

High-Hydrostatic-Pressure Processing in Baked Products

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Contributor: Ángel L. Gutiérrez , Daniel Rico , Felicidad Ronda , Pedro A. Caballero , Ana Belén Martín-Diana

High hydrostatic pressure (HHP) technology can be used to modify various matrices so that they can be used as ingredients in the baking industry. HHP treatments can change the functionality of starches and proteins due to cold gelatinization and protein unfolding. As a result, the resulting ingredients are more suitable for nutrient-rich bakery formulations.

high hydrostatic pressure

plant-based ingredients

physical modifications

functional and nutritional properties

baked products

1. Introduction

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 [\[1\]\[2\]](#). 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 [\[3\]](#). Similarly, commercial gluten-free bakery products are also based on starches and flours, mainly maize and white rice, respectively [\[4\]](#).

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 [\[5\]\[6\]](#). 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 [\[3\]\[5\]\[7\]\[8\]](#). Sources of fortification can also be derived from minor cereals [\[9\]](#), pseudocereals [\[10\]](#), legumes [\[11\]\[12\]](#) or other plant sources such as hemp [\[13\]](#). 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 [14] and more sustainable food chains [11], thus contributing to improving system productivity and sustainability [15].

However, this nutrient-enriching formulation strategy often results in lower sensory quality of the resulting breads [3]. 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 [16][17][18]. High hydrostatic pressure (HHP), also known as cold pasteurisation, has been re-ported to exert significant effects on starch and protein polymers [19][20][21]. 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 [22]. 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 relation to the content of bioactive compounds of starch-rich food ingredients through HHP technology were also addressed.

2. HHP Technology

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 [23], which is approximately 3 °C per 100 MPa for water [24]. In addition, combined pressure and temperature treatments can be carried out using temperature control devices and insulated vessels [25][26][27].

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 [21]. As a consequence of the HHP treatment, the pressurised material may undergo phase transitions, changes in molecular configuration and chemical reactions [28]. 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 [29]. 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 [30]. 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.

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

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 [31][32]. 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 [33]. 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 [34].

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 [35]. 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 [31] as well as with the disappearance of birefringence patterns [21]. The influence of HHP treatment on the ordered state of crystallinity with different amylose/amylopectin ratios in maize starches has been investigated [36]. 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 [37].

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 [30][38]. The extent of gelatinisation can be modulated by processing conditions (starch:water ratio, pressure level, holding time, temperature) [36], allowing intermediate levels of crystalline degradation or partial gelatinisation to be obtained, with different changes in starch functionality.

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 [39]. 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 [40]. The structural thermodynamic equilibrium of proteins depends mainly on three types of interactions: ionic, hydrophobic and hydrogen bonding [39].

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 [41]. 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 [17].

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

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 [33]. 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 [18]. 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 [42] and intrinsic processing conditions such as pressure level, temperature and holding time [33]. 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 [43]. In contrast, in pressure-induced gelatinisation, the starch granules are deformed but retain their granular shape [44].

The degree of gelatinisation achieved by pressure treatment correlates with water-binding capacity (WBC), as reported by Rumpold and Knorr [45], 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 [45]. Increased water retention capacity with the pressure has also been reported for other starch sources, such as corn [46] and quinoa [47]. 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.

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 [48]. 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 [42]. Li et al. [42] 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 [42]. 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 [42].

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 [49]. 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.

Different studies have reported an improvement in the hydration properties of HHP-treated proteins [49][50][51][52]. An increase in the water-holding capacity of pine nut protein fractions [51] and kidney bean protein isolates [49], as well as in the water absorption capacity of rice bran proteins [50] 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 site

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 [53]. It has been reported that pressure, together with enzymatic hydrolysis, can reduce the size of peptides by breaking peptide bonds and thus increase solubility [20]. Zhu et al. [50] 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 [50].

Contradictory results have been reported on the effect of protein unfolding in relation to the foaming capacity of proteins after HHP treatment. Zhu et al. [50] 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 [49], pea protein isolate [54] and soybean protein isolate ($P > 300$ MPa) [52].

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 [49][50].

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% [55]. 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.

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 [2]. 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. [56] 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. Sharma et al. [57] 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 [44].

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 and Arfat and Ahmed, Mulla, Arfat et al., respectively [58][59]. 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 [60]. 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 and Arfat [58] also suggested that pressure favoured damaged starch granules, thus facilitating their swelling.

Unlike other reports [49][50][54], 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 [61]. 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 [51], reducing their flexibility with the cross-linking of disulphide bonds and losing efficacy in emulsion formation, as stated by Cabra et al. [62].

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 [58][63][64], rice [59], waxy rice [46], sorghum [64] and buckwheat [61]. 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 [43]. 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 [65]. In addition, the presence of water is also required [44]. Therefore, the higher the pressure and water availability, the higher the degree of gelatinisation that can be achieved [63], as this allows the infiltration of water into the starch molecule, leading to a partial gelatinisation of the inner regions of the starch granule [64].

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 [58], oat [66], rice [56], buckwheat and tef [65] and chickpea flours [67][68]. The increase in G' with pressure has been attributed to the partial gelatinisation effect combined with protein aggregation [58][69]. Some authors have also pointed out the importance of the flour-to-water ratio (F/W) in increasing the mechanical strength of the gel.

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 [70]. 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.

It has been reported that HHP can modify the strength of gluten [55][71]. Therefore, HHP treatments could improve the functionality of wheat flours with poor breadmaking properties [63]. This technology has been proposed to

improve bread quality in wheat-based formulations with high-fibre ingredients that are prone to promote detrimental effects [19]. Insoluble fibres lead to physical disruption of the gluten network [2] or create break points where gas can more easily escape during proofing [72].

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 [73]. Similarly, Angioloni and Collar [64] 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 [71] or at 500 MPa [64]. 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 [64][74]. Therefore, HHP conditions could lead to an overstructuring effect of the combined action of both structural changes.

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 [43][75]. 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. [66]. 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 [75].

It has been suggested that structure strengthening could be a valid tool to improve the baking performance of gluten-free flour matrices [64]. In these terms, starch pre-gelatinised by HHP treatments has been proposed as a structuring agent [74]. 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. [76] 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 [77]. In both investigations, significant increases in the elastic solid behaviour of the doughs were observed with HHP treatments above 350 MPa [76] and 400 [77]. The increase in dough consistency at high pressure levels, which could impair proper bread development, was attributed by Hüttner et al. [76] to the combined action of protein network formation and starch gelatinisation. However, Vallons et al. [77] 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 [77] or to the weakening of electrostatic and hydrophobic bonds [76].

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 [78]. 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 [8]. 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 [22] 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) [44]. 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 [79]. HPI treatments have been used effectively to promote quercetin enrichment in frozen–thawed cranberries [80], calcium in mango cubes [81] and baby carrots [82], curcuminoids in pineapple slices [83] or anthocyanins in apple slices [84]. 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 [85]. In the study of Balakrishna et al. [86], 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 4-O-(threo-b-

guaiacylglycerol) ether (TTGE), arabinoxylans, γ -aminobutyric acid (GABA) and vitamins such as E and B [87]. Although it has been shown that enzyme activity can be inhibited at pressures above 100 MPa [87], an increase in antioxidant capacity has been observed in germinated brown rice treated with HHP at 100–500 MPa [88]. 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 [89], sweet potato flour [90] and buckwheat flour [91].

8. Conclusions and Future Directions

A range of physically modified ingredients (starches, flours or even grains) could be developed by modulating the HHP processing conditions, potentially having a positive impact on the concentration of bioactive compounds. It has been shown that changes in the functionality of these modified ingredients are associated with changes in the main macromolecules of the flours, such as starch and proteins, as HHP causes gelatinization of starch and unfolding of proteins. Establishing appropriate HHP processing conditions is essential to obtain modified ingredients suitable for bread production. With gluten-containing matrices such as wheat flour, it has been observed that the application of high pressure can have the undesirable effects of excessive gluten strength, increased stiffness and resistance to elongation, as well as reduced cohesion. However, significant improvements have been observed with HHP under mild conditions as well as in combination with other treatments. This could be of industrial significance as current industrial HHP devices can operate in this range of processing conditions. On the other hand, promising results have been reported for ingredients used in GF formulations. Depending on the processing conditions, HHP could modify the pasting and rheological behaviour of the GF flour, increasing the thermal stability and elastic properties of the batters which could improve dough expansion and gas retention during proofing, thus enhancing the sensory properties of the nutrient-rich GF bread. A broad scope of research is required to fully understand the pressure-induced techno-functional changes in complex matrices to produce customized value-added ingredients for bakery products.

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