.05$) lower frequency dependence of the storage modulus for those samples resulting from treatments at 300 MPa for 0 and 5 min and for those resulting from treatments at 600 MPa for 5 and 15 min. However, a significant ($p<0.05$) increase in the frequency dependence of the viscous modulus of all treated samples was found compared to the control. It has been previously reported that HHP treatments can lead to a weaker gel structure (Gutiérrez et al., 2022). Decreasing gel viscoelasticity values with HHP treatments with increasing holding times were observed by Alvarez et al. (2015) after applying pressures of 600 MPa, 50 ºC and for 15 or 25 min to chickpea flour slurries. The authors attributed this effect to increased amylopectin solubilisation with longer holding treatment times and protein denaturation which could affect the rheological properties.

The values of the crossover point related to gel samples from HHP treated grains were higher to those obtained for the gel from a native BW flour, indicating a more elastic predominance in the viscoelastic properties of the gels. A significant ($p<0.05$) increase in the τ_{max} was also observed for these samples. Based on these results, the gel samples from treated flours had a greater ability to maintain the network structure beyond the critical stress value observed for the native gel sample, a behaviour consistent with previous findings (Gutiérrez et al., 2022).

Table 4.2.5. Rheological properties of gel flours samples obtained from native and HHP treated BW grains.

SAMPLE	G'_1 (Pa)	a	G''_1 (Pa)	b	Tan $(\delta)_1$	c	G^*_1 (Pa)	Cross-point (Pa)	τ_{max} (Pa)
Native	1436 c	0.089 c	282 d	0.194 a	0.196 d	0.105 a	1463 d	266 a	133 a
0-300	1306 c	0.075 ab	205 c	0.222 b	0.157 a	0.147 b	1322 c	497 d	312 e
5-300	933 b	0.077 b	160 b	0.249 c	0.172 bc	0.172 cd	947 b	390 c	245 cd
15-300	744 a	0.082 bc	123 a	0.248 c	0.166 b	0.166 c	754 a	331 b	187 b
0-600	900 b	0.083 bc	148 b	0.248 c	0.165 ab	0.165 c	912 b	376 c	227 c
5-600	965 b	0.068 a	162 b	0.244 c	0.167 b	0.176 d	979 b	385 c	255 d
15-600	919 b	0.071 a	164 b	0.245 c	0.178 c	0.174 cd	934 b	330 b	241 cd
SE	40	0.003	5	0.003	0.002	0.003	40	9	7
					Multifactor ANOVA				
Holding time	**	ns	**	**	**	**	**	**	**
Pressure level	ns	ns	ns	*	*	**	ns	**	ns
Holding time x Pressure level	**	*	**	**	*	ns	**	**	**

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. The power law model was fitted to the frequency sweep experimental data ($G' = G'1 \cdot \omega^a$; $G'' = G''1 \cdot \omega^b$; $\tan \delta = (\tan \delta)1 \cdot \omega^c$), where G_1', G_1'', and $\tan(\delta)_1$ are the coefficients obtained from the fitting and represent the elastic, viscous moduli, and loss tangent, respectively, at a frequency of 1 Hz. The a, b, and c exponents quantify the degree of dependence of the dynamic moduli and the loss tangent with the oscillation frequency. G*1 refers to the complex modulus. τ_{max} represent the maximum stress that the samples could tolerate in the linear viscoelastic region (LVR). The crosspoint value represents the stress at G` = G``. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0,05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95% confidence level (p<0.05), ns: not significant.

4.2.7 Phenol content (TPc) and total antioxidant capacity (TAC) of flours

The phenolic content (TPc) and total antioxidant capacity (TAC) of all flour samples were determined in extracts using the following assays: Folin-Ciocalteu (TP), DPPH, ABTS, FRAP and ORAC (TAC). In addition, the antioxidant capacity was evaluated directly on the solid matter of the samples themselves using quencher methods (Q-DPPH, Q-ABTS). The results are shown in Table 4.2.6. Results showed a significant ($p<0.01$) impact of the studying factors and their interaction on the total phenolic content (TPc) of flours. Total phenolic content of the native BW flour was 244 mg GAE/100 g (dw). This result is similar to the one obtained with buckwheat seeds dehulled by Dziadek et al. (2016). Samples from HHP treatments showed a significant ($p<0.05$) decrease in phenol content. A decrease in TP content with HHP treatments was previously reported by Błaszczak et al. (2013) who observed an average decrease of 12.6 % in BW groats treated with HHP at 200 MPa for 2-9 min. The flours resulting from the most intense treatments (600 MPa for 5 and 15 min) resulted in lower TP loss than those from the milder treatments. The sample treated at 300 MPa for 0 min had a TP loss of 21 %, while the flour sample from a 600 MPa for 15 min treatment showed a TP loss of only 12 %**.** It has been reported that high pressures can induce changes in the distribution of phenolic compounds (Vega-Gálvez et al., 2014). These authors observed that increasing the pressure level and holding time resulted in a significant increase in the phenolic content of HHP-treated Cape gooseberry pulp (from 165 mg GAE/100 g dw at 300 MPa/1 min to 268 mg GAE/100 g dw at 500 MPa/5min). They attributed this increase to the effect of the HHP treatment in disrupting the cell walls, increasing their permeability and thus allowing solvent penetration. As a result, a higher content of antioxidant compounds (phenolics, amino acids and proteins) with one or more phenolic hydroxyl groups could be extracted. In buckwheat, the highest concentration of polyphenols is found in the hull (Dziadek et al. 2016). However, non-phenolic organic compounds, such as amino acids or peptides and even some inorganic substances such as iron and manganese compounds could also lead to an apparently increase in phenolic concentration. This is considered a limitation of the Folin-Ciocalteu method for phenolic detection (Prior et al., 2005).

On the other hand, higher TAC values were observed, particularly in the sample obtained from the most intense HHP treatment (600 MPa for 15 min). The extract from this flour sample showed a significant increase ($p<0.05$) compared to the native BW flour (40, 25 and 35 %, for DPPH, ABTS and ORAC, respectively). Yu et al. (2017) has hypothesised that HHP treatment of brown rice would break down the bran cell walls allowing water and soluble pigments to migrate into the grain. A diffusion effect of these pigments from the outer layers to the endosperm was also found to be the reason for flour colour changes after ultrasound treatment of BW kernels, which etched the bran layer (Harasym et al., 2020). These authors also found correlations between colour parameters and antioxidant level. This mechanism of extraction and inward migration of compounds could be related to the observed increase in the antioxidant capacity of the samples resulting from HHP treatments.

Table 4.2.6. Phenol content (TP) and antioxidant capacity (TAC) of flours samples resulting from HHP-treatments and native BW grains.

SAMPLE	TPc (mg GAE/ 100g)	DPPH (mg TE/ 100g)	ABTS (mg TE/ 100g)	FRAP (mg Fe+2/ 100g)	ORAC (mg TE/ 100g)	Q-DPPH (mg TE/100g)	Q-ABTS (mg TE/100g)
Native	244 ^{e}	410 ^{b}	1295 ^{a}	42.6 ^{e}	2292 ^{ab}	576 ^{e}	1715 ^{bc}
0-300	193 ^{a}	337 ^{a}	1470 ^{b}	36.0 ^{a}	1759 ^{a}	198 ^{a}	1200 ^{ab}
5-300	211 ^{cd}	344 ^{a}	1501 ^{bc}	38.5 ^{cd}	1900 ^{a}	311 ^{bc}	1883 ^{c}
15-300	202 ^{b}	361 ^{a}	1573 ^{bcd}	37.4 ^{b}	3055 ^{cd}	434 ^{d}	1852 ^{c}
0-600	207 ^{bc}	423 ^{b}	1583 ^{bcd}	38.6 ^{cd}	2616 ^{bc}	230 ^{ab}	1856 ^{c}
5-600	212 ^{cd}	564 ^{c}	1654 ^{d}	37.9 ^{bc}	1951 ^{a}	386 ^{cd}	1111 ^{a}
15-600	214 ^{d}	576 ^{c}	1624 ^{cd}	38.8 ^{d}	3103 ^{d}	443 ^{d}	1027 ^{a}
SE	2	11	37	0.3	144	26	178
			Multifactor ANOVA				
Holding time	**	**	ns	**	**	**	ns
Pressure level	**	**	*	**	ns	ns	ns
Holding time x Pressure level	**	**	ns	**	ns	ns	*

0-300, 5-300, 15-300: Samples obtained from HHP treated buckwheat grains treated at 300 MPa for 0, 5 and 15 min. 0-600, 5-600, 15-600: Samples obtained from HHP treated buckwheat grains treated at 600 MPa for 0, 5 and 15 min. TPc refers to the total phenol content. Total antioxidant capacity as DPPH, ABTS, FRAP and ORAC on sample flours extracts and Q-DPPH and Q-ABTS of direct (quencher) method on solid flour samples. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.2.8 Rheological properties of doughs

Dynamic oscillatory rheology was carried out on all the doughs containing the different BW flours, native and modified (15-600), according to the different substitution levels studied (15, 30, 50 and 70%). A 100% rice based dough was also used as a reference. The viscoelastic properties of the doughs are shown in Table 4.2.7. The factors BW flour type, replacement level and their interaction were found to be significant ($p<0.01$) for $G`_1$ and $G``_1$. The control dough had the lowest $G`_1$ value, which was significantly ($p<0.05$) different from those of the other doughs. The higher addition of either native or modified BW flour promoted significant increases ($p<0.05$) in $G`_1$ at almost all substitution levels. In addition, significant ($p<0.05$) increases in this parameter were observed when comparing doughs containing the modified BW flour with their counterparts containing the native flour. In particular, the dough at 70 % replacement level with the modified BW flour showed a strong significant ($p<0,05$) increase in $G`_1$ of more than three times the value presented by its counterpart with the native BW flour. The $G``_1$ values also followed this trend. The frequency dependence of the doughs as measured by the coefficients 'a' and 'b', followed a decreasing trend with increasing replacement levels with both the native and the modified BW flour, indicating a higher dough stability as the inclusion of BW flour in the doughs increased.

Strain sweeps at 1 Hz performed on the doughs reported the yield stress or crosspoint where elastic behaviour equals viscous behaviour (Ronda et al., 2017), as well as the maximum stress (τ_{max}) that the doughs can withstand in their viscoelastic structure before breaking (Villanueva et al., 2019). Both the type of BW flour used and the flour replacement level, as well as their interaction, had a significant effect on these two rheological parameters of the doughs. A significant ($p<0.05$) increase in the τ_{max} and crossover point values of the doughs was observed from 30% replacement with the modified wheat flour. On the contrary, no significant difference was observed when comparing the results obtained for the doughs containing the native BW flour with that obtained from 100% rice flour (control dough).

To the best of our knowledge, there is limited literature on the rheological behaviour of doughs containing flours modified by HHP treatments. Angioloni & Collar (2012) reported an increase in the viscoelastic moduli of doughs containing pressure-treated (350 MPa/10 min) oat and wheat flours which partially replaced native wheat flour (at 60% and 50%, respectively) in their dough formulation. The reported increase in dough consistency was associated with the modification of starch and protein caused by the HHP treatment of the flour suspensions. The authors stated that these results could improve the generally poor baking performance associated with the incorporation of significant amounts of high nutritional value flours. Although these authors associated this improvement in structure with disulphide bond formation and gluten strengthening, promising results were also observed in gluten-free flours. White rice, buckwheat and tef batters treated with HHP at pressures above 200 MPa showed increased G* values due to the increasing contribution of the storage modulus value with increasing the pressure (Vallons et al., 2011). Therefore, these authors stated that HHP treatments in such GF flours have the potential to improve their baking performance.

Table 4.2.7. Rheological properties of gluten-free doughs as a function of BW flour replacement level and type of BW flour (native/modified)

Replacement level (%)	BW Flour	G'_1 (Pa)	a	G''_1 (Pa)	b	$(Tan\ \delta)_1$	c	G* (Pa)	Crosspoint (Pa)	τ_{max} (Pa)
0	-	1630 [a]	0.276 [e]	820 [a]	0.29 [d]	0.5 [f]	0.012 [a]	182 [a]	67 [a]	3.1 [a]
15	Native	1945 [b]	0.247 [d]	884 [ab]	0.28 [cd]	0.45 [de]	0.029 [abc]	2137 [b]	63 [a]	2.8 [a]
30	Native	2210 [b]	0.223 [bc]	952 [bc]	0.27 [cd]	0.43 [cd]	0.042 [bcd]	2406 [b]	72 [a]	3.0 [a]
50	Native	2590 [c]	0.208 [b]	1003 [c]	0.26 [bcd]	0.39 [b]	0.047 [cd]	2777 [c]	86 [ab]	3.3 [a]
70	Native	3583 [d]	0.183 [a]	1302 [d]	0.24 [bc]	0.36 [b]	0.061 [d]	3812 [d]	98 [ab]	3.2 [a]
15	Modified	2700 [c]	0.243 [d]	1245 [d]	0.26 [cd]	0.46 [e]	0.021 [ab]	2973 [c]	86 [ab]	4.3 [ab]
30	Modified	3680 [d]	0.234 [cd]	1566 [e]	0.24 [bc]	0.56 [c]	0.01 [a]	3995 [d]	120 [b]	5.4 [bc]
50	Modified	6870 [e]	0.211 [b]	2600 [f]	0.23 [ab]	0.38 [b]	0.016 [a]	7346 [e]	203 [c]	6.4 [c]
70	Modified	11150 [f]	0.183 [a]	3666 [g]	0.2 [a]	0.33 [a]	0.017 [a]	11714 [f]	307 [d]	8.4 [d]
	SE	89	0.006	27	0.01	0.01	0.007	92	13	0.5
					Multifactor ANOVA					
	BW flour	**	ns	**	**	*	**	**	**	**
	Replacement level	**	**	**	**	**	ns	**	**	**
	BW flour x replacement	**	ns	**	ns	*	ns	**	**	*

Replacement level: Percentage of rice flour in a rice flour-based gluten-free dough that is replaced with native/modified BW flour. Native: Flour sample obtained from native BW grains. Modified: Flour sample obtained from BW grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF dough. The power law model was fitted to the frequency sweep experimental data ($G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \omega^c$), where G_1', G_1'', and $\tan(\delta)_1$ are the coefficients obtained from the fitting and represent the elastic, viscous moduli, and loss tangent, respectively, at a frequency of 1 Hz. The a, b, and c exponents quantify the degree of dependence of the dynamic moduli and the loss tangent with the oscillation frequency. G^*_1 refers to the complex modulus. τ_{max} represent the maximum stress at the end of the linear viscoelastic region (LVR) obtained from the strain sweep. The crosspoint value represents the stress at G` = G``. SE: pooled standard error. Values in the same column with no common letters are significantly different. ** $p<0.01$, *$p<0.05$, ns: not significant from multifactor ANOVA.

4.2.9 Bread quality evaluation

Table 4.2.9 shows the results of the bread quality parameters analysed. The flour replacement level, the type of BW flour and their interaction had a significant relevance ($p<0.01$) on the specific volume of bread samples. The inclusion of the native buckwheat flour in the gluten-free formula significantly ($p<0.05$) decreased the specific volume values of the breads at all replacement levels, with values between 2.24 and 2.31 mL/g, lower than those obtained for the control bread made with 100% of rice flour (2.62 mL/g). However, the addition of the modified BW flour at any replacement level, significantly ($p<0.05$) improved the specific volume of the bread compared to its counterparts made with native BW flour. The bread sample made with BW modified flour at 15% of replacement level even showed a significantly ($p<0.05$) higher specific bread volume than the control one made with 100 % with rice flour. These results could be associated with the consistency of the dough. Very weak doughs, characteristic of those without gluten, are unable to retain the gas generated during proofing, resulting in breads with low volume and poor textural properties. This behaviour could be partly remedied with hydrocolloids, which allow an increase in gas retention (Mariotti et al., 2013). In this study, a higher dough consistency has been observed in all samples containing the modified BW flour compared to their counterparts containing the native BW flour (Table 6). The decrease in specific volume, observed at increasing replacement level with the modified BW flour, and in particular at the highest level (70%), could be due to an excessive dough consistency which could limit the gas expansion during proofing and resulted in breads with low development and volume (Villanueva et al., 2019). The use of HHP technology has been suggested to enhance the viscoelastic properties of these doughs with poor breadmaking performance (Angioloni & Collar, 2012b). Positive effects of the inclusion of HHP-modified flours in gluten-free bread formulations have already been observed in the literature, such as an increase in the specific volume of the breads after replacing native oat flour (10%) with an HHP-treated one (200 MPa/10 min) (Hüttner et al., 2010). Better dough processing performance has also been reported for those containing HHP-treated potato starch (200-500 MPa/30min) and HPMC (2%) (D. Zhang et al., 2019). These authors observed improvements in gas retention which they attributed to an increase in viscosity favoured by entanglements between amylose molecules and between amylose and HPMC. An improvement in gas production and dough height was also observed by these authors, which they ascribed to the presence of damaged starch granules, which caused an increase in reducing sugar content and yeast metabolism.

In the case of baking loss, a significant impact ($p<0.01$) of the replacement level and its interaction with the type of BW flour used was found. The ANOVA results showed a significant increase ($p<0.05$) in baking loss only in the breads with low replacement levels with the native (15-30%) and modified (15%) BW flour compared to the bread control.

The textural properties of the bread were significantly influenced by both studied factors ($p<0.01$). A significant increase ($p<0.05$) in crumb firmness was owwwbserved for those breads containing the native BW flour compared to the control bread. However, the use of the modified BW flour resulted in a significant decrease ($p<0.05$) in crumb firmness values compared to the breads using the native flour at each replacement level.

In addition, the bread samples made with 15 and 30 % of the treated BW flour reached a firmness value similar to that of the control bread. This could be related to the higher specific volume values obtained in those breads containing the modified flour, which had a more open crumb structure and thus, a lower crumb deformation resistance (Villanueva et al., 2019). As chewiness is closely related to the textural property of crumb firmness, the same trend as for firmness was observed in the results of the bread samples. Crumb resilience and cohesiveness increased significantly ($p<0,05$) for the breads containing the modified BW flour at any replacement level compared to those made with the untreated flour. Although a decreasing trend was observed for both parameters with increasing replacement levels, the use of the modified BW flour mitigated this decrease. However, significantly ($p<0.05$) higher springiness values were observed for all bread samples containing BW flour compared to the control. Significant ($p<0.05$) increasing values were observed with increasing replacement levels (from 15 % to 50 %) in the breads containing the modified BW flour. All bread samples showed a significant increase in crumb hardness after 7 days. In addition, a hardening trend was observed with increasing the replacement levels for both types of BW flour. However, a significantly lower value ($p<0.05$) was observed in the bread made with the modified flour at 50 % replacement level compared to its counterpart made with the native flour.

Table 4.2.9. Bread quality properties of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified)

Replacement level (%)	BW Flour	Weight loss (%)	Specific volume (mL/g)	Firmness (N)	Firmness (7 days) (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
0	-	16.3 ab	2.62 e	4.1 a	8.8 a	0.788 a	0.57 e	1.8 a	0.27 d
15	Native	16.9 d	2.24 a	6.8 b	15.5 bc	0.888 c	0.53 d	3.2 b	0.28 def
30	Native	17.1 d	2.28 ab	7.1 b	16.2 c	0.89 c	0.51 c	3.2 b	0.26 d
50	Native	16.4 b	2.31 b	7.3 b	24.4 e	0.896 c	0.46 b	3 b	0.21 b
70	Native	15.8 a	2.25 a	10.2 c	22.6 de	0.901 c	0.43 a	3.9 c	0.18 a
15	Modified	16.9 cd	2.74 f	3.8 a	12.5 b	0.873 b	0.59 e	1.9 a	0.3 f
30	Modified	16.3 ab	2.55 d	4.1 a	16 c	0.899 c	0.57 e	2.1 a	0.29 ef
50	Modified	16.4 bc	2.50 d	6.4 b	17 c	0.92 d	0.53 d	3.1 b	0.27 de
70	Modified	16.3 ab	2.38 c	7.3 b	20.3 d	0.931 d	0.48 b	3.3 b	0.24 c
	SE	0.2	0.02	0.5	1	0.004	0.01	0.2	0.01
Multifactor ANOVA									
	BW flour	ns	**	**	**	**	**	**	**
	Replacement level	**	**	**	**	**	**	**	**
	BW flour x replacement	**	**	ns	*	**	ns	*	*

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified BW flour. Native: Flour sample obtained from native BW grains. Modified: Flour sample obtained from BW grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF bread. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0,05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95% confidence level (p<0.05), ns: not significant

Images of the open structure of the loaves are shown in Figure 4.2.2. The influence of replacement level, type of BW flour and the interaction between these two factors on the crumb structure of the breads was significant (p<0.05) for the mean cell size, cell density and uniformity, as can be seen in Table 4.2.10. An overall and significant (p<0.05) increase in uniformity and in the cell wall thickness and a decrease in the mean cell area were observed in the breads containing both types of BW flour with respect to the bread control. Some noticeable differences could also be found by comparing the alveolar surface of the breads made with the modified BW flour with those made with the native flour, especially at high replacement levels. A trend towards an increase in the average cell size was observed in the alveolar structure of the breads containing the modified BW flour, as the replacement level increased from 30 % to 70 %. A significant (p<0.05) decrease in cell density and uniformity values at higher replacement levels was also observed in these breads.

The effect of both studied factors on luminosity was significant (p<0.05). As expected, the addition of both types of BW flour significantly (p<0.05) decreased crust and crumb luminosity values as the replacement level increased (Table 4.2.9). Significant (p<0.05) differences between breads containing native and modified BW flour were found only at the 70% replacement level for crust luminosity and at the 30% and 50% replacement levels for crumb luminosity. The significantly (p<0.05) lower luminosity value found for the modified BW flour (600MPa/15 min) compared to the native flour (Table 1) together with the Maillard reactions that occurred during baking, might have influenced the observed darkening effect of the crust for the bread sample at 70 % of the replacement level with this flour. Breads containing the native BW flour showed a significant (p<0.05) difference from the control in the Chroma (C*) values of the crust. Furthermore, a decreasing trend was observed with increasing replacement levels. A similar decreasing trend was also observed for hue (h) values. Significantly (p<0.05) lower hues and higher Chroma values were observed in the crust colour of the breads containing the modified flour compared to their counterparts made with the native flour at replacement levels of 30 and 50%. Although the colour of the original ingredients is mainly responsible for the values of the crumb colour parameters (Villanueva et al., 2019), the HHP treatment probably modified the browning effect of the BW flour in the bread formulation, leading to the formation of more saturated colour compounds. A significant effect (p<0.01) on Chroma was observed for the factor BW flour used. The significantly (p<0.05) higher Chroma value observed for the modified BW flour compared to the native one (Table 4.2.1) could have affected the crumb Chroma values observed in the breads. The replacement level factor was also significant (p<0.01), showing a clear trend of increasing Chroma values with increasing the replacement percentage with the BW flour.

Table 4.2.10. Crumb grain features and crumb and crust colour parameters of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified).

Replacement level (%)	BW Flour	Average cell size (mm^2)	cell density (nº cell/cm^2)	Crumb grain uniformity	cell wall thickness (mm)	Crust colour			Crumb colour		
						L*	C*	h	L*	C*	h
0	-	0.39 ^{d}	51 ^{de}	5.0 ^{a}	0.70 ^{a}	69.5 ^{f}	33.4 ^{e}	65.1 f	80.8 ^{g}	6.5 ^{a}	86.2 ^{g}
15	Native	0.2 ^{ab}	54 ^{f}	18 ^{cd}	0.83 ^{b}	66.1 ^{ef}	30.9 ^{cd}	64.4 ef	70.2 ^{f}	8.66 ^{b}	70.4 ^{e}
30	Native	0.22 ^{b}	49 ^{cd}	17 ^{cd}	0.88 ^{c}	58.8 ^{cd}	30.4 ^{bc}	62.5 de	64.5 ^{d}	9.97 ^{c}	62.5 ^{c}
50	Native	0.22 ^{b}	48 ^{bc}	18 ^{cd}	0.89 ^{c}	55.9 ^{bc}	29.4 ^{ab}	60.7 cd	57.7 ^{b}	10.7 ^{d}	59.7 ^{a}
70	Native	0.21 ^{ab}	48 ^{bc}	19 ^{d}	0.9 ^{c}	53.9 ^{b}	29.3 ^{ab}	58.9 bc	53.4 ^{a}	11.26 ^{e}	59.1 ^{a}
15	Modified	0.21 ^{ab}	53 ^{ef}	14 ^{c}	0.83 ^{b}	65.5 ^{e}	32.2 de	63.6 ^{ef}	71.9 ^{f}	8.47 ^{b}	72 ^{f}
30	Modified	0.18 ^{a}	52 ^{ef}	21 ^{d}	0.89 ^{c}	60.4 ^{d}	32.4 de	60.6 ^{c}	66.9 ^{e}	10.14 ^{c}	65.2 ^{d}
50	Modified	0.26 ^{c}	46 ^{b}	13 ^{bc}	0.88 ^{c}	54 ^{b}	31.7 de	57.9 ^{b}	61.7 ^{c}	11.89 ^{f}	61.5 ^{b}
70	Modified	0.31 ^{c}	43 ^{a}	8 ^{ab}	0.9 ^{c}	49.6 ^{a}	29.1 a	55.3 ^{a}	55 ^{a}	12.88 ^{g}	61.2 ^{b}
SE		0.01	1	2	0.01	1.2	0.4	0.6	0.6	0.09	0.3
Multifactor ANOVA											
BW flour		**	*	**	ns	*	**	**	**	**	**
Replacement level		**	**	ns	**	**	**	**	**	**	**
BW flour x replacement		**	**	**	ns	*	**	*	ns	**	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified BW flour. Native: Flour sample obtained from native BW grains. Modified: Flour sample obtained from BW grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF bread. L*: luminosity; C*: Chroma; h: hue. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

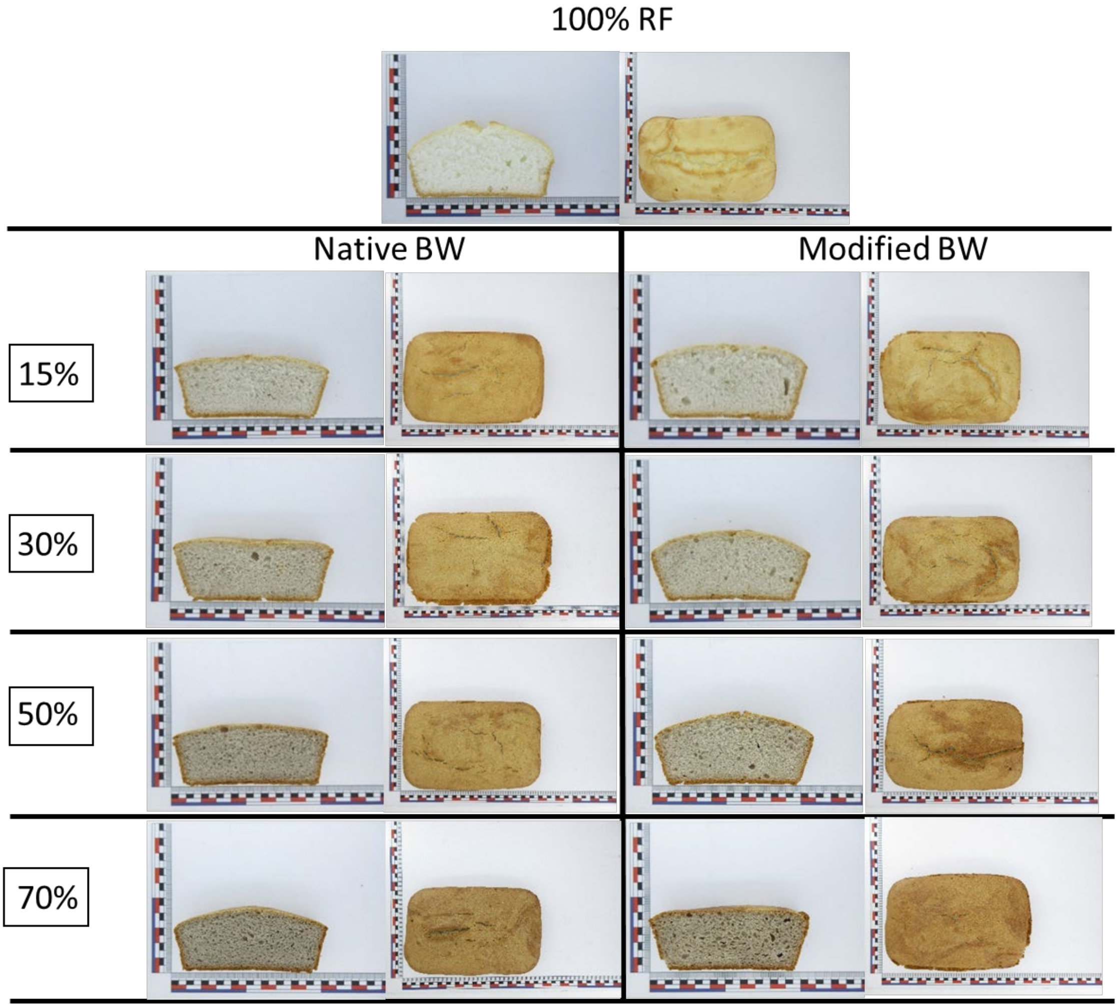

Figure 4.2.2. Photographs of bread loaves (top view) and slices of gluten-free breads made from 100% rice flour (100 %RF) and by replacing rice flour with native buckwheat flour (Native BW) or HHP-modified buckwheat flour (Modified BW) at different replacement levels (15%; 30%; 50%; and 70%)

4.2.10 Phenol content (TPc) and total antioxidant capacity (TAC) of breads

The resulting breads containing the native or modified (15-600) BW flour were also evaluated in terms of their phenol content and antioxidant capacity measured by DPPH, ABTS, FRAP and ORAC in methanolic extracts and DPPH and ABTS by quencher method. The results are presented in Table 4.2.11. A bread made from 100% white rice flour, used as a control, had the lowest phenolic content (TPc) among all the bread samples. Increasing the replacement level resulted in a significant increase ($p<0.05$) in the TPc of the breads. Furthermore, with the exception of the sample at 50 % replacement level, the other breads containing the modified BW flour showed no significant ($p>0.05$) difference compared to their equivalents made with native BW flours. The TPc value of

103 mg GAE/100 g dw obtained for the bread sample at 30 % replacement level with the native BW flour is not far from that reported by Sakac et al. (2011) (115.5 mg GAE/100 g dw) when using a 30 % wholegrain BW flour replacement in a similar gluten-free bread formulation. These authors also reported increased TP values with increasing levels of BW flour replacement in their GF bread samples.

A similar trend was observed when analysing the total antioxidant capacity (TAC) values of the bread samples, except for ORAC and Q-ABTS, where significantly ($p<0.05$) higher values were found for bread samples containing the native BW flour compared to their counterparts containing the modified one. For the other parameters, similar TAC values were observed when comparing the bread samples containing the different BW flours at each replacement level. Significant differences ($p<0.05$) between the samples could only be found comparing breads at different replacement levels, thus a significant ($p<0.01$) effect of the replacement level factor was observed. However, some bread samples containing the modified BW flour also showed a significantly higher TAC value ($p<0.05$) compared to their counterpart containing the native flour. This was the case for bread samples at 15% replacement with the modified BW flour for the DPPH and FRAP antioxidant markers.

It has been reported that fortification of GF breads with BW flours improved their antioxidant properties (Sakac et al., 2011). Previous studies stated that whole BW flour had a better antioxidant capacity than refined BW flour due to the presence of more phenolic compounds from the outer layers of the grains. It has also been noted that some antioxidant compounds may be thermolabile and may be lost during thermal processing such as baking. However, this loss could be masked by some synthesised substances in the bread crust through Maillard reactions, which could manifest the ability to present antioxidant capacity (Pérez-Jiménez et al., 2014), explaining some of the significant differences observed in the baking preparations.

Although the HHP treatment had a positive effect on the antioxidant capacity of flour measured by ORAC (Table 5), an overall decrease in ORAC was observed for all the breads containing the modified flour compared with their native counterparts. This could be related to the modification promoted by the HHP treatment of the antioxidant compounds presented in the buckwheat flours. As previously suggested, the increase observed in some antioxidant markers (DPPH, ABTS, ORAC) from HHP-modified BW flours could be due to a better bioavailability of phenolic and/or non-phenolic compounds (such as amino acids or bioactive peptides) with antioxidant capacity. The antioxidant reactions of amino acids and peptides are mainly driven by hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. Although these reactions can occur in parallel, one of them can dominate depending on the antioxidant structure and the type of assay (Esfandi et al., 2019). In ABTS and DPPH assays, radicals could be neutralised by either HAT or SET reactions. However, the dominant reactivity pattern for ORAC is HAT (Prior et al., 2005). Therefore, some bioactive peptides containing amino acids such as tyrosine, presented in BW flour which can act mainly through HAT (Christa & Soral-´Smietana, 2008; Esfandi et al., 2019), could have expressed a better bioavailability after HHP treatment, thus increasing the ORAC value of the flour (Table 5). However, the antioxidant capacity of these non-phenolic compounds could be affected by thermal treatments such as baking. Therefore, the observed decrease in ORAC values could be explained by the possible loss of these thermolabile compounds during breadmaking process. Decreased antioxidant activity after thermal processing has been previously reported for peptides derived from cowpea (Daliri et al., 2017).

Table 4.2.11. Total phenol content (TPc) and antioxidant capacity (TAC) of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified).

Replacement level (%)	BW Flour	TPc (mg GAE/ 100g)	DPPH (mg TE/ 100g)	ABTS (mg TE/ 100g)	FRAP (mg Fe+2/ 100g)	ORAC (mg TE/ 100g)	Q-DPPH (mg TE/100g)	Q-ABTS (mg TE/100g)
0	-	32 ^{a}	33 ^{a}	92 ^{a}	18.5 ^{a}	169 ^{a}	10.2 ^{a}	68 ^{a}
15	Native	69 ^{b}	97 ^{a}	334 ^{b}	19.7 ^{a}	540 ^{c}	19.9 ^{ab}	202 ^{bc}
30	Native	103 ^{c}	252 ^{cd}	557 ^{d}	25.0 ^{c}	813 ^{de}	34.0 ^{cd}	282 ^{c}
50	Native	145 ^{e}	294 ^{de}	796 ^{e}	27.7 ^{d}	881 ^{e}	41.7 ^{cd}	473 ^{d}
70	Native	167 ^{f}	352 ^{e}	1104 ^{g}	30.8 ^{e}	1050 ^{f}	55.5 ^{e}	494 ^{d}
15	Modified	67 ^{b}	170 ^{b}	434 ^{bc}	21.2 ^{b}	365 ^{b}	18.1 ^{ab}	115 ^{ab}
30	Modified	109 ^{c}	199 ^{bc}	506 ^{cd}	24.3 ^{c}	560 ^{c}	29.7 ^{bc}	143 ^{ab}
50	Modified	129 ^{d}	274 ^{d}	727 ^{e}	28.3 ^{d}	736 ^{d}	39.0 ^{cd}	204 ^{bc}
70	Modified	156 ^{ef}	265 ^{cd}	955 ^{f}	30.7 ^{e}	878 ^{e}	46.6 ^{de}	187 ^{abc}
SE		5	22	32	0.5	42	4.2	38
Multifactor ANOVA								
	BW flour	ns	ns	ns	ns	**	ns	**
	Replacement level	**	**	**	**	**	**	**
	BW flour x replacement	ns	*	*	ns	ns	ns	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified BW flour. Native: Flour sample obtained from native BW grains. Modified: Flour sample obtained from BW grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF bread. TPc refers to the total phenol content. Total antioxidant capacity as DPPH, ABTS, FRAP and ORAC on sample flours extracts and Q-DPPH and Q-ABTS of direct (quencher) method on solid flour samples. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.2.11 Sensory properties

Panellists found significant ($p<0.05$) differences when assessing the sensory properties of the breads containing BW flours compared to the control made with 100% or rice flour, as can be observed in Figure 4.2.3 and in Table 4.2.12. Results showed a significant ($p<0.01$) effect of the replacement level for all sensory attributes, while the type of BW flour was only found to be significant ($p<0.01$) for crust uniformity. The higher crust uniformity scores for the breads with the highest replacement level (50 %) were due to the fact that panellists observed fewer irregularities and a smoother and flatter surface in these breads compared to the control bread and those with a replacement level of 15%. The panellists also observed significant ($p<0.05$) and greater differences in taste, aftertaste and odour intensity between samples at increasing replacement levels with the BW flour compared to the control bread. This could be due to the higher presence of whole BW flour, which has been reported to have a bitter taste due to compounds in the outer layers of the grain (Torbica et al., 2010). In addition, breads with a high proportion of BW flour (50%) had significantly ($p<0.05$) higher crumb hardness and crumb chewiness scores. However, the panellists were unable to find significant ($p<0.05$) differences between the breads containing the modified BW at 15 % replacement level and the control bread in terms of crumb hardness and chewiness.

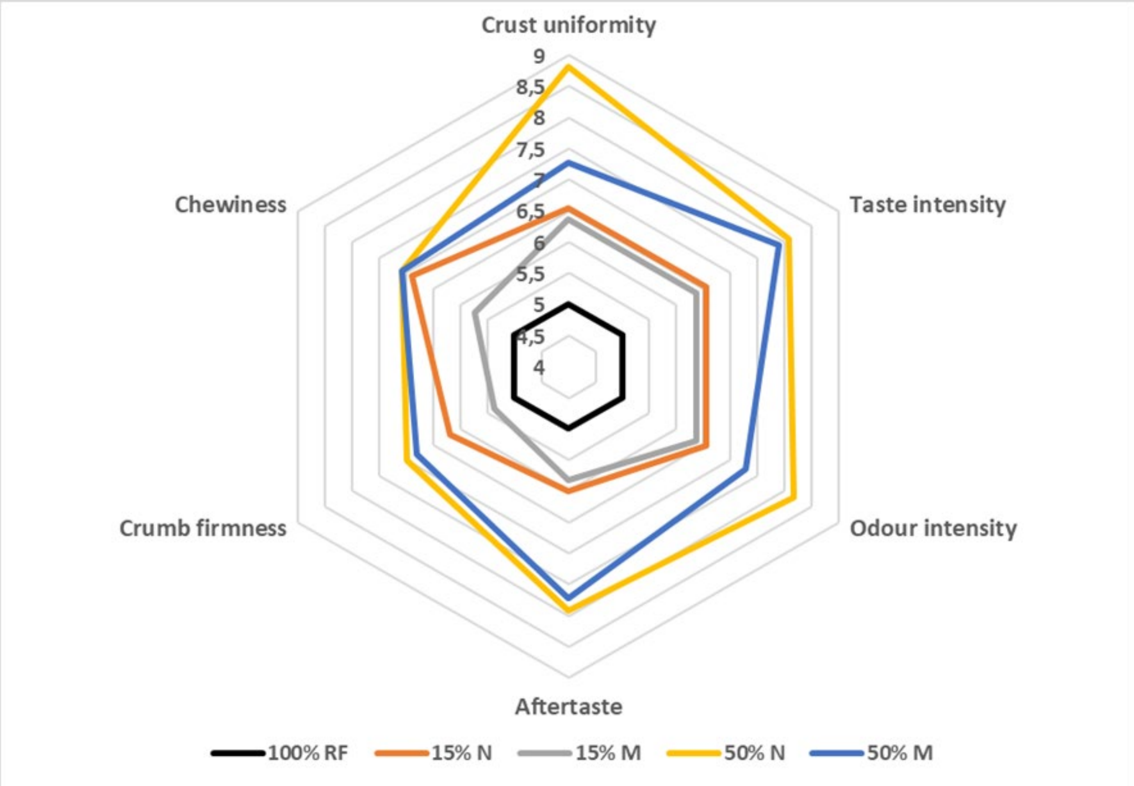

Figure 4.2.3. Sensory properties of a 100% rice-based GF bread (100% RF) and different GF breads obtained by replacing rice flour with BW flour (native –N; modified -M) at different replacement levels (15%, 50%).

Table 4.2.12. Sensory properties of gluten-free breads as a function of BW flour replacement level and type of BW flour (native/modified).

Replacement level (%)	BW Flour	Crust uniformity	Taste intensity	Odour intensity	Aftertaste	Crumb hardness	Chewiness
0	-	5.0 [a]	5.0 [a]	5.0 [a]	5.0 [a]	5.0 [a]	5.0 [a]
15	Native	6.5 [b]	6.5 [b]	6.5 [bc]	6.0 [b]	6.2 [b]	6.9 [b]
15	Modified	6.4 [b]	6.4 [b]	6.4 [b]	5.8 [b]	5.4 [a]	5.7 [a]
50	Native	8.8 [d]	8.1 [c]	8.2 [d]	7.9 [c]	7.0 [c]	7.1 [b]
50	Modified	7.3 [c]	7.9 [c]	7.3 [c]	7.7 [c]	6.8 [bc]	7.1 [b]
	SE	0.2	0.2	0.3	0.3	0.2	0.3
		Multifactor Anova					
	BW flour	**	ns	ns	ns	ns	ns
	Replacement level	**	**	**	**	**	*
	BW flour*replacement	**	ns	ns	ns	ns	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free dough that is replaced with native/modified BW flour. Native: Flour sample obtained from native BW grains. Modified: Flour sample obtained from BW grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF bread. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

CHAPTER 3

Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours. Study of pre-soaking treatment and holding time as processing factors.

4.3 Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours. Study of pre-soaking treatment and holding time as processing factors.

4.3.1 Granulometry and flour colour characteristics.

The granulometry of flours are presented in Table 4.3.1. A significant ($p<0.05$) influence of the holding time factor was found on the median size particle (D_{50}). No significant difference was observed between the native and modified flours. However, the effect of soaking and holding HHP time affected on size dispersion. The values of this parameter in the samples treated at 5 and 15 min without the soaking pre-treatment were significantly higher ($p<0.05$) with respect to the native flour and those from pre-soaking treatments. These values may be associated with the increase in the pressurisation process, as longer treatments had a greater impact than shorter ones. HHP treatments could induce some structural changes on the rice grain that resulted in fracturability changes, leading to fluctuations in particle size distributions. Some variability in the particle size distributions of rice slurries treated with HHP at 600 MPa has been previously reported by Ahmed et al. (2017) at decreasing flour to water ratio.

Resulting flours colour parameters are also showed in table 1. The increase in holding time had a significant impact on the lightness of the samples ($p<0.01$), samples treated at 30 min showed significantly higher values ($p<0.05$) compared to those treated at lower holding times and the native flour. Hue (h) did not show any difference for the holding time on the samples but, Chroma (C*) values were significantly influenced by the holding time factor ($p<0.01$), with these values rising with increasing time. Slight but significant differences in luminosity and chromatic values were previously observed on pressurized brown rice by Yu et al. (2017) who attributed these changes to the migration of water and pigments produced during the treatment due to the depressurisation process that occurred in the last stage of the HHP treatment.

Table 4.3.1. Particle size distribution and colorimetric parameters of flour samples obtained from native and HHP treated paddy rice grains.

Sample	D_{50} (μm)	Size dispersion	L*	a*	b*	ΔE	C*	h
Native	90.1 ^{ab}	1.57 ^{a}	87.71 ^{a}	0.73 ^{a}	5.5 ^{a}	-	5.5 ^{a}	82.4 ^{c}
5U	90.3 ^{ab}	1.69 ^{b}	87.87 ^{ab}	0.84 ^{ab}	5.7 ^{bc}	0.8 ^{a}	5.8 ^{bc}	81.6 ^{bc}
5S	89.7 ^{a}	1.58 ^{a}	87.18 ^{a}	0.87 ^{b}	5.6 ^{ab}	0.6 ^{a}	5.7 ^{ab}	81.2 ^{b}
15U	91.6 ^{b}	2.14 ^{c}	87.10 ^{a}	1.07 ^{c}	5.9 ^{d}	0.9 ^{a}	6.0 ^{de}	79.8 ^{a}
15S	91.4 ^{b}	1.62 ^{ab}	88.77 ^{b}	0.81 ^{ab}	5.8 ^{cd}	1.2 ^{a}	5.9 ^{cd}	82.1 ^{bc}
30U	89.4 ^{a}	1.64 ^{ab}	90.21 ^{c}	0.88 ^{b}	6.0 ^{d}	2.6 ^{b}	6.1 ^{e}	81.6 ^{bc}
30S	89.7 ^{a}	1.67 ^{b}	89.90 ^{c}	1.19 ^{c}	6.3 ^{e}	2.4 ^{b}	6.4 ^{f}	79.2 ^{a}
SE	0.5	0.03	0.32	0.04	0.1	0.2	0.1	0.4
				Multifactor Anova				
Soaking	ns	**	ns	ns	ns	ns	ns	ns
Holding time	*	**	**	**	**	**	**	ns
Soaking x holding time	ns	**	**	**	*	ns	**	**

5U, 15U and 30U: Samples obtained from un-soaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. D_{50}: Median diameter (diameter where 50% of particles had smaller particle size); Size dispersion: $(D_{90}-D_{10})/D_{50}$; L*: luminosity; a* and b*: chromatic colour coordinates; ΔE: Difference of colour between each treated sample and the flour obtained from native grains; C*: Chroma; h: hue. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant.

4.3.2 Scanning electron microscopy

Figure 4.3.1 shows the effect of HHP treatment at longer holding times (15 and 30 min) of pre- or un-soaked rough rice grains in the microstructure of WR flours. The images of the native flour samples (A micrographs, figure 1) showed an irregular and polyhedral appearance of starch granules, organised in packed structures as Zhu et al. (2016) previously observed. However, the starch granular conformation of the HHP treatment resulting flours showed important changes, the polyhedral shapes seem to be attenuated in the samples resulting from the HHP treatment without pre-soaking (B and D images, figure 1). In turn, samples resulting from the soaking treatment prior to HHP processing exhibited more evident changes, towards a more rounded and uniform globular structure (C and E images, figure 1). In addition, it also appeared that the diameter of the starch granules in those flours resulting from the combined treatments was larger than the commonly reported size, which ranged from 2 to 6 μm (W. Li et al., 2012). This is in agreement with previous observations of the swelling of starch granules due to an effect of HHP treatment (Rumpold & Knorr, 2005; Stolt et al., 2000). In the study of Rumpold and Knorr (2005) it was proposed that the initial steps of gelatinisation start with a swelling of the starch granules and the disruption of the crystalline region, followed in a second step by a more open crystalline structure for water. Furthermore, it has also been suggested that, although HHP treatment may preserve the integrity of the starch granule, due to the higher order state of the outer parts of the granule, which are prone to be more resistant to pressure forces, most of the structural changes induced by HHP occur in the inner parts of the granule (Li et al., 2012; Zhu et al., 2016). The greater changes observed on the presoaked samples could be due to the higher moisture reached during soaking, which further diffused into the inner parts of the grain under high pressure. Therefore, more starch granules were brought into contact with water to initiate a pressure-induced gelatinisation process (Balakrishna et al., 2020). Ravichandran et al. (2018) also reported a higher degree of gelatinisation in pre-soaked HHP-treated paddy grains than in un-soaked ones.

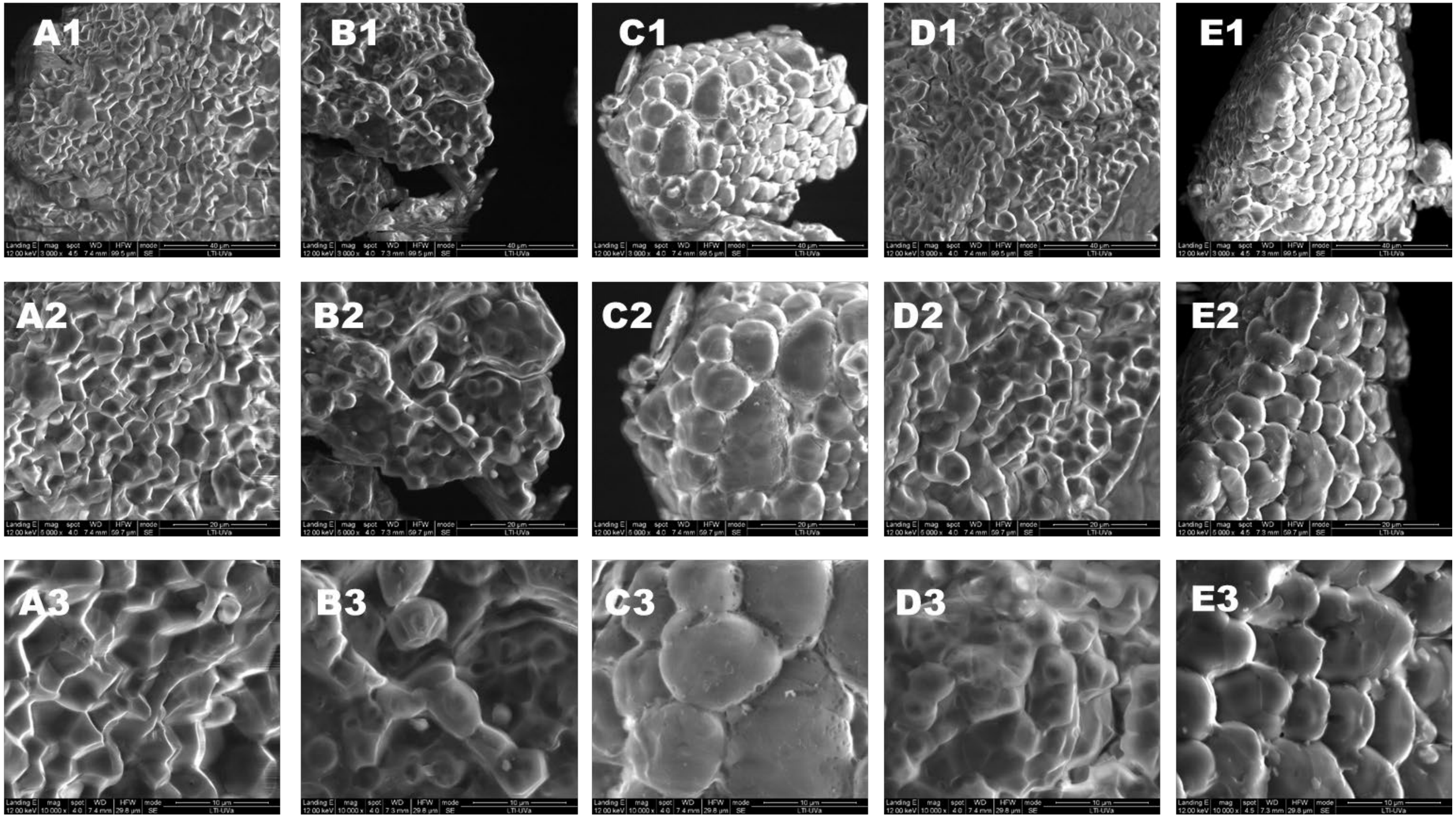

Figure 4.3.1. Scanning electron microscopy (SEM) photomicrographs of whole rice flour samples obtained from native and HHP-treated grains at different magnifications: 3000x (1), 5000x (2) and 10000x (3). A: native flour; B, C: flours obtained from HHP treatment at 15 min of un-soaked and pre-soaked grains, respectively; D, E: flours obtained from HHP treatment at 30 min of un-soaked and pre-soaked grains, respectively.

4.3.3 Functional properties

The results obtained for the functional properties are presented in Table 4.3.2. A significant ($p<0.05$) effect of the soaking pre-treatment on the water and oil absorption capacity of the flour samples was observed. The pre-soaked samples showed significantly ($p<0.05$) higher increase in WAC compared to the native and un-soaked ones. Regarding the OAC parameter, the pre-soaked and HHP-treated samples for 5 and 15 min showed significant increases ($p<0.05$) compared to the value of the native flour. Previous reports also observed higher WAC values in pre-soaked samples compared to un-soaked ones when studying the effect of HHP treatments on whole buckwheat grains (Gutiérrez et al., 2022). In the study of Cappa et al. (2016) a significantly ($p<0.05$) higher water retention capacity (WRC) was observed with HHP treatment (600 MPa) of rice flour slurries compared to the untreated samples. However, they reported that increasing the holding times from 5 to 10 min did not result in an increase of the WRC value. The WAC of the flours depends on the hydrophilic parts of the main biomolecules of which they are composed. Proteins, as well as starch, affected by processing technologies can be structurally modified, leading to present alterations in their water binding sites (Vela et al., 2021). The application of pressures above 300 MPa will lead to changes in the conformational structure of the protein. The modified protein can exhibit some variations in its functional properties. These variations are function of HHP treatment condition and therefore dependent on factors such as pressure level and holding time (V. M. Balasubramaniam et al., 2017). Zhu et al. (2017) has already reported an increase in the ability of proteins to bind water with increasing treatment intensity, as they reported higher WAC values in rice pressure-treated bran proteins with increasing pressure levels. They attributed this improvement to the HHP treatment given, which promoted an unfolding of the protein structures and the exposure of more hydrophilic groups. In a similar fashion, conformational changes of the protein could also lead to improvements in the oil binding capacity of the flours, as it was ascribed by Lin & Fernández-Fraguas (2020a) to explain the increase observed in common beans flours HHP-treated at 450 MPa for 10 min.

The factors soaking treatment and holding time significantly ($p<0.05$) affected the results of water absorption index (WAI), swelling power (SP) and water solubility index (WSI) of the resulting flours. Pressure-treated samples showed a significant reduction ($p<0.05$) in WAI and SP values compared to the native one (6-16%). Pre-soaking treatment also affected these parameters, but only at 5 min of holding time, where soaked samples (5S) showed significantly lower values ($p<0.05$) than their un-soaked counterparts (5U). Some authors have attributed the reduction of SP values after HHP treatments (at 50 °C) in brown rice to an inhibition of swelling promoted by the dispersed fibres in brown rice flour (S. M. Zhu et al., 2016). Other authors also suggested that a higher degree of amylose molecular rearrangement promoted by HHP at high temperatures (70-90 °C) would limit starch hydration and thus decrease granule swelling (W. Li et al., 2012). A significant ($p<0.05$) decrease in the WSI index was only observed for the 5 min pre-soak sample, while significant increases ($p<0.05$) were observed for the 15 min HHP-treated samples and the un-soaked samples treated with HHP for 30 min. Reduced solubility in rice starch has been attributed to the aggregation of amylose molecules during HHP treatment (W. Li et al., 2012). In brown rice treated with HHP, the observed solubility increases were related to the ability of starch molecules to diffuse out of the granule (S. M. Zhu et al., 2016).

The pre-soaking treatment had a particular relevance on the interface properties of the resulting flour. In particular, emulsion activity and stability were significantly affected (p<0.01). Significantly (p<0.05) higher values in emulsion activity were observed for all un-soaked samples compared to the control at different holding time. On the other hand, the pre-soaked samples had significantly lower emulsion activity and stability compared to the control with treatments longer than 5 min. With regard to foaming properties, significant increases in the foaming capacity of the flour samples were observed with increasing treatment time. However, no foam stability could be found in any of the flour samples. Protein is known to be important for its role in the emulsifying and foaming properties of flours (Yada, 2004). Zhu et al. (2017) reported higher activity and emulsion stability in rice bran protein dispersions treated with HHP (100 MPa). They suggested that this effect was caused by a higher number of exposed hydrophilic and hydrophobic groups due to a more open state of the protein structure triggered by the HHP treatment. The same authors also observed a reduction in stability at 500 MPa pressure and attributed this impairment to a decreased molecular flexibility with increasing pressure intensity.

Table 4.3.2. Functional properties (hydration, emulsion and foaming) of flour samples obtained from native and HHP treated paddy rice grains.

Sample	WAC (g/g)	OAC (g/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)	EA (%)	ES (%)	FC (mL/g)
Native	1.38 ^{a}	1.36 ^{a}	8.18 ^{d}	3.20 ^{b}	8.45 ^{e}	6.8 ^{c}	6.1 ^{b}	2 ^{a}
5U	1.39 ^{a}	1.34 ^{a}	7.67 ^{c}	3.63 ^{c}	7.95 ^{cd}	8.7 ^{d}	7.3 ^{c}	3 ^{b}
5S	1.50 ^{cd}	1.49 ^{b}	6.89 ^{a}	2.39 ^{a}	7.06 ^{a}	6.6 ^{c}	5.6 ^{b}	3 ^{b}
15U	1.44 ^{b}	1.41 ^{ab}	7.78 ^{c}	4.44 ^{d}	8.14 ^{de}	9.4 ^{d}	5.5 ^{b}	3 ^{b}
15S	1.50 ^{d}	1.48 ^{b}	7.57 ^{bc}	3.64 ^{c}	7.85 ^{bcd}	4.5 ^{b}	2.9 ^{a}	4 ^{c}
30U	1.45 ^{bc}	1.41 ^{ab}	7.26 ^{b}	3.73 ^{c}	7.54 ^{b}	9.7 ^{d}	5.4 ^{b}	4 ^{c}
30S	1.56 ^{e}	1.39 ^{ab}	7.48 ^{bc}	3.19 ^{b}	7.72 ^{bc}	3.2 ^{a}	3.0 ^{a}	4 ^{c}
SE	0.02	0.03	0.13	0.13	0.12	0.4	0.2	0
				Multifactor Anova				
Soaking	**	*	*	**	**	**	**	*
Holding time	*	ns	*	**	**	ns	**	ns
Soaking x Holding time	ns	ns	**	ns	**	**	ns	*

5U, 15U and 30U: Samples obtained from unsoaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. WAC: Water absorption capacity, OAC: Oil absorption capacity, WAI: Water absorption index, WSI: Water solubility index, SP: Swelling power, FC: Foaming capacity, EA: Emulsion activity. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0.05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95% confidence level (p<0.05), ns: not significant.

4.3.4 Pasting properties

In Fig. 4.3.2 the RVA pasting curves and in table 4.3.3 the results of the pasting parameters are showed. Although little effect of the factors studied (pre-soaking and holding time) could be observed, some clear trends in the pasting profiles were noted. A significant ($p<0.05$) increase was only observed for sample 15S in pasting temperature. A higher PT is related to a higher resistance to starch granule swelling (Solaesa et al., 2021). The flours resulting from the HHP treatments also showed significantly ($p<0.05$) lower (5-11 %) peak viscosity (PV) values than the native flour. During continuous heating and stirring of the flour suspensions in the RVA tests, the increase in viscosity is greatly caused by the swelling of the starch granules before physical breakdown (W. Li et al., 2012). Hence, the decrease observed for the SP in the functional properties (Table 2) would be in line with those decreasing values of PV. Previous observations have reported decreased PV values in rice starch treated with HHP at 600 MPa (30 min) (W. Li et al., 2012). These authors suggested that at this pressure level an amylose-lipid complex was generated and intertwined with the amylopectin molecules, limiting the swelling of the starch granules. Cappa, Lucisano, et al. (2016) also found decreasing PV values for rice flour treated with HHP at 400 MPa. They indicated that these values could be due to the samples were partially gelatinised. In addition, those authors also reported rice flour samples treated at 600 MPa (5 min) had a decrease in the enthalpy of gelatinisation that could suggest the samples achieved a certain degree of gelatinization (Vallons & Arendt, 2009b). However, in this study, no significant difference in gelatinisation enthalpy was found between the native flours and those resulting from the HHP treatments (values ranged from 8.75 J/g to 8.01 J/g, corresponding to the native and 15S sample respectively, data not shown). Other authors have found a small decrease in gelatinisation enthalpy with increasing time (15 to 30 min) and temperature (50 to 70 °C) but no significant differences were also observed ($p>0.05$) in high-pressure treated (600 MPa) paddy rice (Balakrishna & Farid, 2020). These authors explained that the husk provided a certain degree of resistance to the applied HHP treatment, so the pressure-induced gelatinisation phenomenon could not be detected. The reduction in PV could also be caused by an increase in water competition from the other major components of whole flours: fibre and protein. If those molecules showed a preference for water binding, the availability of water for swelling of the starch granules could be reduced and thus their maximum viscosity could be lowered (Ronda et al., 2017). The increase in the WAC values (see Table 4.3.2) of the flours resulting from the HHP treatments could be indicative of some modifications exerted by the pressure treatment on the water binding capacity of the flour components.

The significant ($p<0.05$) lower values in the breakdown viscosity of the flours resulting from the HHP treatments compared to the native ones, gave indications that those modified flours had yet improved their paste thermal stability. This improvement of paste stability has been reported in previous studies of HHP treatments on whole buckwheat grains (Gutiérrez et al., 2022). A reinforced crystalline structure and starch/protein/fibre cross-linking reactions caused by the pressure treatments could have increased the starch granular resistance as was suggested by Lin and Fernández-Fraguas (2020). In contrast, similar viscosity values were observed between the samples, at the end of the cooling period (final viscosity). Only those flours from 15 min of HHP treatments showed significantly ($p<0.05$) lower values compared to the native flour.

Furthermore, with the exception of 15U flour sample, the rest of the modified flours had a significant (p<0.05) increase in setback viscosity values (5-12 %). These data contrast with those reported by others where a decreased final viscosity value for rice starch (W. Li et al., 2012) or no significant changes in rice flour slurries treated with HHP were found (Cappa, Lucisano, et al., 2016).

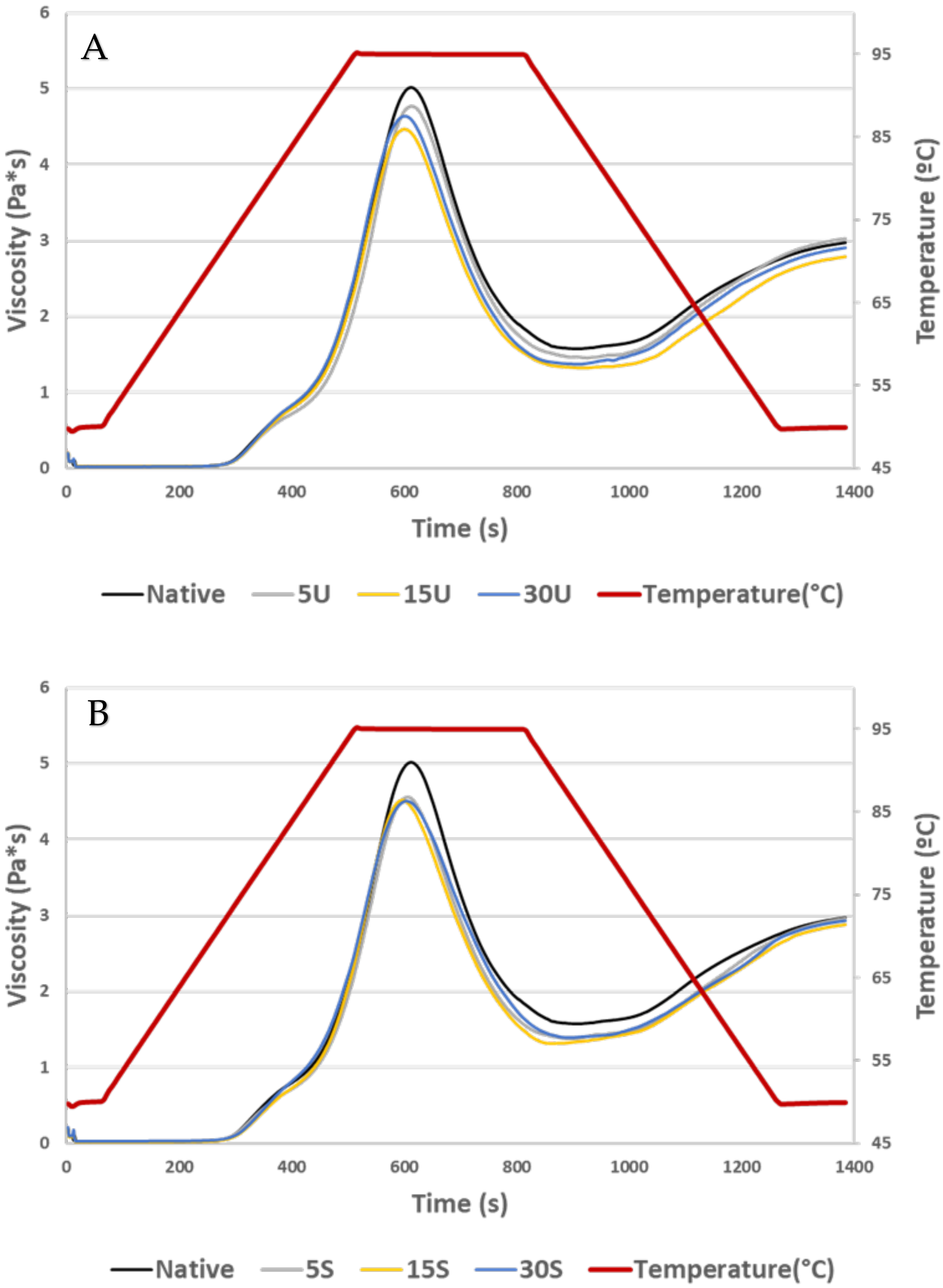

Figure 4.3.2. Pasting profiles of flour samples, A: Native and samples obtained from un-soaked HHP-treated paddy rice grains at 600MPa for 5, 15 and 15 min (5U, 15U and 30U). B: Native and samples obtained from pre-soaked and HHP-treated paddy rice grains at 600MPa for 5, 15 and 15 min (5S, 15S and 30S).

Table 4.3.3. Pasting properties of flour samples obtained from native and HHP treated paddy rice grains.

Sample	PT (ºC)	PV (Pa · s)	TV (Pa · s)	BV (Pa · s)	FV (Pa · s)	SV (Pa · s)
Native	72.49 ab	5.02 d	1.58 c	3.45 d	2.98 cd	1.40 a
5U	72.77 bc	4.77 c	1.46 b	3.32 c	3.03 d	1.57 c
5S	71.80 a	4.58 ab	1.38 ab	3.20 ab	2.97 bcd	1.59 c
15U	72.79 bc	4.47 a	1.32 a	3.14 a	2.79 a	1.47 ab
15S	73.50 c	4.52 ab	1.35 ab	3.17 ab	2.88 b	1.53 bc
30U	72.61 abc	4.65 bc	1.37 ab	3.27 bc	2.91 bc	1.54 bc
30S	72.91 bc	4.50 ab	1.39 ab	3.12 a	2.93 bc	1.54 bc
SE	0.27	0.05	0.03	0.03	0.03	0.03
			Multifactor Anova			
Soaking	ns	ns	ns	*	ns	ns
Holding time	ns	*	ns	ns	**	ns
Soaking x Holding time	ns	ns	ns	ns	ns	ns

5U, 15U and 30U: Samples obtained from unsoaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. PT: Pasting Temperature, PV: Peak Viscosity, FV: Final Viscosity, TV: Trough Viscosity, BV: Breakdown Viscosity, SV: Setback Viscosity. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0,05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95% confidence level ($p<0.05$), ns: not significant

4.3.5 Rheological properties of gels

The results of the rheological properties from the dynamic oscillatory tests performed on the gels prepared with the flour samples are presented in Table 4.3.4. The frequency sweeps showed the elastic G_1\` and viscous $G_1$\`\` coefficients were significantly influenced ($p<0.01$) by the soaking factor. At this regard, small differences were found comparing the gel samples from modified flour with the native one, where only the 15S sample showed a significantly higher value. However, significant differences ($p<0.05$) were observed between the gels of the pre-soaked samples than the un-soaked ones, as higher complex modulus (G*) values were obtained for those samples treated for more than 5 min. Increases in the elastic moduli values have also been reported with increasing holding time (7.5 - 15 min) with HHP treatment on Basmati rice flour dispersions at constant pressure levels (350-550 MPa) (Ahmed et al., 2007). An enhance of the gel strength of rice starch after HHP treatments was also reported by Jiang et al. (2015). Those authors attributed this effect to changes in the structure of the rice starch granule due to the pressure, which affected the hydrogen bonds between starch molecules. In the study by Lin and Fernández-Fraguas (2020), greater viscoelastic moduli values were observed with HHP treatments of bean flour slurries at increasing pressure levels (300 - 600 MPa) and increasing holding times (5 - 15 min). Those authors stated that at a high-pressure level (600 MPa) a strong network was formed by the presence of gelatinised starch and/or denatured protein. Results also showed a trend of increasing loss tangent values with increasing treatment time, hence this factor had a significant ($p<0.01$) relevance in this parameter. Increasing the treatment intensity led to a larger increase in the viscous component with respect to the rise of the elastic one. At the studying conditions, all gels samples had a solid-like behaviour as loss tangent values were lower than 1. Concerning the a and b values, small differences were found. Only the 30S gel sample exhibited a significant ($p<0.05$) decrease in the frequency dependence of the viscous component G''_1 with respect to the native b value.

Through the strain sweeps performed outside the linear viscoelastic region (LVR), the ability of the gel samples to withstand deformation forces until they lose their elastic prevalence can be observed (crosspoint). Holding time and soaking factors were significant ($p<0.01$; $p<0.05$, respectively) in this respect. A general decrease in crossover point values was observed with increasing holding time for all HHP-treated samples. The end of LVR of each sample gel is expressed by the τ_{max} value. Treatment conditions (pre-soaking and holding time) had a significant effect ($p<0.01$) on this parameter. A significant ($p<0.05$) sharp increase (more than two-fold) was observed for the sample resulting from the least intensity treatment (5S). It was also revealed an increase in the holding time led to a significant ($p<0.05$) decrease in the τ_{max} values of the un-soaked samples. As for the pre-soaking ones, only the most intensively treated (30S) showed a significant decrease compared to the native. Therefore, the ability to withstand the strain forces of the gel samples was at least maintained up to 15 minutes of holding time treatments.

Table 4.3.4. Rheology properties of gel flour samples obtained from native and HHP treated paddy rice grains.

Sample	G'_1 (Pa)	a	G''_1 (Pa)	b	Tan $(\delta)_1$	c	G^*_1 (Pa)	Cross-point (Pa)	$\tau_{máx}$ (Pa)
Native	77 ^{ab}	0.195 ^{ab}	23.6 ^{a}	0.333 ^{b}	0.308 ^{a}	0.138 ^{bc}	80 ^{abc}	111 ^{d}	14 ^{bc}
5U	80 ^{abc}	0.185 ^{a}	24.3 ^{ab}	0.332 ^{b}	0.304 ^{a}	0.147 ^{c}	83 ^{abc}	106 ^{cd}	37 ^{d}
5S	83 ^{bc}	0.196 ^{ab}	25.8 ^{bc}	0.329 ^{ab}	0.312 ^{ab}	0.134 ^{abc}	87 ^{bc}	98 ^{bc}	15 ^{bc}
15U	75 ^{a}	0.203 ^{b}	24.6 ^{ab}	0.331 ^{b}	0.329 ^{bc}	0.128 ^{ab}	79 ^{a}	91 ^{ab}	14 ^{bc}
15S	85 ^{c}	0.197 ^{ab}	26.6 ^{cd}	0.329 ^{ab}	0.315 ^{abc}	0.132 ^{abc}	89 ^{c}	98 ^{bc}	16 ^{cd}
30U	75 ^{a}	0.202 ^{b}	24.7 ^{ab}	0.330 ^{ab}	0.329 ^{bc}	0.127 ^{ab}	79 ^{a}	94 ^{ab}	10 ^{a}
30S	84 ^{bc}	0.205 ^{b}	27.8 ^{d}	0.324 ^{a}	0.332 ^{c}	0.119 ^{a}	88 ^{c}	87 ^{a}	11 ^{a}
SE	2	0.531	0.0	0.002	0.005	0.005	2	2	0
					Multifactor Anova				
Soaking	**	ns	**	ns	ns	ns	**	*	**
Holding time	ns	*	ns	ns	**	*	ns	**	**
Soaking x Holding time	ns	ns	*	ns	ns	ns	ns	ns	**

5U, 15U and 30U: Samples obtained from un-soaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. The power law model was fitted to the frequency sweep experimental data ($G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \omega^c$), where G_1', G_1'', and $\tan(\delta)_1$ are the coefficients obtained from the fitting and represent the elastic, viscous moduli, and loss tangent, respectively, at a frequency of 1 Hz. The a, b, and c exponents quantify the degree of dependence of the dynamic moduli and the loss tangent with the oscillation frequency. G^*_1 refers to the complex modulus. τ_{max} represent the maximum stress that the samples could tolerate in the linear viscoelastic region (LVR). The crosspoint value represents the stress at G` = G``. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95 % confidence level ($p<0.05$), ns: not significant.

4.3.6 Nutritional properties of the flours

4.3.6.1 Vitamin and mineral content

The measurement of the thiamine, niacin and pyridoxine content is shown in Table 4.3.5. The value observed for thiamine in the native sample were in the range of those previously reported by Balakrishna and Farid (2020). Soaking pre-treatment, holding time and the interaction between them significantly ($p<0.01$) affected the thiamine content in the results observed for the modified WR flours. In contrast, these factors had no significant influence on niacin and pyridoxine content. Among the modified WR flours, no significant losses of vitamins were observed. On the other hand, these showed significant increases in thiamine (up to 88% for samples 15S and 30S), niacin (18 % for sample 5U only) and pyridoxine (up to 32% for sample 30S). An increase in thiamine content in paddy rice has also been found with increasing pressure level from 450 MPa to 600 MPa and HHP treatment temperature from 50 to 70 °C (Balakrishna & Farid, 2020). These authors did not observe an increase in thiamine content with longer treatment times (from 15 min to 30 min), which is in agreement with the results observed in the present investigation, although an increase in pyridoxine content with increasing holding time from 5 to 15 min was observed. Balakrishna and Farid (2020) attributed for the observed increases to a diffusion effect of thiamine from the outer to the inner layers of the grain. However, unlike that research, this study measured the vitamin content of whole (brown) rice flours instead of white rice. Therefore, the significant difference observed among the samples could be attributed to a higher bran content in the whole flour of the resulting flours from HHP treatments. Since the same milling procedure was followed for both the native and the HHP-treated samples, the HHP treatment could have promoted a different milling behaviour of the last ones, increasing the recovery percentage of the bran fraction. This could be due to the pressure promoted a firmer adhesion of the bran layer to the endosperm, similar to the parboiling process. Increases in thiamine content were also observed in milled rice after steaming following soaking of paddy rice. This was related to the inner bran layers being embedded in the gelatinised surface endosperm and thus prevented from being removed during milling (Rao & Bhattacharya, 1966).

In table 4.3.5 is also showed the mineral content of the WR flour samples. The values found for the native sample were in the range reported by F. Wu et al. (2013) in brown rice for calcium, phosphorus, manganese and potassium. However, the values obtained for zinc (2.3 mg/100g) and iron (9.3 mg/100g) were higher than those previously reported by F. Wu et al. (2013), who established a zinc range of 1.5 - 2.2 mg/100g and an iron range of 0.7 - 5.4 mg/100g. Differences in mineral content in the reports of others could be attributed to differences in rice variety and/or environmental and growing conditions (Xia, Tao et al., 2017). The soaking pre-treatment factor only significantly affected ($p<0.05$) the iron results. On the other hand, the holding time and its interaction with the pre-soaking significantly ($p<0.05$) influenced the mineral content of calcium, iron, and manganese. The phosphorus, potassium and zinc content of the modified WR flours was not significantly different from the result found in the native WR flour. However, a significant ($p<0.05$) reduction (5-14 %) in manganese content was found in almost all modified WR flours. In addition, those flours resulting from HHP treatments of 30 min holding time or those resulting from a pre-soaking treatment with 15 min holding time showed a significant decrease ($p<0.05$) in calcium and iron content. It was suggested that increased bioavailability of minerals in brown rice after HHP treatment (300 MPa) could be attributed to the effect of disrupting the pressure on the cells, releasing minerals into the extracellular environment. However, at higher pressure levels, the bioavailability of minerals decreased because gelatinised starch under pressure could act as a mineral encapsulating agent (Ding et al., 2021). It was also assumed that the variability found in the mineral content of HHP-treated brown rice depended on the spatial distribution, chemical form, ligand composition and reactive activity of the compounds in which they are present (Xia et al., 2017).

Table 4.3.5. Vitamin and mineral content of flour samples obtained from native and HHP treated paddy rice grains.

Sample	Thiamine (mg/100g)	Niacin (mg/100g)	pyridoxine (mg/100g)	Ca (mg/100g)	P (mg/100g)	Fe (mg/100g)	Mn (mg/100g)	K (mg/100g)	Zn (mg/100g)
Native	0.17 [a]	5.05 [ab]	0.185 [a]	20 [b]	255 [ab]	9.3 [c]	1.44 [c]	265 [a]	2.3 [ab]
5U	0.27 [ab]	5.95 [c]	0.199 [ab]	15 [ab]	240 [a]	7.5 [b]	1.32 [b]	255 [a]	2.8 [b]
5S	0.30 [b]	5.03 [ab]	0.211 [abc]	20 [b]	255 [ab]	8.3 [bc]	1.36 [b]	270 [a]	2.3 [ab]
15U	0.30 [b]	5.19 [abc]	0.216 [bc]	20 [b]	270 [b]	9.0 [c]	1.42 [c]	260 [a]	2.2 [ab]
15S	0.32 [b]	5.58 [abc]	0.235 [cd]	10 [a]	260 [ab]	6.0 [a]	1.24 [a]	250 [a]	2.0 [a]
30U	0.28 [b]	4.83 [a]	0.206 [ab]	10 [a]	260 [ab]	6.0 [a]	1.24 [a]	250 [a]	2.0 [a]
30S	0.32 [b]	5.70 [bc]	0.245 [d]	10 [a]	255 [ab]	6.3 [a]	1.30 [b]	260 [a]	1.9 [a]
SE	0.02	0.05	0.007	0.01	6	3.0	0.02	6.00	2
		Multifactor Anova							
Soaking	**	ns	ns	ns	ns	*	ns	ns	ns
Holding time	**	ns	ns	*	ns	**	*	ns	ns
Soaking x Holding time	**	ns	ns	*	ns	**	**	ns	ns

5U, 15U and 30U: Samples obtained from un-soaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95 % confidence level ($p<0.05$), ns: not significant

4.3.6.2 Total phenol content and antioxidant capacity

The phenol content (TPC) and total antioxidant capacity (TAC) measured by DPPH, ABTS, FRAP, ORAC, Q-DPPH and Q-ABTS tests of WR flour samples are presented in Table 4.3.6. Both factors (soaking and holding time) significantly affected the results of TPC and TAC of the sample extracts ($p<0.01$), as well as the results of Q-DPPH and Q-ABTS ($p<0.05$). The phenolic content of the native sample extract was 107 mg GAE/100 g dw. This result was higher than that reported by Goffman and Bergman (2004) (69 mg GAE/100 g dw) and lower than that observed by Shen at al. (2009) (197.5 mg GAE/100 g dw). However, the value shown by the latter authors was an average of a large number of analysed genotypes (n=481) whose values ranged from 108.1 to 1244.9 mg GAE/100 g (dw) with the lowest values belonging to uncoloured bran genotypes and close to the outcome observed for the native extract in this investigation. All the rice samples in the present study came from the same cultivar, hence the variability in the data of the different samples studied must have come from the different treatment conditions. The influence of the processing condition factors was significant for the phenol content parameter ($p<0.01$). Significant ($p<0.05$) improvements (up to 23 %) were observed in the phenol content of the WR flour samples coming from treatments compared to the native flour (except for 5U sample). The phenol content values of the physically modified WR flour samples, ranged from 107 to 132 mg GAE/100 g dw, with significantly ($p<0.05$) increasing values for those samples with the pre-soaking treatment and holding times of up to 15 min. Increasing the holding time further than 15 min did not increase the TPC value of the resulting flours. It has been suggested that HHP treatment may enhance the release of antioxidant compounds by altering cell walls and organelles and thereby delivering them to the extracellular environment (Xia, Wang, et al., 2017). Furthermore, significant increases ($p<0.05$) in the phenol content of rough rice were reported under increasing levels of pressure (from 10 to 100 MPa) and after germination (M. Y. Kim et al., 2017). They attributed this increase to a change in phenolic distribution due to changes in the microstructure of the rice. Mass transfer phenomena have also been reported through HHP treatments, as pressure can enhance solvent penetration through disrupted cell walls with increased permeability (Balakrishna et al., 2020). Increases in soluble phenolic compounds in rough rice following hydrothermal treatment have also been associated with cell wall instability (Min et al., 2014). Other authors have also reported the diffusion of coloured pigments and polyphenols from rice bran into the endosperm with soaking and/or steaming treatments. They also informed that the pigment diffusion could also come from the seed coat, as observed in millet (Rocha-Villarreal et al., 2018).

With regard to the significant ($p<0.05$) increase observed in phenol content in those soaked samples compared to the un-soaked ones, it could be suggested that the soaking pre-treatment might have increased the permeabilisation of the cell wall and thus made them more susceptible to the disruptive effect promoted by the HHP treatment. A significant ($p<0.05$) increase in phenol content was reported for pre-soaked buckwheat rough grains followed by two cycles of an HHP treatment in comparison with the un-soaked and HHP-treated ones (Gutiérrez et al., 2022).

Total antioxidant capacity (TAC) results were also highly influenced ($p<0.01$) by the soaking and holding time factors. In general, the modified WR flours did not show lower values than the native one, except for the 5U and 15U samples which showed significantly lower values in DPPH and Q-DPPH and 5U in ORAC. On the other hand, an improvement in TAC values was observed with increasing holding time and, in particular, in those samples coming from a pre-soaking treatment. Significant ($p<0.05$) increases in DPPH and Q-DPPH were observed for all pre-soaked samples and the un-soaked ones with 30 min of holding time (30U). In addition, significant ($p<0.05$) increases (8 - 36 %) in ABTS, ABTS-Q and FRAP were observed for all WR flour samples resulting from HHP treatments. But in the ORAC test, only those samples resulting from 30 min of holding time, or the 15S sample showed significantly ($p<0.05$) higher values than the native sample. It was previously suggested that the phenol content is mainly responsible for the anti-radical efficacy of rice bran extracts (Goffman & Bergman, 2004). Hence, the higher TAC values observed in the HHP-resulting WR flours could be attributed to the increase observed in phenolics for those samples. It was reported that higher antioxidant activity due to HHP treatment in brown rice could also be caused by promoting better bioaccessibility of free antioxidants, which show higher antioxidant activity than those presented in bound forms (Xia, Wang, et al., 2017).

Table 4.3.6. Total phenol content (TPC) and total antioxidant capacity (TAC) of flour samples obtained from native and HHP treated paddy rice grains.

Sample	**TPC (mg GAE/ 100g)**	**DPPH (mg TE/ 100g)**	**ABTS (mg TE/ 100g)**	**FRAP (mg Fe+2/ 100g)**	**ORAC (mg TE/ 100g)**	**Q-DPPH (mg TE/ 100g)**	**Q-ABTS (mg TE/100g)**
Native	107 ^{a}	48.0 ^{a}	147 ^{a}	122 ^{a}	246 ^{bc}	826 ^{b}	252 ^{a}
5U	107 ^{a}	48.1 ^{a}	160 ^{b}	141 ^{c}	200 ^{a}	794 ^{a}	275 ^{b}
5S	119 ^{b}	51.9 ^{b}	176 ^{c}	142 ^{c}	263 ^{cd}	879 ^{c}	293 ^{c}
15U	120 ^{b}	48.2 ^{ab}	158 ^{b}	138 ^{b}	230 ^{b}	785 ^{a}	293 ^{c}
15S	132 ^{c}	56.7 ^{c}	192 ^{d}	142 ^{c}	277 ^{d}	925 ^{d}	297 ^{cd}
30U	120 ^{b}	52.0 ^{b}	177 ^{c}	143 ^{c}	285 ^{d}	885 ^{c}	289 ^{c}
30S	132 ^{c}	62.0 ^{d}	201 ^{e}	147 ^{d}	324 ^{e}	952 ^{d}	307 ^{d}
SE	2	1.3	3	1	9	10	4
				Multifactor Anova			
Soaking	**	**	**	**	**	**	**
Holding time	**	**	**	**	**	**	*
Soaking x Holding time	ns	ns	*	ns	ns	**	ns

5U, 15U and 30U: Samples obtained from un-soaked HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. 5S, 15S and 30S: Samples obtained from pre-soaked and HHP-treated paddy rice grains at 600 MPa for 5, 15 and 30 min. TPC refers to the total phenol content. Total antioxidant capacity as DPPH, ABTS, FRAP and ORAC on sample flours extracts and Q-DPPH and Q-ABTS of direct (quencher) method on solid flour samples. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95 % confidence level ($p<0.05$), ns: not significant

4.3.7 Rheological properties of the doughs

Table 4.3.7 summarises the effects of replacement level and the use of the modified whole rice flour (15S, selected on the basis of observed nutritional and techno-functional outcomes) instead of the native one on the rheological properties of gluten-free doughs. A significant impact ($p<0.01$) of both factors was observed on the coefficients ($G`_1$, $G``_1$) obtained through the frequency sweep of the oscillatory tests performed. However, the interaction between the factors did not significantly affect the results. In all the cases the storage modulus values (G`) were greater than those for the loss modulus (G``) meaning doughs were more elastic than viscous, as was previously reported in rice doughs (Sivaramakrishnan et al., 2004; Ronda et al., 2017). The G'_1 and G''_1 values of the control dough made with 100% white rice flour were significantly ($p<0.05$) lower than the rest of the doughs tested. Increasing the replacement level with WR flour resulted in significantly ($p<0.05$) higher values for the corresponding viscoelastic coefficient values. Furthermore, replacing the white rice flour of the GF dough formula with the modified WR instead of the native one, led to significantly higher values ($p<0.05$) in $G`_1$ from the 30% to 70% of the replacement levels. The values of the complex modulus (G*) also followed the same trend observed for the elastic modulus. Concerning the loss tangent values $(\tan\delta)_1$, a significant difference was detected only at the 30% of replacement level among the different WR flours-containing-doughs. At that replacement level, the doughs with the modified WR flour exhibited a more elastic behaviour than its counterpart formulated using native WR flour. The replacement level factor also had a significant impact ($p<0.05$) on the a, b and c exponents. The higher the replacement level, the lower values were found for these exponents, which means that the less frequency dependence the dough structures were. A number of studies have associated the inclusion of fibre-rich ingredients in dough formulations to lead an increase in the solid elastic-like behaviour, whether in gluten or wheat-based (Angioloni & Collar, 2008) or gluten-free doughs (Föste et al., 2014). Tsatsaragkou et al. (2014) observed an increase in the elastic character of the dough after the addition of carob flour to partially replace the rice flour in the GF formula. Those author claimed that fibre-rich ingredients could act as strength and elasticity promoters in dough structures. In addition, they attributed the increase in the elastic behaviour to a reduced water availability which caused water competition between the dough constituents and affected the rheological behaviour of the dough, decreasing the frequency dependence of the storage and viscous modulus. Therefore, the increase in elastic and complex modulus observed in the doughs containing the modified WR flour could be related to the higher water absorption capacity given by the modified WR flour compared to the native WR flour (see WAC in Table 4.3.2). Other authors have also observed an increased elasticity after HHP treatment of white rice batters (200-600 MPa, 10 min) (Vallons et al., 2011) and oat batters (>350 MPa, 10 min) (Hüttner et al., 2009b). The latter authors attributed this increase to a combined effect of starch gelatinisation and protein denaturation promoted by the pressure treatment.

The strain sweeps carried out allowed us to observe the significant impact ($p<0.01$) of the factors on the τ_{max} and the crossover point. The ANOVA results of τ_{max} determined that from the 50 % replacement level onwards, a significant ($p<0.05$) increase in the linear viscoelastic region (LVR) was observed. At that replacement level in addition, greater stress that the doughs could withstand until the viscous modulus equalled the elastic modulus (G` = G``) could be noted. A further increase in the stability of the dough structure against shear stresses has also been reported by Hüttner et al. (2009) with HHP-treated oat doughs.

Table 4.3.7. Rheological properties of the gluten-free flour dough samples as a function of WR flour replacement level and type of WR flour (native/modified).

Replacement level (%)	WR Flour	G`$_1$ (Pa)	a	G``$_1$ (Pa)	b	(Tan δ)$_1$	c	G* (Pa)	Crosspoint (Pa)	τ_{max} (Pa)
0	-	384 ^{a}	0.438 ^{e}	308 ^{a}	0.49 ^{f}	0.80 ^{e}	0.050 ^{a}	492 ^{a}	2.7 ^{a}	0.8 ^{a}
15	Native	672 ^{b}	0.36 ^{d}	418 ^{b}	0.43 ^{e}	0.62 ^{d}	0.071 ^{ab}	792 ^{b}	10.1 ^{a}	1.0 ^{a}
30	Native	945 ^{c}	0.323 ^{c}	518 ^{cd}	0.43 ^{e}	0.55 ^{c}	0.106 ^{cd}	1078 ^{c}	14.0 ^{ab}	1.5 ^{ab}
50	Native	1808 ^{e}	0.278 ^{b}	834 ^{e}	0.37 ^{cd}	0.46 ^{b}	0.09 ^{bcd}	1991 ^{e}	40.2 ^{c}	2.4 ^{c}
70	Native	2734 ^{g}	0.220 ^{a}	953 ^{f}	0.33 ^{ab}	0.35 ^{a}	0.115 ^{d}	2895 ^{g}	84.2 ^{e}	3.6 ^{d}
15	Modified	741 ^{b}	0.354 ^{cd}	444 ^{bc}	0.43 ^{e}	0.60 ^{cd}	0.075 ^{abc}	864 ^{b}	12.3 ^{a}	1.1 ^{a}
30	Modified	1161 ^{d}	0.286 ^{b}	559 ^{d}	0.39 ^{d}	0.48 ^{b}	0.109 ^{d}	1288 ^{d}	25.1 ^{bc}	2.2 ^{bc}
50	Modified	2037 ^{f}	0.259 ^{b}	875 ^{e}	0.35 ^{bc}	0.43 ^{b}	0.096 ^{bcd}	2217 ^{f}	56.0 ^{d}	3.1 ^{d}
70	Modified	3034 ^{h}	0.225 ^{a}	1105 ^{g}	0.32 ^{a}	0.36 ^{a}	0.097 ^{bcd}	3229 ^{h}	112.4 ^{f}	4.8 ^{e}
	SE	55	0.01	23	0.01	0.02	0.011	59	3.8	0.2
						Multifactor Anova				
	WR flour	**	ns	**	*	ns	ns	**	**	**
	Replacement level	**	**	**	**	**	*	**	**	**
	BW flour x Replacement level	ns	ns	ns	ns	ns	ns	ns	ns	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free dough that is replaced with native/modified WR flour. Native: Flour sample obtained from native paddy rice grains. Modified: Flour sample obtained from pre-soaked paddy rice grains treated with HHP at 600 MPa for 15 min. A 0 % replacement level refers to 100 % rice-based GF dough. The power law model was fitted to the frequency sweep experimental data ($G' = G'_1 \cdot \omega^a$; $G'' = G''_1 \cdot \omega^b$; $\tan \delta = (\tan \delta)_1 \cdot \omega^c$), where G_1', G_1'', and $\tan(\delta)_1$ are the coefficients obtained from the fitting and represent the elastic, viscous moduli, and loss tangent, respectively, at a frequency of 1 Hz. The a, b, and c exponents quantify the degree of dependence of the dynamic moduli and the loss tangent with the oscillation frequency. G^*_1 refers to the complex modulus. τ_{max} represent the maximum stress at the end of the linear viscoelastic region (LVR) obtained from the strain sweep. The crosspoint value represents the stress at G` = G``. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95 % confidence level ($p<0.05$), ns: not significant.

4.3.8 Bread quality properties

Specific volume, bake loss

The specific volume, bake loss and textural parameters of the breads studied are shown in Table 4.3.8. The replacement level significantly (p<0.01) affected weight loss in bread samples with WR flour present. The values of this parameter ranged from 18.8 % to 21.5 % and although slight but also significant differences (p<0.05) were observed between the breads containing WR flour and the bread control, no clear trend could be discerned. It was noted a significant (p<0.01) effect of both factors and the interaction between them on the specific bread volume. The breads made with the WR native flour showed a significant (p<0.05) decrease compared to the white rice control bread. Moreover, the higher the replacement level the greater the decline (up to 16 %). On the other hand, the breads containing the modified WR flour resulted with a significant (p<0.05) increase up to a replacement level of 50 % (with an increase of 11 %) with respect to the white rice control bread. The 70 % of replacement level meant a significant (p<0.05) drop on the specific bread volume comparing to the control (11%). Higher specific bread volumes have been linked to improvements in the consistency of weak doughs (Villanueva et al., 2019), as higher viscosity values could help the dough to strengthen its structure to retain the gas generated during fermentation and therefore allow better gas expansion in the oven. Although higher viscoelasticity values were observed in the rheological properties of the doughs with the addition of both kinds of WR flour (see Table 7), only those containing the modified flour resulted in a higher specific bread volume. Therefore, apart from improvements in consistency, other properties induced by HHP treatment in WR flour might have helped inducing better baking performance. It was claimed that HHP processing can modify the structure of native starch and thus its pasting behaviour (Cappa, Barbosa-Cánovas, et al., 2016). In the present study, a significantly higher pasting temperature (p<0.05) was observed for the modified flour selected for bread making (15S, see fig 4.3.2 and Table 4.3.3). Such an increase in PT could allow for further development of the dough during the baking step before the solid breadcrumb structure is established (Ronda et al., 2017). It was also reported that protein polymerisation could improve the elastic behaviour of GF doughs and consequently improve baking performance. Polymerisation of the free sulphydryl groups presented in the white rice batters after HHP treatments was observed and attributed to disulphide bonding, which favoured the appearance of urea-insoluble aggregates (Vallons et al., 2011). Hüttner et al. (2010) reported positive effects on the specific volume of GF bread at replacing 10% of a native oat flour with an HHP-modified one (200 MPa, 10 min). Those authors attributed this improvement to pressure-weakened protein effect that allowed less interference of the oats proteins in the uniformity of starch gel during baking (Hüttner et al., 2010). The significant (p<0.05) and great decrease in the specific volume of those bread at the 70 % of the replacement level with both kind of WR flour, could be attributed to an excessive consistency of the dough which limited the gas expansion and thus, did not allow for a proper loaf development during baking (Ronda et al., 2017).

Texture

A significant (p<0.01) effect of the replacement level was found in all texture parameters of the bread samples. However, the use of modified WR in the GF bread formulation only had a significant impact on crumb firmness (p<0.01) and springiness (p<0.05). Meanwhile, the interaction between the two factors studied only significantly affected crumb firmness at 7 days (Table 8). It was observed that the replacement of white rice flour by WR flour did not increase the crumb firmness of the breads except for those containing the maximum level of substitution (70 %). In addition, the use of the modified WR flour at 50 % replacement level significantly (p<0.05) reduced (29%) the crumb firmness compared to the control. This observed decrease could be attributed to the increase in bread volume observed for that specific bread sample, as a lower resistance to crumb deformation of the texture probe should be detected in a bread with a higher amount of entrapped air (Villanueva et al., 2019). In the case of chewiness, as this parameter is affected by firmness, it followed a similar trend. A significant (p<0.05) increase in chewiness was observed as the replacement level of the native WR flour was increased. However, at their counterpart breads made with the modified WR, a significant (p<0.05) increase in chewiness was only detected at the highest replacement level. The crumb firmness at 7 days of the breads containing WR flour rose with increasing replacement level. However, this increase was only significant (p<0.05) in comparison with the control at 70 % of the replacement level. This results contrasted with other authors reports, as positive effects on the use of HHP-treated rice flour to decrease the staling of GF bread were found (Cappa, Barbosa-Cánovas, et al., 2016). No significant differences in springiness were observed when comparing the breads containing WR flour with the control bread, with the exception of the bread sample at 50 % replacement level with the modified WR flour, which resulted in a significant (p<0.05) increase in crumb springiness. Nevertheless, this increase was desirable as this quality parameter was related to an elastic and airy crumb structure in fresh breads (Boulemkahel et al., 2022). Similarly, the significant (p<0.05) higher resilience and cohesiveness observed in breads containing WR flour from 30 % of the replacement level indicated a more integrated crumb structure with increasing recovery capacity.

Table 4.3.8. Bread quality properties of gluten-free breads as a function of WR flour replacement level and type of WR flour (native/modified)

Replacement level (%)	WR Flour	Weight loss (%)	Specific volume (mL/g)	Firmness (N)	Firmness (7 days) (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
0	-	19.9 b	3.33 e	1.46 bcd	5.7 bc	0.896 ab	0.46 a	0.60 ab	0.178 ab
15	Native	20.7 de	3.29 d	1.68 de	3.9 a	0.878 a	0.48 a	0.70 bc	0.177 ab
30	Native	21.5 f	3.24 c	1.65 d	5.9 bc	0.871 a	0.54 b	0.78 c	0.213 cd
50	Native	20.6 cd	3.23 c	1.60 cd	6.9 c	0.931 bc	0.56 bc	0.85 c	0.208 bc
70	Native	18.8 a	2.79 a	2.01 f	8.7 d	0.935 bc	0.61 d	1.15 d	0.229 cd
15	Modified	20.7 cde	3.49 f	1.31 abc	6.3 bc	0.883 a	0.45 a	0.52 a	0.168 a
30	Modified	19.2 a	3.50 f	1.26 ab	4.8 ab	0.939 bc	0.52 b	0.62 ab	0.205 bc
50	Modified	21.3 ef	3.71 g	1.03 a	5.0 ab	0.961 c	0.59 cd	0.59 ab	0.228 cd
70	Modified	20.1 bc	2.97 b	1.97 ef	9.9 d	0.937 bc	0.61 d	1.13 d	0.243 d
	SE	0.2	0.01	0.11	0.5	0.016	0.01	0.05	0.011
					Multifactor Anova				
WR flour		ns	**	**	ns	*	ns	**	ns
Replacement level		**	**	**	**	**	**	**	**
BW flour x Replacement level		**	**	ns	**	ns	ns	ns	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified WR flour. Native: Flour sample obtained from native paddy rice grains. Modified: Flour sample obtained from pre-soaked paddy rice grains treated with HHP at 600 MPa for 15 min. A 0 % replacement level refers to 100 % rice-based GF bread. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0.05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95 % confidence level (p<0.05), ns: not significant

Crust and crumb colorimetric parameters

The crust and crumb colour parameters are shown in Table 4.3.9. The replacement level significantly ($p<0.01$) affected all of the colorimetric parameters of the WR flours-containing breads. Increasing the replacement level from 30 % onwards with both types of WR flour significantly ($p<0.05$) decreased the crust luminosity compared to the control bread. This darkening effect, with increasing replacement level with WR flour could be attributed to the rice bran constituents in WR flour. Rice bran is considered a vegetable protein source (Issara & Rawdkuen, 2016). The presence of increasing levels of WR flour in the GF bread formula and hence, an increase in the protein content could have boosted the Maillard reactions occurred during baking, as they are mainly triggered by reducing sugars and amino acids. It was yet reported protein-enriched GF breads increased Maillard reactions and thus, led to darker crusts (Villanueva et al., 2015). The presence of modified WR flour in place of native flour was not particularly relevant regarding crust luminosity. A similar trend was observed for Chroma (C*) results, as significant ($p<0.05$) and decreasing values were detected with increasing replacement levels. The changes observed in crust colour difference (ΔE) were related to significant ($p<0.05$) increasing values with increasing the replacement level. From the 30 % replacement level, values ranged from 7.9 to 14.2 hence, the difference regarding with the control bread crust was sensory perceptible (Vicente et al., 2023b). As ΔE was determined by the combination of L*, a* and b*, the increasing values observed at higher replacement levels could be attributed to the variations observed in L* where the largest differences were noted.

Crumb colour parameters were affected by replacement level ($p<0.01$), treatment factor ($p<0.05$) and the interaction between them ($p<0.05$). The colour of the crumb is well related to the colour of the ingredients in the formula (Villanueva et al., 2019). Therefore, the increased level of replacement of the white flour by the WR one could be the reason for the decrease (14 % ~ 17 %) in the luminosity values of the WR flour-containing breads compared to the control. Among the bread samples, those with the modified WR flour had a significant ($p<0.05$) decrease in luminosity values compared to their counterparts with the native flour at the 15 % and 30 % replacement levels. At higher replacement levels there were no significant differences when comparing breads containing the different types of WR flour. Gluten-free breads often show too white colouration and diminished appealing compared the wheat-based breads (Conte et al., 2019) therefore, this darkening effect could help to increase their sensory acceptability. Significantly ($p<0.05$) higher C* values were also found in bread samples at higher replacement levels. This difference could be attributed to the fact that the colour of the crumb varied with the higher redness and yellowness values that would be provided by the WR flour. As mentioned above, the colour of the crumb is related to the characteristic colour of the ingredients. However, the higher Chroma values observed for the breads containing native WR compared to their counterparts with the modified WR flour did not correspond with the values reported for the native flour and 15S (table 4.3.1), as significantly higher C* values were observed for the modified flour compared to the native. This could be possible due to the possibly different nature of the pigments that could have come by diffusion from the outer layers of the rice grain and increased the colorimetric parameters but had a lower thermal stability. Coloured pigments are reported to be well correlated with phenolic compounds and antioxidant capacity (Shen et al., 2009). In turn, phenolic and flavonoid compounds could be affected by heat treatments (Chaaban et al., 2017; Ertosun et al., 2023). Ainhoa et al. (2023) has speculated on thermal oxidation phenomena to explain the lower colour intensity values of microwave-treated quinoa grains. The difference in crumb colour with the control bread (ΔE) also resulted in higher values with increasing replacement level with the WR flour, with values above 5 observed at the lowest replacement level in those breads containing the modified WR flour.

Table 4.3.9. Crumb grain characteristics and colorimetric parameters of the crust and crumb gluten-free bread samples as a function of WR flour replacement level and type of WR flour (native/modified).

		Crumb grain			Crust colour				Crumb colour			
Replacement level (%)	**WR Flour**	**Average cell size (mm²)**	**cell density (nº cell/cm²)**	**uniformity**	**L***	**C***	**h**	**ΔE**	**L***	**C***	**h**	**ΔE**
0	-	0.46 ^{a}	25 ^{e}	4.3 ^{d}	59.5 ^{ef}	36.8 ^{f}	62.5 ^{c}	-	62.9 ^{e}	5.9 ^{a}	85.5 ^{e}	-
15	Native	0.45 ^{a}	26 ^{e}	4.0 ^{d}	60.5 ^{f}	32.4 ^{e}	64.0 ^{de}	4.7 ^{a}	58.6 ^{cd}	7.4 ^{cd}	82.1 ^{d}	4.6 ^{a}
30	Native	0.55 ^{ab}	23 ^{cd}	3.2 ^{bc}	53.2 ^{c}	29.7 ^{c}	61.5 ^{b}	9.9 ^{c}	59.7 ^{d}	9.0 ^{e}	77.9 ^{a}	4.5 ^{a}
50	Native	0.86 ^{c}	17 ^{b}	2.2 ^{a}	51.7 ^{bc}	29.1 ^{b}	61.3 ^{b}	11.0 ^{c}	53.9 ^{ab}	9.7 ^{f}	79.1 ^{b}	9.8 ^{cd}
70	Native	1.17 ^{d}	15 ^{a}	2.1 ^{a}	48.7 ^{a}	27.7 ^{a}	61.8 ^{bc}	14.2 ^{d}	52.5 ^{a}	11.8 ^{h}	78.0 ^{a}	12.1 ^{d}
15	Modified	0.53 ^{ab}	23 ^{d}	3.7 ^{cd}	57.3 ^{de}	32.5 ^{e}	63.4 ^{d}	5.0 ^{a}	54.6 ^{ab}	6.5 ^{b}	81.9 ^{d}	8.3 ^{bc}
30	Modified	0.62 ^{b}	21 ^{c}	2.7 ^{ab}	55.9 ^{d}	30.2 ^{d}	64.2 ^{e}	7.9 ^{b}	56.1 ^{bc}	7.1 ^{c}	79.9 ^{c}	7.0 ^{ab}
50	Modified	1.24 ^{de}	15 ^{a}	2.4 ^{a}	51.5 ^{bc}	29.6 ^{c}	59.8 ^{a}	11.0 ^{c}	53.8 ^{ab}	7.7 ^{d}	80.0 ^{c}	9.3 ^{bc}
70	Modified	1.35 ^{e}	14 ^{a}	2.4 ^{a}	50.3 ^{ab}	28.0 ^{a}	61.3 ^{b}	12.8 ^{d}	53.3 ^{ab}	10.5 ^{g}	78.7 ^{b}	10.8 ^{cd}
	SE	0.05	1	0.2	0.8	0.1	0.2	0.5	1.0	0.2	0.2	0.9
						Multifactor Anova						
WR flour		**	**	ns	ns	**	ns	ns	*	**	**	ns
Replacement level		**	**	**	**	**	**	**	**	**	**	**
WR flour x Replacement level		*	ns	ns	**	ns	**	ns	*	**	**	*

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified WR flour. Native: Flour sample obtained from native paddy rice grains. Modified: Flour sample obtained from pre-soaked paddy rice grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100 % rice-based GF bread. L*: luminosity; ΔE: Difference of colour between each bread sample with the 100 % rice-based bread; C*: Chroma; h: hue. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly ($p<0.05$) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99 % confidence level ($p<0.01$), * The factor studied or its interaction with other factor is significant at >95 % confidence level ($p<0.05$), ns: not significant.

Crumb grain characteristics

The cross-section of the slices of each bread sample is presented in Fig. 4.3.3. The crumb grain characteristics were also analysed and presented in Table 4.3.9. Replacement level and treatment factor (except for the uniformity parameter) had a significant effect ($p<0.01$) on the crumb grain structure of the breads. An increasing mean cell area with increasing replacement level was observed, with values ranged from 0.45 and 0.53 mm^2 at the lowest replacement level (15 %) to 1.17 and 1.35 at the highest replacement level (70 %) corresponding to the breads containing the native and the modified WR flour, respectively. Furthermore, an additional and significant ($p<0.05$) increase in this parameter was observed for those breads containing 50 % and 70 % modified WR flour compared to the counterpart breads made with native WR flour. It was previously reported that the addition of fibre-rich ingredients could lead to an association of air bubbles by altering the crumb structure (Perez-quirce et al., 2018). The emergence of these large cells together with the presence also of small and characteristic cells resulted in a decrease of homogeneity in the crumb structure, as can be seen through the decrease of the uniformity and cell density parameter values, particularly significant ($p<0.05$) from 30 % replacement levels onwards.

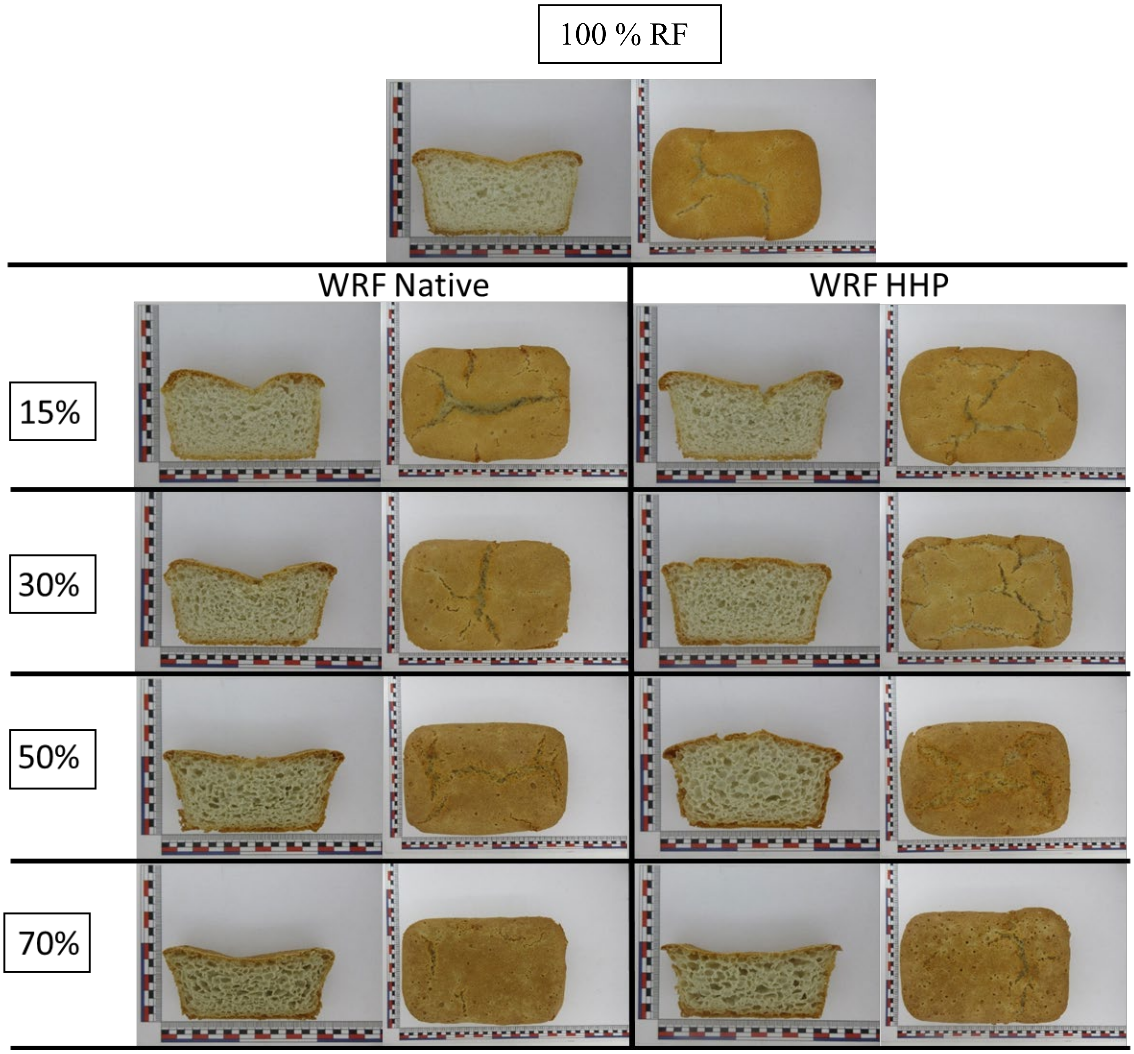

Figure 4.3.3. Photographs of bread loaves (top view) and slices of gluten-free breads made from 100 % rice flour (100 % RF) and by replacing rice flour with native (WRF Native) or modified (WRF HHP) WR flour at different replacement levels (15, 30, 50 and 70 %).

4.3.9 Sensory evaluation

Figure 4.3.4 and table 4.3.10 shows the sensory test results of the GF breads at two replacement levels (15 – 50 %) with the modified or native WR flour. The rice-based GF bread was placed in the middle of the scale with a score of 5. Only the replacement level significantly ($p<0.05$) affected the evaluation of the WR flour breads, although the interaction with the replacement level and the type of WR flour used also significantly ($p<0.01$) influenced the crust uniformity results. In all sensory attribute results, the bread containing WR native flour at 15 % of the replacement level was scored very similar to the reference rice-based GF bread (control). In addition, the breads containing the modified WR flour did not score significantly differently compared to their counterparts containing native WR flour at the same replacement levels, except in the crust uniformity attribute. In this respect, the lower scores obtained with the breads made with the modified WR flour could be due to the more open and therefore less uniform crust structure, as they had a higher specific volume. The panellists scored with significantly ($p<0.05$) higher values the crumb humidity of those breads at 50 % replacement level.

This could be due to the presence of fibre in the WR flour, which showed a relevant water binding capacity, as was also describe Föste et al., (2014). The increased humidity of these breads may have contribute to score with lower values the crumb hardness attribute. Breads with this sensation were reported to be easy to swallow (Kuèerová et al., 2013).

The panellists also found a significantly ($p<0.05$) cohesiveness on the crumb structure at these higher replacement levels. Although no significant differences were found among breads containing the native or the modified WR flour with respect to flavour intensity, taste intensity and aftertaste, higher and significant ($p<0.05$) scores were found in those breads at 50 % replacement level. Sabanis et al. (2009) reported that the addition of high-fibre ingredients in gluten-free bread affected texture release, mouthfeel and taste. In this regard, it was reported that an excess of quinoa bran promoted bitterness that could affect the overall acceptability of GF breads (Föste et al., 2014). However, the addition of 10 % rice bran in the white rice-based GF formulas scored better on sensory attributes in the investigation of Phimolsiripol et al. (2012) compared to the control. The authors attribute these results to panellists who are accustomed to whole bread consumption.

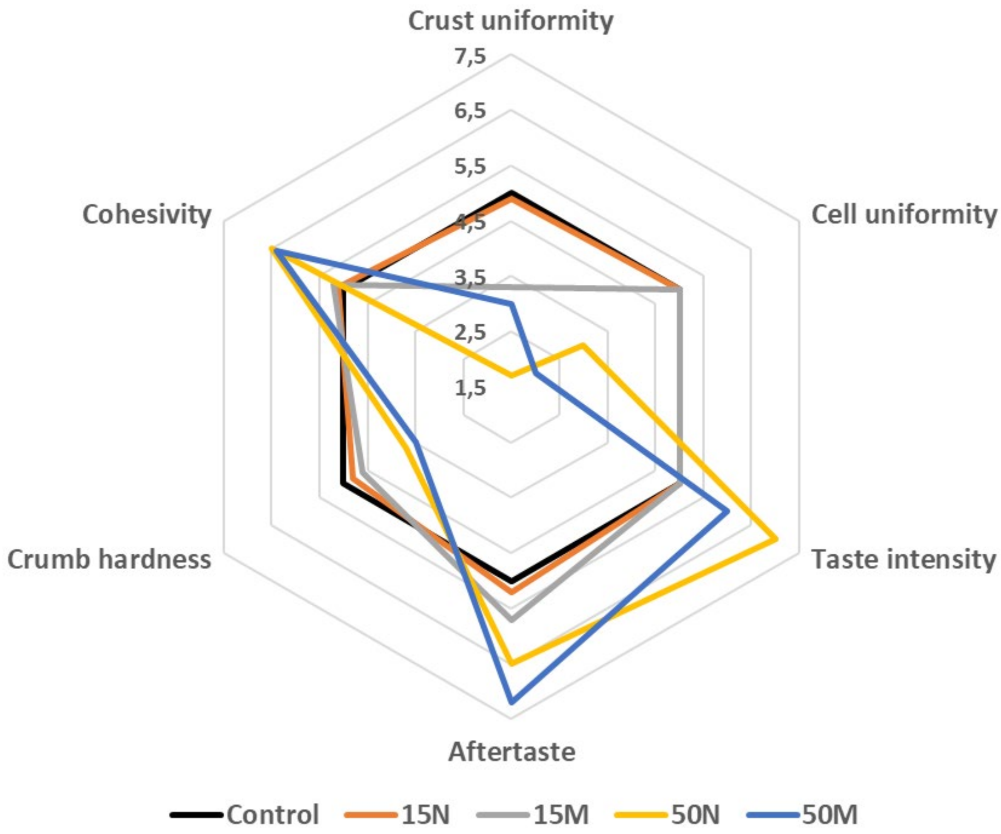

Figure 4.3.4. Sensory properties of gluten-free breads according to the replacement level (15/50 %) of the rice flour of the bread formula with whole rice flour (N-native; M-modified by HHP). A bread made with 100 % rice flour (control) was used as reference.

Table 4.3.10. Sensory evaluation of gluten-free breads as function of WR flour (native/modified) replacement level (15% - 50%).

Replacement level (%)	**WR Flour**	Crust uniformity	Cell uniformity	Taste intensity	Crumb humidity	Aftertaste	Crumb hardness	Cohesivity	Chewiness
0	-	5.0 ^{c}	5 ^{b}	5 ^{a}	5.0 ^{a}	5.0 ^{a}	5.0 ^{c}	5.0 ^{a}	5.0 ^{a}
15	Native	4.9 ^{c}	5 ^{b}	5 ^{a}	4.6 ^{a}	5.2 ^{a}	4.8 ^{c}	5.1 ^{a}	5.2 ^{ab}
15	Modified	3.3 ^{b}	5 ^{b}	5 ^{a}	4.7 ^{a}	5.7 ^{ab}	4.6 ^{bc}	5.2 ^{ab}	5.5 ^{abc}
50	Native	1.7 ^{a}	3 ^{a}	7 ^{b}	6.3 ^{a}	6.5 ^{bc}	3.7 ^{ab}	6.5 ^{c}	6.1 ^{bc}
50	Modified	3.0 ^{b}	2 ^{a}	6 ^{b}	6.1 ^{a}	7.2 ^{c}	3.5 ^{a}	6.4 ^{bc}	6.2 ^{c}
	SE	0.2	0	0	0.4	0.4	0.4	0.4	0.3
					Multifactor Anova				
	WR flour	ns	ns	ns	ns	ns	ns	ns	ns
	Replacement level	**	**	**	ns	**	**	**	*
	WR flour x Replacement level	**	ns	ns	ns	ns	ns	ns	ns

Replacement level: Percentage of rice flour in a rice flour-based gluten-free bread that is replaced with native/modified WR flour. Native: Flour sample obtained from native paddy rice grains. Modified: Flour sample obtained from pre-soaked paddy rice grains treated with HHP at 600 MPa for 15 min. A 0% replacement level refers to 100% rice-based GF bread. SE: Pooled standard error from ANOVA. Mean values in the same column with different letters are significantly (p<0,05) different. Multifactor Analysis of Variance: ** The factor studied or its interaction with other factor is significant at >99% confidence level (p<0.01), * The factor studied or its interaction with other factor is significant at >95% confidence level (p<0.05), ns: not significant

CHAPTER 4

Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread

4.4 Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread

4.4.1. Rheological Properties of Doughs

The effects of the buckwheat hull particles (BH) addition at different particle sizes (fine, F- and coarse, C-) and at two addition levels in the gluten-free (GF) doughs were evaluated using dynamic oscillatory tests. The results presented in Table 4.4.1 showed that both factors and their interaction had a significant effect ($p < 0.01$) on the viscoelastic properties of the dough except for τ_{max}. A significant increase ($p < 0.05$) in the elastic (G′) and viscous (G″) moduli was observed for all BH-containing doughs compared to the control. The G'_1 values of the fine BH (FBH)-containing doughs increased by two and five times at addition levels of 3% and 6%, respectively, whereas a smaller increase was observed for the coarse BH (CBH)-containing doughs. Similarly, the G''_1 values of the dough containing 6% FBH increased by three and a half times compared to the control dough. These results led to a significant decrease ($p < 0.05$) in the loss tangent for the doughs containing BH compared to the control, indicating a more elastic behaviour.

Table 4.4.1. Rheological properties of gluten-free doughs.

Sample	G'_1 (Pa)	a	G''_1 (Pa)	b	(tan δ) 1	c	G^*_1 (Pa)	Crosspoint (G′ = G″) (Pa)	τ_{max} (Pa)
C	712 ^{a}	0.326 ^{d}	400 ^{a}	0.384 ^{c}	0.561 ^{d}	0.058 ^{a}	817 ^{a}	24 ^{a}	1.67 ^{a}
FB3	1480 ^{c}	0.274 ^{b}	682 ^{c}	0.364 ^{b}	0.462 ^{b}	0.090 ^{b}	1629 ^{c}	42 ^{c}	2.07 ^{a}
CB3	1027 ^{b}	0.299 ^{c}	509 ^{b}	0.384 ^{c}	0.495 ^{c}	0.086 ^{b}	1147 ^{b}	35 ^{b}	1.85 ^{a}
FB6	3738 ^{e}	0.211 ^{a}	1385 ^{e}	0.307 ^{a}	0.37 ^{a}	0.096 ^{b}	3985 ^{e}	83 ^{e}	3.25 ^{b}
CB6	1973 ^{d}	0.272 ^{b}	896 ^{d}	0.359 ^{b}	0.454 ^{b}	0.087 ^{b}	2166 ^{d}	50 ^{d}	2.19 ^{ab}
SE	76	0.003	28	0.004	0.004	0.003	57	1	0.86
Analysis of variance and significance of factors (*p-values*)									
BH particle size	**	**	**	**	**	ns	**	**	ns
BH addition level	**	**	**	**	**	ns	**	**	ns
Particle size × Addition level	**	**	**	**	**	ns	**	**	ns

C: Control dough. FB3/CB3: Doughs containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Doughs containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. G'_1 and G''_1 represent the elastic and viscous coefficients at a frequency of 1 Hz after fitting to the power law model. G^*_1 refers to the complex modulus. τ_{max} represents the maximum stress in viscoelastic lineal region (LVR) obtained from the strain sweep. The crosspoint value represents the stress at G′ = G″. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0.05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant.

Previous studies conducted in gluten-free (GF) systems have reported that the addition of insoluble fibre particles led to an increase in dough firmness and consistency (Sciarini et al., 2017; Sabanis et al., 2009) . As it was reported by Sciarini et al. (2017), dough viscosity is related to the high water holding capacity (WHC) of fibres. Furthermore, F. Zhu et al. (2014) showed that the finer the BH particle, the more WHC it had. L. Wang et al. (2023) verified higher water absorption of wheat doughs enriched with cell-scale BH (50–10 μm) than those enriched with tissue-scale BH (500–100 μm).

Thus, the higher viscoelastic moduli for FBH-containing doughs compared to the coarse ones, indicating a higher dough consistency (Ronda et al., 2017), could be explained by variations in the WHC of the different BH fractions used in this study.

The dependence of the viscoelastic moduli with the frequency, measured with the exponents "a" and "b", also followed a similar trend. All fibre-enriched doughs had significantly ($p < 0.05$) lower values for these parameters than the control, with the greatest difference corresponding to the 6% FBH dough, indicating that the addition of BH improved the structural stability of the dough, particularly for the FBH doughs.

The maximum stress value (τ_{max}) that the dough can withstand before the elastic behaviour begins to decline showed a significant ($p < 0.05$) increase with the addition of 6% FBH compared to the control. In turn, the crossover point, or the stress level at which the elastic behaviour of the dough changes to a viscous one, revealed significant differences ($p < 0.05$) in all BH-supplemented doughs compared to the control. Following the same trend as the elastic modulus, the 6% FBH dough showed an increase of three and a half times the value observed for the control. The increase in dough mechanical strength with the addition of insoluble fibres has previously been observed by C. Wang et al. (2021), who attributed the internal rigidity of the dispersed fibres in the dough for the observed increase in dough resistance to kneading.

4.4.2. Proximal Composition of Bread Samples

Table 4.4.2 shows the nutritional composition of breads. As expected, the addition level of BH was significant ($p < 0.01$) for total dietary fibre (TDF). The TDF content in bread increased significantly ($p < 0.05$) with the increasing BH addition. The lowest TDF values corresponded to the control sample, followed by breads with a 3% hull addition (the GF bread containing the fine and coarse BH–FB3 and CB3 samples, respectively), while the highest TDF content was found for those breads at 6% of addition level of CBH and FBH (CB6 and FB6 samples, respectively). The addition of BH, regardless of particle size, increased the TDF content of GF breads (GFB) from three to five times more than that of the control samples. Phimolsiripol et al. (2012) *(Phimolsiripol et al., 2012)* observed a significant increase in the TDF of a rice-based GFB with the addition of 10% defatted rice bran flour as a fibre-enriching ingredient, from 2.29% in the control bread to 5.97% for bran-enriched bread. In this research, the contribution to TDF of rice flour was minimal (its dietary fibre content was 1.15% *w/w*, dry basis), so several factors could have played an important role in increasing the DF content of buckwheat-hull-containing breads (BHBs). As it was expected, the most important factor was the addition of BH in the GF bread formula. However, the amount of HPMC added, which is considered a fibre (Pérez-Quirce et al., 2014), might also have contributed to that increase in the TDF content. The value obtained showed that it could be possible to obtain the "high fibre" claim (EU Regulation No 1924/2006) (Stephen et al., 2017) even with BHBs formulated with the lowest BH addition.

Table 4.4.2. Proximal composition of gluten-free breads.

Sample	Carbohydrates (dw) (%)	T.D.F. (dw) (%)	Protein (dw) (%)	Ash (dw) (%)	Fats (dw) (%)
C	85 [b]	2.8 [a]	9 [a]	1.9 [a]	4.4 [a]
FB3	84 [ab]	8.0 [b]	9 [a]	1.9 [a]	5.7 [a]
CB3	84 [ab]	8.5 [b]	9 [a]	1.9 [a]	5.7 [a]
FB6	83 [ab]	13.1 [c]	11 [a]	1.9 [a]	4.7 [a]
CB6	81 [a]	12.5 [c]	13 [a]	2.1 [a]	4.5 [a]
SE	1	0.4	1	0.1	0.5
Analysis of variance and significance of factors (*p*-values)					
BH particle size	ns	ns	ns	ns	ns
BH addition level	ns	**	ns	ns	ns
Particle size × Addition level	ns	ns	ns	ns	ns

C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. TDF: Total dietary fibre. Protein content was measured as N content * 6.25. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0,05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant, dw: dry weight basis.

With regard to the other components analysed (fat, protein and ash), no significant ($p < 0.05$) differences were observed. The low presence of these components in BH, together with the relatively low addition level of BH in the GFB formula, would explain the lack of significant differences in the breads tested.

4.4.3. Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC)

Total phenol content (TPC) and total antioxidant capacity (TAC) were evaluated in all GFB samples (Table 4.4.3). TPC was significantly ($p < 0.01$) affected by the BH addition level and ranged from 0.3 to 2.1 mg GAE/100 g. Significantly ($p < 0.05$) higher TPC values were also found for BHBs enriched at 6% compared to those enriched at 3%. These are expected results as a high phenol content was reported in the BW hull (F. hua Li et al., 2013; W. Zhang et al., 2017). The particle size also showed a significant ($p < 0.05$) effect on TPC. Regardless of the concentration of BH used, higher TPC was observed in those breads enriched with smaller particle size. This is in line with the findings reported by Zhu et al., who reported a significant ($p < 0.05$) higher phenol content for BH particles after undergoing an ultrafine grinding treatment (F. Zhu et al., 2014)*(F. Zhu et al., 2014)*.

Table 4.4.3. Total phenol content and total antioxidant capacity of gluten-free breads.

Sample	TPC (mg GAE/ 100 g dw)	DPPH (mg TE/ 100 g dw)	ABTS (mg TE/ 100 g dw)	ORAC (mg TE/ 100 g dw)	FRAP (mmol Fe $^{+2}$/ 100 g dw)	Q-DPPH (mg TE/ 100 g dw)	Q-ABTS (mg TE/ 100 g dw)
C	n.d.	19 ^{a}	162 ^{a}	78 ^{a}	13.3 ^{a}	2 ^{a}	287 ^{a}
FB3	0.6 ^{a}	40 ^{b}	191 ^{ab}	135 ^{b}	13.5 ^{ab}	24 ^{bc}	966 ^{cd}
CB3	0.3 ^{a}	38 ^{b}	235 ^{bc}	135 ^{b}	13.8 ^{b}	17 ^{b}	878 ^{bc}
FB6	2.1 ^{b}	54 ^{c}	279 ^{c}	290 ^{d}	15.1 ^{d}	52 ^{d}	1058 ^{d}
CB6	1.8 ^{b}	60 ^{c}	261 ^{c}	222 ^{c}	14.6 ^{c}	30 ^{c}	799 ^{b}
SE	0.3	3	20	4	0.2	3	43
	Analysis of variance and significance of factors (*p*-values)						
BH particle size	ns	ns	ns	**	ns	**	**
BH addition level	**	**	*	**	**	**	ns
Particle size × Addition level	ns	ns	ns	**	**	*	ns

C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. TPC: Total phenol content. DPPH and Q-DPPH: Antioxidant capacity against the DPPH* radical in sample extracts and in solid samples, respectively. ABTS and Q-ABTS: Antioxidant capacity against the ABTS*+ radical in sample extracts and in solid samples, respectively. ORAC: Oxygen radical absorbance capacity of sample extracts. FRAP: Ferric reducing antioxidant power of sample extracts. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0.05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant, dw: dry weight basis.

With regard to antioxidant parameters, the results obtained from the extracts showed a significant ($p < 0.05$) increment in antioxidant activity with increasing BH addition. Additionally, in the ORAC and FRAP tests, the effect of particle size was only significant ($p < 0.05$) in bread enriched at 6%, where bread enriched with CBH had significantly ($p < 0.05$) lower antioxidant activity compared to its FB's counterpart. However, when the antioxidant activity was evaluated directly on the samples (Q-DPPH and Q-ABTS tests) the hull particle size showed a significant ($p < 0.01$) effect, probably due to the non-soluble phenolic compounds associated with fibre. This effect was also previously reported by Zhu et al. (2014), who suggested that the grinding could cause damage to the fibre matrix, favouring the extractability of phenolic compounds, which are mainly responsible for the antioxidant activity. Although the use of BH to improve the antioxidant capacity of bread has already been proposed (Sedej et al., 2012), the literature on this application of this BW by-product is still scarce. However, the use of other edible parts of the BW grain is more widespread (Sakac et al., 2011). A previous study showed higher TPC and TAC values for breads containing buckwheat flour (Żmijewski et al., 2015). The observed increases in antiradical activity could be attributed to the release of antioxidant compounds from cell wall degradation during dough fermentation and also to the Maillard reaction products associated with the baking process.

4.4.4. Bread Microstructure

Figure 4.4.1 shows the photomicrographs of the different breads obtained by BH fortification. Compared to the uniform and smooth structure of the control crumb (Figure 1(1a,2a), the crumb of the BHBs showed a more irregular surface, with heterogeneous structures that exposed some starch granules (Figure 1(1b,1c,2b,2c)). BH particles are mainly composed of non-starch polysaccharides such as hemicellulose, a typical component of cell walls in dicotyledonous plants (Hromádková et al., 2007) and lignin (Steadman et al., 2001). Due to their brittle nature, the BW dehulling process could promote an irregular, sharp and sheet-like appearance of BH particles, which would contribute to the irregular structure observed in the crumb of BH-enriched breads. In contrast, the starch granules in these samples were more regular and well-defined. The preservation of the granular starch structure could be associated with the lower availability of water in the medium, as the hull particles would exhibit a high water retention capacity (F. Zhu et al., 2014) and would compete with starch for water.

HPMC is a well-known hydrocolloid used to provide a network structure in gluten-free systems, helping to stabilise gas cell expansion (Pérez-Quirce et al., 2014) and develop a continuous matrix (Sabanis et al., 2009)(Sabanis et al., 2009). As can be seen in Figure 1(2c,2d), hull particles stood out from the crumb structure, which could have hindered the HPMC network, leading to air bubble collapse. Previous studies proved that insoluble fibre could promote gas cell interference, destabilising the aerated structure of the dough (Martínez et al., 2014)*(Martínez et al., 2014)*. The results obtained in this study showed that this effect was highly dependent on the size and the level of BH addition, with a stronger effect when using a higher BH particle size and a higher BH concentration.

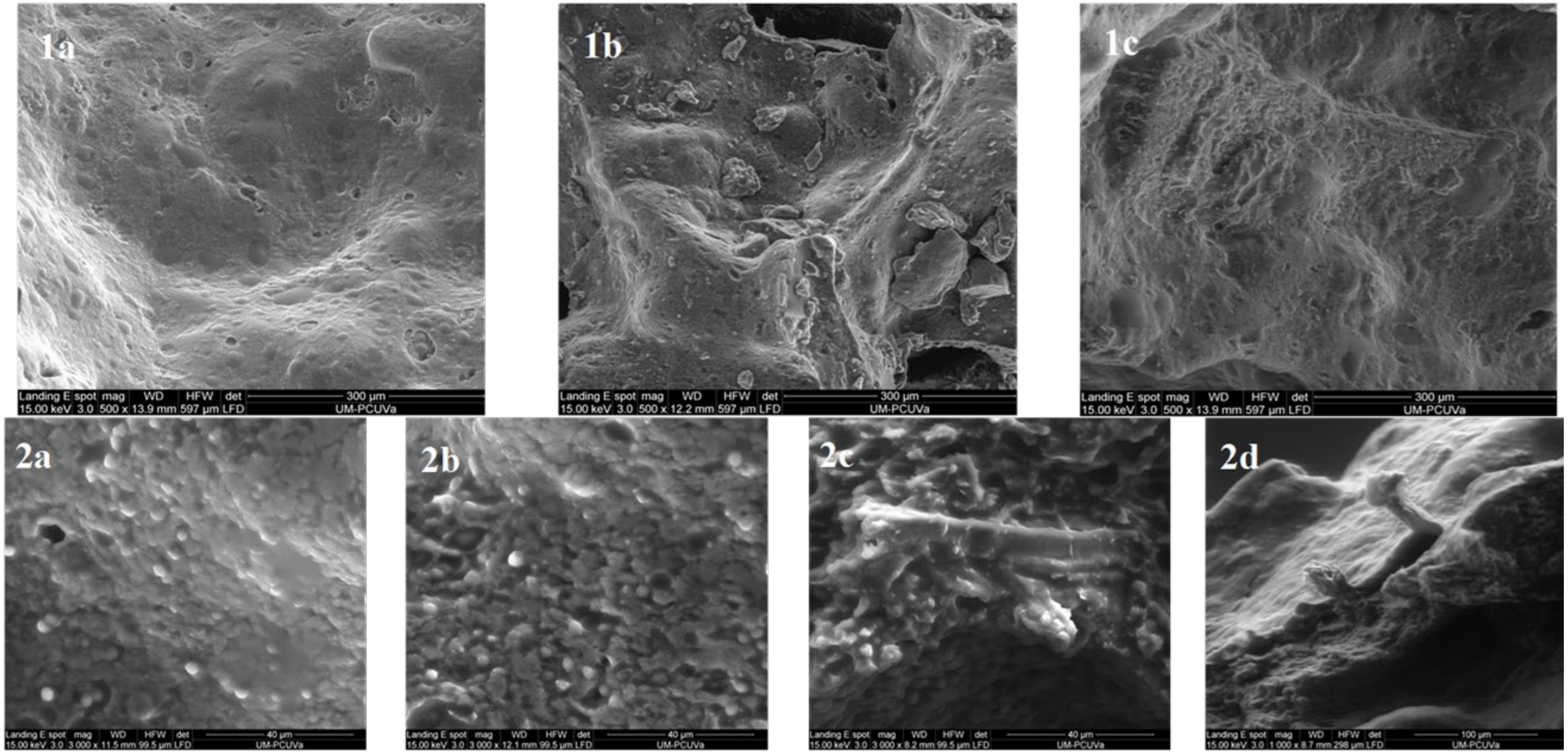

Figure 4.4.1. Scanning electron microscope images of the bread crumbs. (1a,2a): control bread sample; (1b,2b): bread containing coarse buckwheat hull particles at an addition level of 3%; (1c, 2c and 2d): breads containing coarse buckwheat hull particles at an addition level of 6%.

4.4.5. Bread Quality Evaluation

The BH particle size and its interaction with the BH addition level showed a significant effect ($p < 0.01$ and $p < 0.05$, respectively) on the bread volume (Table 4.4.4). The use of CBH at both addition levels resulted in a significant ($p < 0.05$) reduction in the bread volume and specific volume, with a significant ($p < 0.05$) reduction of 17% in bread specific volume for CB3 samples compared to the control bread. In contrast, no significant differences were found for FBs at any level of addition compared to the control bread. As compacted bread could be perceived in an undesirable way by consumers (Wronkowska et al., 2013), the use of FBH in GFB formulations at the doses tested in this study could be used for appropriate GFB formulation. The reduction in bread volume of GFBs as a result of the addition of large and insoluble fibre particles was previously reported by Martínez et al. (2014), who suggested that these particles interacted with the internal structure of the dough and promoted a reduced gas retention capacity. Conversely, the addition of BH particles to wheat bread affected bread specific volume when cell-scale BH (50–10 μm) was added instead of the coarser tissue-scale BH (500–100 μm) (L. Wang et al., 2023).

Table 4.4.4. Bread quality properties of gluten-free breads.

Sample	Volume (mL)	Specific Volume (mL/g)	Weight Loss (%)	Hardness (N)	Hardness (Day 7) (N)	Chewiness (N)	Resilience	Cohesiveness	Springiness
C	457 ^{c}	3.0 ^{b}	22.8 ^{d}	1.8 ^{a}	4.63 ^{ab}	0.7 ^{ab}	0.19 ^{b}	0.45 ^{bc}	0.85 ^{a}
FB3	470 ^{c}	2.8 ^{b}	17.1 ^{a}	2.2 ^{a}	3.88 ^{a}	0.8 ^{ab}	0.21 ^{c}	0.47 ^{c}	0.80 ^{a}
CB3	396 ^{a}	2.5 ^{a}	19.1 ^{bc}	3.2 ^{b}	6.81 ^{d}	1.1 ^{b}	0.17 ^{a}	0.41 ^{a}	0.81 ^{a}
FB6	460 ^{c}	2.8 ^{b}	18.6 ^{b}	3.0 ^{b}	5.65 ^{bc}	1.1 ^{b}	0.18 ^{ab}	0.43 ^{ab}	0.82 ^{a}
CB6	418 ^{b}	2.6 ^{a}	19.8 ^{b}	3.1 ^{b}	6.53 ^{cd}	1.1 ^{b}	0.17 ^{ab}	0.41 ^{a}	0.81 ^{a}
SE	6	0.2	1.8	0.7	0.31	0.2	0.02	0.03	0.04
Analysis of variance and significance of factors (p-values)									
BH particle size	**	**	*	ns	**	ns	**	**	ns
BH addition level	ns	ns	**	**	**	**	ns	*	*
Particle size × Addition level	*	ns	ns	ns	**	ns	**	*	ns

C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0.05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant.

The addition of BH particles in the bread formulation had a remarkable effect on the weight loss of GFBs as both factors, the BH particle size and BH addition level, were significant ($p < 0.05$ and $p < 0.01$, respectively). According to the ANOVA results, significantly ($p < 0.05$) lower values of weight loss were found for all BHBs compared to the control. Among BHBs, the FB3 sample had the lowest percentage of weight loss. These results were similar to those obtained by Zanoletti et al. (2017), who previously reported that ingredients with water retention sites, such as buckwheat bran particles, have the ability to increase the moisture content of wheat dough and reduce the baking loss of breads. Complementarily, Zhu et al. (2014) showed that the reduction of hull particle size increased the water holding capacity of these fibres, which would reduce the weight loss of bread during baking.

The level of BH addition showed a significant ($p < 0.01$) effect on bread crumb hardness. All BHBs exhibited significantly ($p < 0.05$) higher hardness values ($p < 0.05$) than the control, with the exception of the FB3 sample. The observed increase was particularly noticeable for the CB samples as the hardness increased from 1.8 N for the control to 3.2 N and 3.1 N for the CB3 and CB6 samples, respectively. A significant increase in crumb hardness with increasing levels of insoluble fibres in GFB has been previously reported (Sciarini et al., 2017). Martínez et al. (2014) also reported a significant ($p < 0.05$) increase in hardness when using coarse fibres instead of finer ones. Increased crumb firmness in bread formulations after the addition of buckwheat fibre was also previously observed in the study of Zanoletti et al. (2017), where fine and coarse buckwheat bran particles at different doses enriched a wheat-based bread.

All breads analysed showed an increase in crumb hardness after 7 days, but the FB3 sample exhibited the lowest value, followed by the control bread and the FB6 sample. Higher and significant ($p < 0.05$) crumb hardness values were observed for the CB samples compared to the control and regardless of the addition level. Increase in firmness during storage was related to the water retention capacity of the fibre, as Sciarini et al. (2017) observed. Those authors indicated that the addition of fibre reduced water loss during storage, resulting in the hardening rate of the crumb to slow down.

A significant effect of the BH addition level factor was found regarding crumb springiness and chewiness ($p < 0.05$ and $p < 0.01$, respectively), although any statistical difference was observed for the BHBs with respect to the control.

Conversely, the effects of the BH addition level, BH particle size and the interaction between the two factors were found to be significant for cohesiveness. CBs showed significantly ($p < 0{,}05$) lower crumb cohesiveness than the control bread, but this detrimental effect was not found in FBs. The reduction in crumb cohesiveness with the addition of buckwheat bran particles was also reported by Zanoletti et al. (2017) in a wheat bread formulation. This reduction was attributed to the appearance of micro-fractures in the air cell walls during the crushing. Micro-fractures could be promoted by the inclusion of coarse solid particles, which would promote the apparition of discontinuities in the crumb network. This hypothesis was consistent with the changes in crumb microstructure observed in this study (Figure 1, subfigure 2d), as CBH could become intertwined with the polymeric crumb network, thus promoting the appearance of micro-fractures. The resilience of the breads, which is related to the crumb recovery ability after a compression cycle, followed a similar trend to the cohesiveness. However, in this study, the effect of the BH addition level was not found to be significant.

The effect of BH particle size, BH addition level and the interaction between the two factors were significant ($p < 0.05$) for all crumb characteristics of the GFBs tested, except for uniformity and cell density for the BH particle size factor (Table 4.4.5 and Figure 4.4.2). With the exception of the FB6 sample, all BHBs showed a significant ($p < 0.05$) decrease in mean cell area, with the decrease ranging from 33% to 43%. In contrast to this parameter, an opposite trend was found for cell density, where all BHBs showed a significant ($p < 0.05$) increase compared to the control. The void fraction, which indicates the percentage of the cross-sectional area of the crumb that holds the gas cells, showed a significant ($p < 0.05$) decrease in BHBs compared to the control, except for the FB6 sample. These results were consistent with those obtained by Martínez et al. (2014).

Table 4.4.5. Crumb grain characteristics of gluten-free breads.

Sample	Mean Cell Area (mm²)	Crumb Grain Uniformity	Cell Density (cells/cm²)	Void Fraction (%)
C	0.30 ᵇ	7 ᵃ	44 ᵃ	13.7 ᵈ
FB3	0.19 ᵃ	19 ᵃᵇ	52 ᶜ	10.0 ᵇ
CB3	0.17 ᵃ	35 ᵇ	48 ᵇ	8.3 ᵃ
FB6	0.29 ᵇ	8 ᵃ	50 ᵇ	15.7 ᵉ
CB6	0.20 ᵃ	14 ᵃ	56 ᵈ	11.6 ᶜ
SE	0.01	5	1	0.4
Analysis of variance and significance of factors (*p*-values)				
BH particle size	**	ns	ns	**
BH addition level	**	*	**	**
Particle size × Addition level	**	ns	**	**

C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0.05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant.

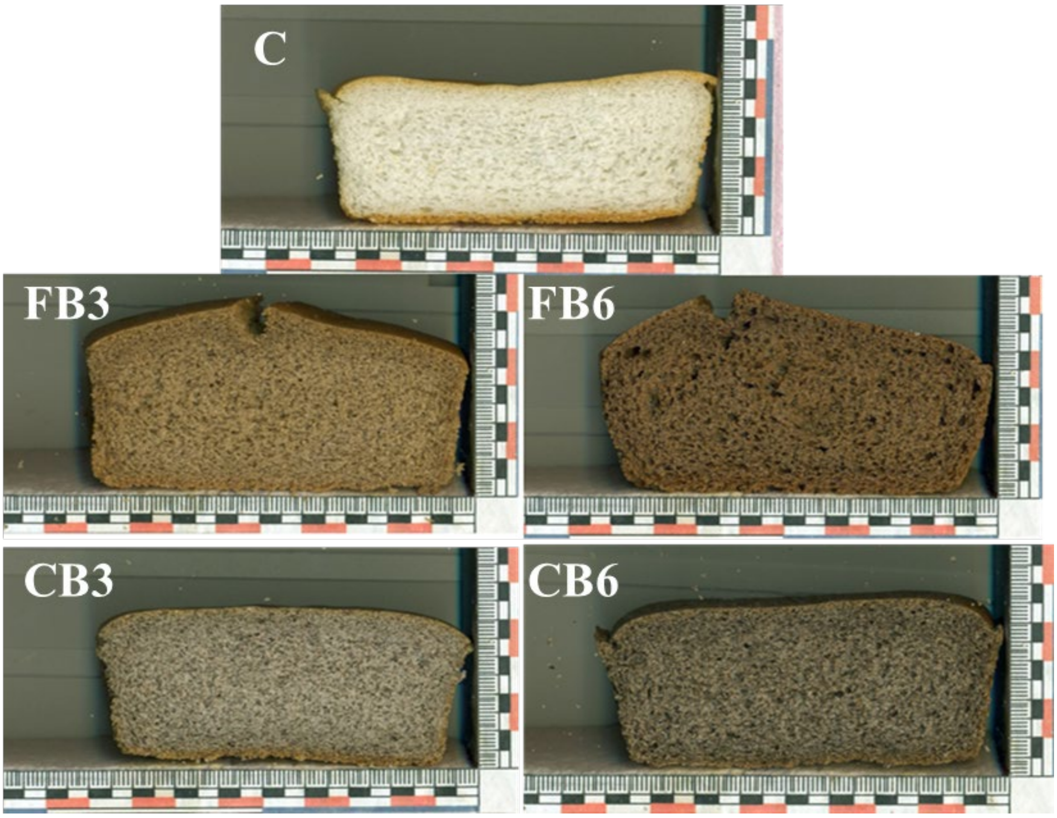

Figure 4.4.2. Images of the internal crumb structure of gluten-free breads. C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%.

Crust and crumb colour parameters (L*, a*, b*, C*, h, ΔE) of BHBs are shown in Table 4.4.6. Both investigated factors (BH addition level and particle size) had a significant ($p < 0.01$) effect on the crumb luminosity (L*), whereas only the BH addition level had a significant ($p < 0.05$) effect on the same parameter of the crust. All BHBs showed significantly ($p < 0.05$) lower values than the control bread for crust and crumb luminosity and chromaticity (C*), as could also be noted from the colour difference (ΔE). Significantly ($p < 0.05$) lower crust luminosity was observed with increasing BH addition. In turn, the FBH addition induced a significant ($p < 0.05$) darkening effect with respect to the use of the coarse BH fraction. Lower L* values with fibre addition in bread have already been reported (Sabanis et al., 2009; Martínez et al., 2014; Ktenioudaki & Gallagher, 2012). However, a darker GFB may not be perceived as negative, in contrast to the GFB commonly seen in market, characterised by a lighter colour than wheat bread (Sabanis et al., 2009).The effect of the studied factors in the crust and crumb colour coordinates (a* and b*) was significant ($p < 0.05$). Significantly ($p < 0.05$) lower a* and b* values were found for the crust of all BHBs compared to the control. Furthermore, the addition of CBH promoted the reduction of a* values more than the addition of FBH, as did the use of a 6% addition level compared to 3%. As crust chroma values are mainly determined by the Maillard reaction, the addition of the higher levels of fibres could have hindered this reaction. Anil demonstrated that breads with a lower dosage of hazelnut testa particles were more influenced by Maillard and caramelisation reactions than those with the higher dosage (Anil, 2007). The a* coordinate of the crumb was significantly ($p < 0.05$) higher in all BHBs than in the control. The addition of FBH resulted in a more reddish crumb colour than the coarse BH. Since the temperature of the crumb is not as high as in the crust, the crumb colour is mainly related to the colour of the ingredients (Villanueva et al., 2019a). Therefore, the native BH colour could have determined the colorimetric characteristics of the crumb.

Table 4.4.6. Crumb and crust colour parameters of gluten-free breads.

Sample	Crust Colour						Crumb Colour					
	L*	**a***	**b***	**C***	**h**	**ΔE**	**L***	**a***	**b***	**C***	**h**	**ΔE**
C	61 ^{c}	13.3 ^{e}	23 ^{d}	27 ^{e}	60 ^{e}		74 ^{e}	−0.5 ^{a}	5.4 ^{b}	5 ^{a}	95 ^{c}	
FB3	49 ^{b}	10.8 ^{d}	11 ^{c}	15 ^{d}	45 ^{c}	17.0 ^{a}	50 ^{c}	8.3 ^{d}	10 ^{d}	13 ^{d}	50 ^{b}	26 ^{b}
CB3	49 ^{b}	7.8 ^{bc}	10 ^{b}	12 ^{c}	51 ^{d}	18.9 ^{b}	53 ^{d}	4.5 ^{b}	5.8 ^{b}	7 ^{b}	52 ^{b}	22 ^{a}
FB6	44. ^{a}	9.8 ^{c}	5 ^{a}	11 ^{b}	26 ^{a}	24.7 ^{c}	42 ^{a}	8.9 ^{e}	6.6 ^{c}	11 ^{c}	35 ^{a}	33 ^{d}
CB6	45 ^{a}	7.1 ^{a}	5 ^{a}	8 ^{a}	33 ^{b}	25.3 ^{c}	45 ^{b}	5.8 ^{c}	4.0 ^{a}	7 ^{b}	37 ^{a}	29 ^{c}
SE	1	0.2	1	1	1	0.3	1	0.1	0.3	1	2	1
Analysis of variance and significance of factors (p-values)												
BH particle size	ns	**	*	**	**	*	**	**	**	**	ns	**
BH addition level	**	**	**	**	**	**	**	**	**	**	**	**
Particle size × Addition level	ns	ns	*	ns	ns	ns	ns	**	**	**	ns	ns

C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. L*, a*, b*: CIELAB colour coordinates, C*: chroma, h: hue, ΔE: difference of colour of each BHB sample with the control. SE: Pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different ($p < 0.05$). ** $p < 0.01$, * $p < 0.05$, ns: not significant.

4.4.6. Sensory Evaluation

Figure 4.4.3 and Table 4.4.7 show the results of the sensory attributes of GFBs that were evaluated by consumers. With the exception of the CB6 sample, consumers rated all the sensory attributes studied higher than five, with small variations between the different types of bread. Overall, consumers scored FBs better than CBs and the lower addition level better than the higher one. The effect of FB particle size was significant ($p < 0.01$) on crust and crumb colour. For these parameters, the panellist considered the crust and crumb colour of the control bread to be more appealing. In contrast, the flavour of the BHBs (except for the CB6 sample) was rated better than that of the control bread. As the addition level of BH particles was low, the characteristic bitter taste and aromatic intensity of BH (Torbica et al., 2010) were not perceived as unpleasant by the panellists. Torbica et al. (2010)*(Torbica et al., 2010)* observed that rice-based breads with no more than 10% inclusion of unhulled BW flour resulted in higher scores than breads containing dehulled BH flour. Panellists also gave similar scores to both kinds of FB samples, CB3 and the control bread in the following attributes: taste, aftertaste and hardness. However, CB6 samples showed a significant ($p < 0.05$) decrease in taste, aftertaste and overall acceptability, indicating that at this level of addition and using BH at this particle size, the GF bread resulted in impaired sensory attributes. On the other hand, no significant differences were found between the control and FB3 samples in terms of overall acceptability, proving that the FB3 samples had sensory attributes very similar to those of the control GF bread. Sabanis et al. (2009) also reported higher scores for GF breads enriched with 3% cereal fibre added than those enriched with higher levels.

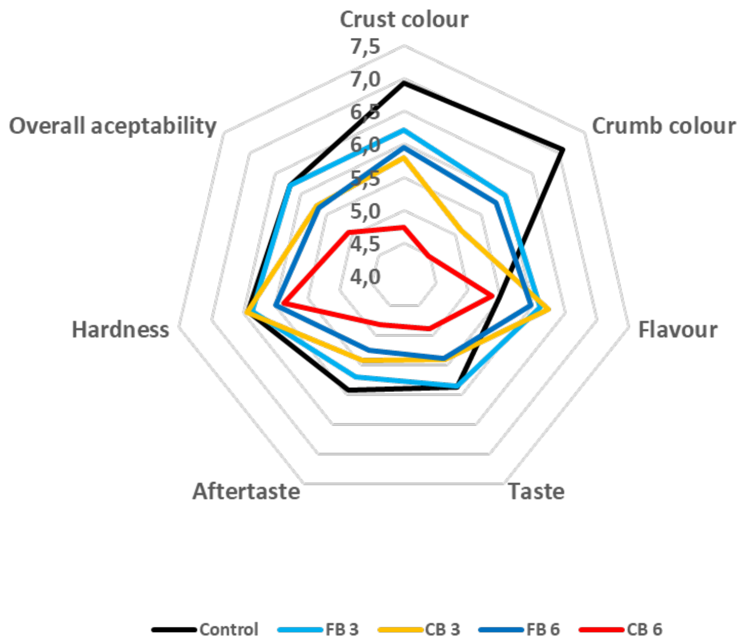

Figure 4.4.3. Effect of the addition of buckwheat hulls on the sensory properties of gluten-free breads. C: Control bread. FB3/CB3: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: Breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%.

Table 4.4.7. Sensory properties of gluten-free breads

Sample	Crust colour	Crumb colour	Flavour	Taste	Aftertaste	Hardness	Overall acceptability
C	6.9 ^{c}	7.1 ^{d}	5.5 ^{ab}	5.9 ^{b}	5.9 ^{c}	6.43 ^{ab}	6.20 ^{c}
FB3	6.2 ^{b}	6.0 ^{c}	6.1 ^{bc}	5.9 ^{b}	5.7 ^{bc}	6.37 ^{ab}	6.20 ^{c}
CB3	5.8 ^{b}	5.1 ^{b}	6.2 ^{c}	5.4 ^{ab}	5.4 ^{abc}	6.44 ^{b}	5.70 ^{bc}
FB6	5.9 ^{b}	5.8 ^{c}	6.0 ^{abc}	5.4 ^{ab}	5.2 ^{ab}	6.00 ^{ab}	5.65 ^{b}
CB6	4.7 ^{a}	4.5 ^{a}	5.4 ^{a}	4.9 ^{a}	4.8 ^{a}	5.87 ^{a}	5.07 ^{a}
SE	0.2	0.2	0.2	0.2	0.2	0.20	0.19
Analysis of variance and significance of factors (*p-values*)							
BH particle size	**	**	ns	*	ns	ns	**
BH addition level	**	ns	*	*	*	*	**
Particle size x Addition level	ns	ns	ns	ns	ns	ns	ns

C: Control bread. FB3/CB3: breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 3%. FB6/CB6: breads containing fine (FB) or coarse (CB) buckwheat hull particles at an addition level of 6%. SE: pooled standard error obtained from ANOVA. Values in the same column with different small letters are significantly different (p<0.05). **p < 0.01, *p < 0.05, ns: not significant).

V. CONCLUSIONES

V. CONCLUSIONES

La tecnología de altas presiones hidrostáticas (APH) aplicada sobre granos enteros de cereales y pseudocereales resultó eficaz para la obtención de harinas integrales modificadas físicamente cuyas propiedades se adecúan a los requerimientos de los procesos de elaboración de productos horneados sin gluten. Además, a partir del control de las variables del tratamiento, se pudo modular la respuesta tecno-funcional y nutricional de las harinas resultantes, lo que permite adaptar estos ingredientes a la formulación de diferentes productos sin gluten. Asimismo, la cascarilla de trigo sarraceno, subproducto generado del procesamiento del grano para la obtención de granos pelados y harinas, demostró ser adecuada para su empleo como ingrediente alimentario en las formulaciones de pan sin gluten.

A partir de los resultados obtenidos en los estudios que constituyen los capítulos de esta tesis, se pudieron extraer las siguientes conclusiones particulares:

- El número de ciclos de tratamiento APH no ejerció un efecto significativo sobre las propiedades nutricionales y tecno-funcionales de las harinas procedentes de granos tratados. Sin embargo, la realización de un pretratamiento de remojo del grano influyó notablemente en las mencionadas propiedades, denotando la importancia de alcanzar una humedad interna crítica para mejorar la eficacia de la transmisión de la presión y, consecuentemente, el efecto del tratamiento.

- El impacto del tratamiento APH demostró ser también dependiente, tanto del origen botánico de la matriz tratada, como de los factores de control propios del tratamiento, como es el caso del nivel de presión alcanzado y el tiempo de mantenimiento de la presión.

- Los tratamientos APH combinados con un pretratamiento de remojo, aplicados a 300 MPa sobre granos enteros de trigo sarraceno, dieron lugar a harinas con una menor capacidad de absorción de agua, pero con una actividad emulsionante y capacidad de formación de espuma notables. Asimismo, las harinas obtenidas con el menor tiempo de tratamiento (0 min) mostraron un aumento en la entalpía de gelatinización, mientras que los geles obtenidos a partir de éstas, experimentaron un incremento significativo de su capacidad para soportar esfuerzos cortantes, efecto que también fue observado en las harinas obtenidas en los tratamientos realizados a 600 MPa.

- El tratamiento APH aplicado a 600 MPa sobre las matrices de trigo sarraceno y arroz, dio lugar a un incremento en la capacidad de absorción de agua de las harinas resultantes. Además, se observó que las harinas de arroz modificadas sin tratamiento de remojo previo, mostraron una mayor actividad emulgente, pudiendo ser aptas como ingrediente para la formulación de cremas, salsas, masas batidas o productos cárnicos.

- Las harinas de trigo sarraceno procedentes de granos con remojo previo y tratamiento APH durante 15 o 30 min, se caracterizaron por una mayor estabilidad térmica durante su empastado. Dichas harinas mostraron además un descenso en su entalpía de gelatinización, lo que sugiere que el tratamiento APH dio lugar a una gelatinización parcial del almidón. Asimismo, mostraron una reducción en el intervalo de temperaturas de gelatinización, que fue indicativo de la aparición de una estructura más homogénea y organizada.

- Las masas de panes sin gluten elaboradas a partir de las harinas resultantes del tratamiento combinado de remojo y APH a 600 MPa durante 15 min se caracterizaron por valores de los módulos elástico y viscoso más elevados, así como una mayor capacidad para resistir esfuerzos cortantes, en comparación con las masas elaboradas con harinas nativas. Además, los panes resultantes desarrollaron un mayor volumen específico y tuvieron una miga de textura más blanda.

- Desde un punto de vista nutricional, los tratamientos APH a 600 MPa dieron lugar a incrementos en el contenido fenólico y la capacidad antioxidante de las harinas, que fueron mayores conforme aumentó el tiempo de tratamiento. Este incremento fue también observado en los panes elaborados con las harinas modificadas de trigo sarraceno. Asimismo, se observaron incrementos en el contenido de tiamina y piridoxina de las harinas de arroz modificadas.

- La inclusión de partículas de cascarilla de trigo sarraceno en fórmulas básicas de pan sin gluten supuso una estrategia efectiva para incrementar su contenido en fibra dietética. Su adición en un porcentaje del 3% (p/p) y una granulometría reducida (D_{50} = 62,7 μm) no produjo alteraciones significativas en las propiedades físicas de los panes, dando lugar a un producto con un perfil sensorial similar al desarrollado mediante una formulación básica de harina de arroz refinado. En cambio, el contenido fenólico y la capacidad antioxidante de los panes suplementados con dicho ingrediente fue significativamente superior al del pan control.

V. CONCLUSIONS

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The high hydrostatic pressure technology (HHP) applied on whole cereal and pseudocereals grains was effective in obtaining physically modified whole flours with properties that meet the requirements of gluten-free production processes. By controlling the treatment variables, the techno-functional and nutritional response of the resulting flours can be modulated, making it possible to adapt these ingredients to the formulation of different gluten-free baked products. Besides, buckwheat hulls, a by-product generated from the processing of the grain into peeled kernels and flours, proved to be suitable for use as a food ingredient in gluten-free bread formulations.

From the results obtained in the studies that compose the chapters of this thesis, the following particular conclusions could be established:

- ❖ The number of HHP treatment cycles did not lead to a significant effect on the nutritional and techno-functional properties of flours from treated grains. However, the performance of a soaking pre-treatment of the grain had a significant influence on the aforementioned properties, indicating the importance of reaching a critical internal moisture to improve the efficiency of pressure transmission and consequently, the effect of the treatment.

- ❖ The impact of the APH treatment was also shown to be dependent on factors such as the botanical origin of the treated matrix, as well as on controlling factors specific to the treatment, such as the pressure level reached and the pressure maintenance time.

- ❖ HHP treatments combined with a soaking pre-treatment, conducted at a pressure level of 300 MPa and applied on whole buckwheat kernels, resulted in flours with a lower water absorption capacity, but with remarkable emulsifying activity and foaming capacity. Likewise, the same flours obtained with the shortest treatment time (0 min) showed an increase in the enthalpy of gelatinisation, while the gels produced from these flours showed a significant increase in their capacity to withstand shear stresses, an effect that was also observed in the flours resulting from treatments carried out at 600 MPa.

- ❖ The HHP treatment applied at 600 MPa on the buckwheat and rice whole grain matrices increased the water absorption capacity of the resulting flours. Modified rice flours without pre-soaking treatment showed higher emulsifying activity. They can therefore be added to cream formulas, batters or meat products.

- ❖ Modified buckwheat flours from HHP treatments with pre-soaking and holding times of 15 min or longer of the grains were characterised by a higher thermal stability during pasting. In addition, it was also observed a decrease in the gelatinization enthalpy of that flours, suggesting that the HHP treatment triggered a partial gelatinisation of the starch. A reduction of the gelatinisation temperature range was also observed, indicating the appearance of a more homogeneous and organised starch structure.

- Gluten-free doughs made from wholemeal flours resulting from the combined treatment of pre-soaking and HHP at 600 MPa for 15 min were characterised by higher values of the elastic and viscous modulus as well as a higher capacity to resist shear stresses compared to doughs made from native flours. In addition, the resulting breads developed a higher specific volume and had a softer crumb texture.

- From a nutritional point of view, HHP treatments at 600 MPa resulted in increases in phenolic content and antioxidant capacity of the flours, which were higher as treatment time increased. Increases in thiamine, niacin and pyridoxine content were also observed in rice flours resulting from APH treatments and in particular in those resulting from combined treatment with pre-soaking.

- The inclusion of buckwheat hull particles in basic gluten-free bread formulations was an effective strategy to increase their dietary fibre content. Its addition at a percentage of 3% (w/w) and at a reduced particle size (D50 = 62.7 μm) did not lead to significant alterations in the physical properties of the breads, resulting in a product with a sensory profile similar to that developed using a basic refined rice flour formulation. In contrast, the phenolic content and antioxidant capacity of the breads supplemented with this ingredient was significantly higher than that of the control bread.

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VI. REFERENCES

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VII. APÉNDICE

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Relación de publicaciones derivadas de la presente tesis.

Conforme a los requisitos expuestos en la RESOLUCIÓN de 12 de mayo de 2022, del Rectorado de la Universidad de Valladolid, por la que se ordena la publicación del Reglamento de Estudios de Doctorado de la Universidad de Valladolid y específicamente en lo referente al artículo 19 sobre la tesis doctoral en modalidad ordinaria, el presente documento refrenda dicha modalidad en la propia estructura del documento, cuyo planteamiento se encuentra definido como índice, introducción, objetivos, materiales y métodos, un cuerpo de resultados y discusión por capítulos bien diferenciados, conclusiones y bibliografía , tal como se expone en el apartado 1 de dicho artículo. Así mismo como requisito planteado para dicha modalidad, y detallado en el apartado 2 de dicho artículo, se presentan diferentes aportaciones que acreditan la calidad de la tesis. A continuación, se indican los datos de las diferentes publicaciones derivadas de la presente tesis doctoral, detallándose su vinculación con los diferentes epígrafes y capítulos de la presente tesis doctoral:

1) “*The Application of High-Hydrostatic-Pressure Processing to Improve the Quality of Baked Products: A Review*”. Gutiérrez, Á.L.; Rico, D.; Ronda, F.; Caballero, P.A.; Martín-Diana, A.B. Foods 2024, 13, 130. https://doi.org/10.3390/foods13010130.

   Esta publicación ha sido originada a partir de la información contenida en el apartado I “Introduction” y particularmente en los apartados del 1.1 al 1.7.

2) "*Development of a gluten-free whole grain flour by combining soaking and high hydrostatic pressure treatments for enhancing functional, nutritional and bioactive properties*". Gutiérrez, Á. L., Rico, D., Ronda, F., Martín-Diana, A. B., & Caballero, P. A. (2022). Journal of Cereal Science, Volume 105, 103458. https://doi.org/10.1016/j.jcs.2022.103458.

   Este artículo de investigación fue generado a partir de la información presentada en el capítulo 1 del apartado 4.1 “Results and discussion” y los correspondientes materiales y métodos citados en el apartado III.

3) “*Valorisation of Buckwheat By-Product as a Health-Promoting Ingredient Rich in Fibre for the Formulation of Gluten-Free Bread*”. Gutiérrez, Á.L.; Villanueva, M.; Rico, D.; Harasym, J.; Ronda, F.; Martín-Diana, A.B.; Caballero, P.A. Foods 2023, 12, 2781. https://doi.org/10.3390/foods12142781

   Esta publicación fue propiciada a raíz del material presentado en el capítulo 4 del apartado 4.4 “Results and discussion” y los pertinentes materiales y métodos presentados en el apartado III.

4) *"High hydrostatic pressure processing of whole buckwheat grains to obtain functional gluten-free ingredients: effect of pressure and holding time"* Gutiérrez, Á. L., Rico, D., Ronda, F., Martín-Diana, A. B., and Caballero, P. A. Food Hydrocolloids (2024) (enviado para su publicación)

   A partir de la información presentada en el capítulo 2, apartado 4.2 "Results and discussion" y mediante los materiales y métodos pertinentes proporcionados en el apartado III, se ha originado dicho manuscrito adaptado y enviado para su publicación a la revista de impacto: "*Food Hydrocolloids*"

5) *"Application of high hydrostatic pressure on whole rice grains for the improvement of nutritional and techno-functional properties of gluten-free flours"* Gutiérrez, Á. L., Rico, D., Ronda, F., Caballero, P. A., and Martín-Diana, A. B. Trends in Food Science & Technology (2024) (Enviado para su publicación).

   El contenido aportado en el capítulo 3, apartado 4.3 "Results and discussion" y elaborado a partir de los materiales y métodos correspondientes del apartado III, fue adaptado para su preparación como artículo de investigación a fin de enviarse a la revista *de* impacto: *"Trends in Food Science & Technology"*