The Effect of High-Pressure Pre-Soaking on the Water Absorption, Gelatinization Properties, and Microstructural Properties of Wheat Grains

Ivan Shorstkii 1,*, Maxim Sosnin 1, Emad Hussein Ali Mounassar 1, Ute Bindrich 2, Volker Heinz 2 and Kemal Aganovic 2

1 Advanced Technologies and New Materials Laboratory, Kuban State Technological University, Moskovskaya 2, 350072 Krasnodar, Russia
2 German Institute of Food Technologies DIL e.V., Professor-von-Klitzing-Straße 7, 49610 Quakenbrück, Germany
* Correspondence: thegector@mail.ru

Abstract: High-pressure processing (HPP) is a novel technology that is used in many food processing operations to increase both the efficiency and a reduction in the energy and time required to modify and improve the physical and chemical properties of traditional food products. The objective of this study was to investigate the impacts of applying three treatments of a high hydrostatic pressure (HHP) and a control, i.e., 0, 100, 300 and 600 MPa, on the water absorption, gelatinization properties, and microstructural changes of wheat grains. The results indicated that the HHP treatments with a pressure of 300 and 600 MPa resulted in an increase of 16.7–24.8% in the mass of the grains; however, the pressure of 600 MPa did not result in a mass increase through water uptake. Further, the transition enthalpy increased with the HHP pressure, with 600 MPa defined as the threshold value for pressure. The results from this study demonstrated that a HHP treatment may enhance the soaking process of wheat grains and, thus, positively affect their gelatinization properties. These preliminary results may be used to improve the processing efficiency and quality of wheat-based products.

Keywords: high hydrostatic pressure; soaking; structure; starch; wheat grains; gelatinization

1. Introduction

Currently, high hydrostatic pressure (HHP) is used in different fields of industrial food production to obtain innovative products based on traditional or new raw materials, as well as to obtain products with improved physical, chemical and techno-functional properties [1,2]. Industrial applications of HHP in the food industry became possible as a result of intensive research carried out since the end of the 19th century [3]. The first areas of research were focused on the main structural substances of a living cell, changes in their chemical and physical properties, morphology and other relevant reactions and transformations. In evaluating the effectiveness of HHP treatments on food products in relation to other technologies, it is necessary to consider the gentler effect on the substances of food products compared with heat treatment; therefore, the possibility of using this method for processing food products sensitive to thermal denaturation needs to be evaluated [4].

Considerable material has been accumulated to study the biological effect of high pressure on proteins, carbohydrates, fats, nucleic acids, enzymes and the tissues of animal and plant cells and on seed germination, as well as on the processes of division and fertilization [5–7]. A pressure of several thousand atmospheres causes significant changes in the physicochemical state and properties of proteins and nucleic acids. Meanwhile, the denaturation of these substances depends on the amount of pressure, duration of exposure, temperature factor, pH of the medium and other experimental conditions [8].
Pressure-gelatinization does not induce the formation of a thermal degradation; thus, resulting in less grains swelling and amylose leaching [9]. In comparison, heat-gelatinization, which has been studied much more extensively, is influenced by the heating temperature and duration, water content, starch source and shear forces [10]. In the case of the pressure-gelatinization of solids, the most important factors are the holding time and pressure level [2].

The global production of starch is roughly 120 million metric tons annually and is growing by 5% annually [11]. The increasing role of starch in the economy of developed countries is caused by its unique properties as a final product and the raw materials for modified starch production [12]. Another global challenge is the significant amount of countries which are classified as arid and the challenges in these regions are exacerbated by a rising food demand due to population increases [13].

The process of germination is divided into the stage of preparation of the material and germination itself. The preliminary preparation of grain consists of the removal of extraneous bodies, grain sorting, washing and soaking which lasts from 12 to 72 h, significantly increasing the time of germination. The objective of this study was to introduce HHP technology into the soaking process of wheat samples and then to investigate the effect of the HHP pre-soaking treatments on the water absorption and gelatinization properties of wheat grains. Additionally, microscopic observations by scanning electron microscopy was performed to analyze the microstructural changes.

2. Materials and Methods

2.1. Raw Materials

A non-hulled Hard Red Spring wheat (Triticum durum) was chosen as the material in this study, and was obtained from a local wholesale retailer (Quakenbrück, Germany). The average initial moisture of the grain samples was 9.2 ± 0.3% and was measured gravimetrically using the oven method at 103 °C as described [3].

2.2. Samples Preparation and Analysis

Unpeeled wheat grains were prepared and analyzed as described in the scheme of the trials and analysis (Figure 1). Briefly, unpeeled wheat grains were treated by HPP at pressure levels (from 0 to 600 MPa), further soaked in distilled water for 2 h, dried in an oven and analyzed using a differential scanning calorimetry technique, and optical and scanning electron microscopy.

![Figure 1. Scheme for the course of the trials and analysis.](image-url)
2.3. HHP Treatment

For each experiment, 200 g of wheat grains were packed in a polyethylene pouch \((8 \times 20 \text{ cm})\) together with 500 mL of distilled water. The pack was sealed in a manner to keep the headspace in the pouch to a minimum. This was due to the different compressibility of air and water because air in the pouch needed to be avoided so as to not break the packages. The polyethylene pouches were submerged in the vessel of the HHP unit. The pressure transmitting media was a mixture of water and frigigel. The pressure treatments were performed at pressure levels between 100 and 600 MPa, for 1 min of holding time after the targeted processing pressure was reached. A pilot-scale high-pressure machine (Isostatic press 6500 bar, Nova Swiss, France) had a vertical high pressure vessel with a vessel treatment volume of 2 L. During the HHP treatments, the temperature of the unit was recorded by the machine, and this was in the range of 20–25 °C. The pressure build-up rate was 100 MPa/min, and the pressure-decreasing rate was 3000 MPa/min. Due to compression heating, an increase of the temperature of the pressurizing fluid was recorded by up to a maximum of 5 °C at 600 MPa. After the HHP treatment (i.e., the pressure come-up time and 1 min of holding), the samples were left for soaking at atmospheric pressure to reach a total soaking time including the HHP treatment of 120 min. Experiments at atmospheric pressure (0.1 MPa), set under the same conditions of time and temperature, served as the control samples. A comparison analysis was also provided with an untreated sample.

2.4. Water Absorption during HHP Treatment

After the HHP treatment, the samples containing grains and distilled water were kept at the temperature of 20 °C in a water bath (Grant OLS 200, Wiltshire, UK). The total soaking time including the HHP treatment was 120 min. To obtain the water absorption kinetic curves, all the samples were measured in intervals of 5, 10, 30, 60 and 120 min. For that purpose, the hydrated samples were periodically removed and filtered through a coarse filter cloth and placed on 2 layers of paper towels, quickly blotted 4 to 5 times to remove the surface water by gently rolling the grains on the towel, analyzed, and returned to the containers. During the filtering and massing operations, the soaking procedure time was paused for 30 s. The mass uptake was determined gravimetrically at the scheduled time. An electrical balance (error of ±0.01 g) was used to weigh the grains, which were then returned to the containers and reweighed at the scheduled time. The mass uptake was calculated from Equation (1):

\[
\% \text{ Weight uptake} = \frac{W - W_0}{W_0} \times 100
\]

where \(W_0\) is the initial grains mass (200 g) and \(W\) is the grains mass at time \(t\).

2.5. Convective Drying

After the water uptake, the grain samples were blotted with a paper towel. The grain samples of each pre-treatment were ground and used for the determination of the initial moisture content \((M_0)\) before they were dried. The measurement of the moisture content was performed using a moisture scale (VPB-10, Allscales Europe, Veen, The Netherlands) and was run in duplicates. For the drying, the samples were evenly spread in a monolayer (equal to the grains’ thicknesses) into drying containers with a perforated structure, allowing for improved air circulation and moisture removal. The drying containers and samples were weighed with a precision scale (Kern 440-49A, Kern & Sohn GmbH, Balingen, Germany). The drying process was performed in a preheated drying oven (FP 240, Binder GmbH, Tuttingen, Germany) at a constant temperature of 90 °C and an air velocity of 0.2 m/s. The drying procedure was run until an equilibrium moisture content was reached.
2.6. Differential Scanning Calorimetry (DSC)

To observe changes in the properties as a function of the temperature, differential scanning calorimetry (DSC 250; TA Instruments, Eschborn, Germany) was performed: single grain samples $8 \pm 0.7\, \text{mg}$ were placed together with $15.7 \pm 2.8\, \text{mg}$ of demineralized water into aluminum pans and were heated from $5\, ^\circ\text{C}$ to $95\, ^\circ\text{C}$, at a heating rate of $5\, \text{K/min}$, then, the heat flow was determined. The measured transition enthalpy ($H$) was converted into $J/\text{g}$ of the dry mass. The data was averaged with a minimum of 3 replicates for each sample.

2.7. Stereo Microscope

A morphological characterization of the HHP pre-treated and untreated wheat grains were analyzed by a stereo-microscope (Wild M8, Leica Microsystems GmbH, Wetzlar, Germany). For the observations, the preparation of single grain cross section was performed using a steel blade. The grain specimens were transferred to a $0.9\, \mu\text{m}$ thin polyethylene terphthalate film using adhesive labels (PLANO GmbH) and fine preparation needles. A digital camera, a Leica EC 3, Heerbrugg, was used to capture the images.

2.8. Scanning Electron Microscopy (SEM) Analysis

The grain specimens were fixed in a SEM sample holder with a special optimal cutting temperature (OCT) compound glue, cryogenically frozen in super-cooled liquid nitrogen and inserted in a cryogenic preparation system (Emitech K 1250), where the samples were broken down, and the free water was removed by sublimation. Afterwards, the surface was sputtered with gold in a deep frozen state. The prepared grain specimens were transferred into the SEM (jeol JSM 6460 LV) at $(-180)\, ^\circ\text{C}$.

An electron beam was generated in the scanning electron microscope (SEM) which was accelerated to $15\, \text{kV}$ voltage. In general, this primary electron beam is directed in an isometric pattern at a sample’s surface. The secondary electron signal caused by the primary beam will then change according to the state of the sample surface and the blade angle of the beam; thus, the image which is built up in the Braun tube arises from light spots whose brightness is changed. Since the secondary electrons can be forced by magnetic lenses to follow a curved course, it is possible to make the surface profiles visible which lie outside of the direct target line of the collector. Consequently, a large depth of the field was possible and, in addition, a tri-dimensional effect could be produced. The generated images were saved electronically.

2.9. Statistical Analysis

A moisture analysis of each sample was measured 5 times, and the mean values were calculated. The DSC analysis was performed for three samples of each HHP treatment mode. Differences in the mean values were evaluated using a Tukey test (with a 95% probability), where the Design Expert software (version 13.0.5.0, Stat-Ease Inc., Minneapolis, MN, USA) was used for the analysis.

3. Results and Discussion

In the following sections, the results on the effects of the HHP treatment on the moisture and mass uptake of wheat grains is discussed, followed by a further elucidation of the microscopic images and visualization of the moisture migration path. Finally, the gelatinization properties are reported and discussed.

3.1. Effects of HHP on Mass Uptake

The curves on the mass uptake of the untreated and HHP-treated wheat grain samples for the selected pressure conditions (the control, 100, 300 and 600 MPa) are presented in Figure 2. As it can be seen, after the first 20 min of soaking, a positive linear behavior was observed for all the samples; however, for the pressure treatment of 100 MPa, the mass uptake increased by around 153 %. Conversely, the mass uptake of the wheat grain samples...
treated at 300 MPa and 600 MPa increased as well by up to 157 and 145%, respectively. This was, at the same time, the treatment condition that exhibited the maximum mass uptake among the investigated conditions. Apparently, at the highest measured pressure level of 600 MPa, the mass uptake was lower, compared to 100 and 300 MPa. Such a difference might have been due to the viscosity enhancement, as well as a reduction in the fluidity [13,14]. According to the literature data, the dynamic viscosity of water may increase from 0.001 Pa·s to 0.0014 Pa·s with an increasing pressure up to 600 MPa at 20 °C [15,16]. Notably, the viscosity began to exponentially increase starting at 200 MPa.

![Figure 2](image-url)

**Figure 2.** Curve of mass gain for wheat grains during hydration for different HHP treatment intensities with an indication of the mass uptake as a function of the pressure at 120 min.

This effect might be also related to the effects of HHP on starch, but also as a result of certain pressure-related structural changes within the grain seed, and consequential changes in the water binding properties. The HHP can also have an effect on starch granules [17], but this effect largely depends on the source and the structure of the starch granule [18]. The HHP can also have an impact on starch gelatinization, that can be induced by pressure at a lower temperature than a typical thermally-induced gelatinization, due to pressure-induced modifications of the amorphous and crystalline regions of a starch granule [14,19,20]. It is also possible that pressure is involved in the creation of non-preferential disruption of crystallites, resulting in cracks and channels in a wheat kernel structure, as reported in one previous study on the pressure gelatinization of wheat starch [21]. The appearance of cracks and spaces between granules was observed by scanning electron microscopy and reported by [22] for cooked rice grains. In comparison with our study, [22] reported appearance of cracks on the ventral side of the grain in a low-moisture content area. In our study, because of an all-round hydrostatic compression, most of the cracks were observed on the middle part (half-thickness) of the grain kernel. From the author’s point of view in comparison with rice, with a solid oval structure at the cross-section, wheat grains could demonstrate a different behavior. The cross-section of a wheat kernel has a crease [23,24], which might decrease the stress factor during a high pressure treatment.

The measured soaking kinetic results (Figure 2) were in agreement with a study of Zhu et al. [25], who reported an increase in the final moisture content for brown, medium-milled and fully-milled rice pre-treated with high pressure, independent of the soaking
time. It was found that a high-pressure treatment (>200 MPa) resulted in high water absorption characteristics. Yu et al. [24] referred to the final moisture content as the equilibrium moisture content (EMC) for brown rice, while in the case of wheat grains it was unclear whether the water absorption was thermodynamically an equilibrium process. Possible reasons for a higher moisture content as a result of a high pressure treatment could be due to the formation of more cracks on the grains, which would allow for an easier water penetration by capillary pressure. Zhu et al. [25] have stated that the processing of grains causes the removal or destruction of protective outer bran layers, especially the hydrophobic lipid layer, thereby increasing the water diffusion during high pressure soaking. Irrespective of this, it can be concluded that the rate for attaining a maximum moisture content was higher for a high pressure compared to untreated samples, but only to a mild pressure level, in this case 300 MPa. Other processing windows would need more attention and further studies should be conducted to further elucidate the effect of pressure on wheat grain structures, the mechanism of water absorption and the optimal processing conditions.

3.2. Microscopic Analysis of Untreated and HHP-Treated Wheat Grains

Images of the cross sections of the untreated and pressure-treated wheat grain samples are shown in Figure 3. From the figure, a characteristic xiphoid arrangement of cells with a very elongated shape, typical for vitreous hard grains, can be seen. The main visual difference between the differently treated grain cross section images was the starch whiteness. It is obvious that for a higher pressure treatment, the wheat grain samples (c-e) exhibited more intensive whiteness compared to the untreated (a) and control samples (b). In order to elucidate this effect at a microscopic level, further images using scanning electron microscopy (SEM) were taken and discussed.

![Figure 3. Cross section images of wheat grains of untreated (a), control (b) and after high-pressure treatment at 100 MPa (c), 300 MPa (d) and 600 MPa (e).](image)

The SEM was used to optically observe the moisture migration path and to determine if significant differences in the moisture distribution among the grains soaked after the HHP treatments could be observed. In Figure 4, the SEM images of untreated, control and HHP-treated wheat grain samples at the same area are shown. A relatively smooth surface with less wrinkles was observed for an untreated sample compared to those of the control and HPP-treated samples. Additionally, an analysis of transversally cleaved grains indicated that less and smaller cavities occurred in the pericarp and crease regions of the untreated wheat.
3.3. Effect of HHP on the Gelatinization Properties of Wheat Grains

Figure 4. SEM images of wheat grains of untreated (a–c), control (d–f) and after high-pressure treatment at 100 MPa (g–i), 300 MPa (j–l) and 600 MPa (m–o) of wheat grains at different magnifications (30×, 250×, 1000×).

Figure 4a shows the whole cross section of a soaked wheat grain, with visible cracks in the endosperm. A low number of cracks was distributed mostly along the cells’ surfaces, from the skin to the inner area which can be better seen in Figure 4b,c. The observed micro cracks most likely represent the penetration path of water during the soaking. In
Figure 4d–f, an enlarged version of the three regions where the cracks were most substantial can be observed. A similar distribution of cracks in hot-air dried grains were reported in other studies [24–26]. The enhancement of the number of cracks might have been due to the thermal deformations during the drying procedure [22]. Further images in Figure 4g–i, indicate cracks in different forms, including wide, longitudinal and cross cracks originating from the surface of the wheat grains, as well as many small irregular cracks and bursting cracks in the inner area of a wheat grain. Figure 4h,i show the microstructure of the soaked wheat grains in more detail using a larger magnification. A certain number of finer cracks can be observed that were connected with larger cracks. Additionally, the size of the cracks increased (Figure 4g). These observations support the assumption that water mainly penetrated via wide cracks into the inner area of the wheat grains, formed during soaking [25]. The pressure enhancement shown in Figure 4j–l,m–o demonstrates a further increase in the size and number of cracks. By examining the microstructure of a grain, a heterogeneous penetration path of water during soaking can be confirmed. Similar to other grains as also in the wheat grains, starch granules are bound to one another by a cementing phase (corresponding to the protein matrix) and are typically located in the endosperm [26]. However, due to the strong hydration effect of cell walls and the aleurone layer, and the numerous small and large cracks within a grain (Figure 4o), this allowed for the penetration of water throughout a grain. It can be concluded that under increased pressure, water penetrated into the pores and cracks, as well as into the solid state of the wheat grain, and this was easier than when no pressure was used; thus, resulting in a faster and enhanced mass increase during soaking compared to the samples without using any pressure.

3.3. Effect of HHP on the Gelatinization Properties of Wheat Grains

Differential scanning calorimetry (DSC) has been considered to be an effective way for gaining information about the pasting properties of starch. The DSC curves of the untreated and pressure-treated wheat grain samples in a measurement range of 20–95 °C are shown in Figure 5, and the numerical DSC data (T<sub>peak</sub>, and Q<sub>peak</sub>) are presented in Table 1.

| Sample          | Untreated | Control | HHP          |
|-----------------|-----------|---------|--------------|
|                 |           |         | 100 MPa | 300 MPa | 600 MPa |
| Transition temp. | 60.6 ± 0.4| 59.9 ± 0.3| 61.9 ± 0.1| 60.4 ± 0.1| 60.9 ± 0.1|
| Heat flow, Q<sub>peak</sub> [W/g] | 0.3 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 |
| Total enthalpy, ∆H [J/g] | 1.2 ± 0.1 | 1.4 ± 0.5 | 1.8 ± 0.1 | 1.7 ± 0.5 | 1.9 ± 0.1 |

For all the investigated samples, a small peak may be observed at the temperature of around 60 °C (Figure 5). This observed peak is related to the thermal effects on the starch and proteins, and the related gelatinization. The enthalpy value (∆H) is the energy required for the complete gelatinization of starch granules. The larger the ∆H value, the more energy that is required for the gelatinization, the greater is the starch recrystallization area, and the greater the degree of retrogradation. The values of the transition temperature T<sub>peak</sub> of the wheat grains fluctuated irregularly, indicating that the HHP treatment had little effect on the crystalline structure of the starch (Table 1). At the same time, the wheat grains soaked at a higher pressure showed a higher enthalpy than the samples soaked at a lower pressure.

When the gelatinization occurred, its degree was measured to increase with the pressure until the gelatinization was complete. The highest enthalpy measured was 1.96 J/g for the treatment at 600 MPa at the temperature of 60.9 °C (Figure 5e). This is consistent with earlier reports by Huang et al. (2009), where it was reported that a HHP treatment at 600 MPa and 70 °C for 120 min induced the highest degree of starch gelatinization (almost 100%) in glutinous rice. Conversely, Zhu et al. (2016) measured that HHP treatments at 500 MPa would not cause the complete gelatinization of wheat starch suspensions.
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Figure 5. Heat flow as a function temperature (DSC curves) of untreated, control, and high-pressure-treated wheat grain samples at 100 MPa, 300 MPa and 600 MPa.

4. Conclusions

The aim of this work was to investigate if there is and to what extent, an impact of a HHP treatment has on the moisture uptake of wheat grains. The results indicated that by applying the HHP, a facilitated mass and moisture uptake in wheat grains was possible. Increasing the applied pressure from 100 MPa to 300 MPa indicated a significant increase in the moisture content; however, a further increase in pressure up to 600 MPa did not result in a further mass uptake. This could be related to structural changes within the wheat grain kernel and endosperm, or be related to water immobilizing properties.

As expected, the HHP treatment enhanced the gelatinization of the wheat grain with a high swelling degree. The HHP treatment at a low pressure of 100 MPa was sufficient to result in a wheat grain with a high water absorption. The degree of gelatinization at 600 MPa was highest among the treated samples; however, other questions arise from these measurements, in the first place, and the optimal high-pressure processing conditions for soaking and the gelatinization properties should be identified. Furthermore, more studies from a mechanistic point of view are needed to fully elucidate and understand the effects of HHP on the soaking and water absorption. Finally, the impacts of such a treatment on the final grain properties, such as the milling, yields or baking properties should be investigated to fully evaluate the suitability of high pressure for this type of application.

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