

Microscopic Study of Starch Gelatinization under High Hydrostatic Pressure

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Starch gelatinization corresponds to a melting phase transition in aqueous medium. Such a transition involves important mass transfer of water. Using a high-pressure bomb including optical ports, the volume variation of starch granules in suspension was related to gelatinization during a high-pressure treatment up to 420 MPa. Microscopic observations of wheat and potato starch granules were compared with macroscopic measurements of compressibility up to 600 MPa and gelatinization intensity using differential scanning calorimetry on treated suspensions. Wheat starch gelatinization started below 300 MPa and was completely achieved at 600 MPa. Potato starch was not altered under 600 MPa. The behavior of the volume variation of starch granules under pressure compared with starch suspensions compressibility could be explained by simultaneous compression and hydration mechanisms.

Keywords: *Starch gelatinization; high hydrostatic pressure; image analysis*

INTRODUCTION

Starch gelatinization consists of a biopolymer melting in an aqueous medium. When a water–starch suspension is heated, a phase transition is observed for starch between 60 and 70 °C. Optical properties of starch granules, such as light polarization or iodine coloration, are changed.

Granule swelling increases with temperature and becomes irreversible when gelatinization occurs. The swelling corresponds to a mass transfer of bulk water in the suspension to water linked with starch components (amylose and amylopectin). Generally, granule structure is dispersed when heat treatment reaches a certain level, and the solubilization of amylose chains is responsible for an increase in viscosity.

Starch gelatinization is an endothermic transition which is well identified with differential scanning calorimetry (DSC). This method allows the characterization of melting parameters, i.e., enthalpy of gelatinization (ΔH_f) and temperature of melting (endothermic peak temperature).

High-pressure technology has been known since the beginning of the 20th century (Bridgman, 1914), but applications in the food industry have only been developed in the past 20 years, particularly in Japan and Europe (Suzuki and Tanigushi, 1972; Weber and Drickamer, 1983; Gekko and Fukamizu, 1991a,b; Dumay et al., 1994).

Since 1980, several papers have indicated that high-pressure gelatinization of starch is possible with a heat treatment before compression (Muhr and Blanshard, 1981; Hayashi, 1992; Kudla and Tomasik, 1992; Hibi et al., 1993). Some research involved high-pressure differential thermal analysis (HPDTA), which allowed a simultaneous application of pressure and heating

(Muhr et al., 1982). These authors have calculated the volume variation of starch during gelatinization under pressure by using Clapeyron's equation (1), which is valid for any phase transition at equilibrium:

$$\left. \frac{\partial T}{\partial P} \right|_{\Delta G=0} = T \frac{\Delta V}{\Delta H} \quad (1)$$

T is the absolute melting temperature, P is the pressure, and ΔG , ΔV , and ΔH are the specific Gibbs free energy, volume, and enthalpy changes, respectively. ΔV is the volume change of 1 g of starch crystallites dissolved in an infinite reservoir of the water–starch mixture. ΔH , dP/dT , and T_m measurements permit one to calculate the corresponding ΔV . Results from these calculations indicated a reduction of starch volume of 0.5 mm³/g of crystallites at 400 MPa and an increase in volume of about 1 mm³/g under atmospheric conditions.

Recent research on starch gelatinization under pressure showed a better enzymatic digestibility of treated starch (Hayashi and Hayashida, 1989). Pressure could also delay starch gel retrogradation.

The aim of this paper was to compare mass transfer observations from microscopic study of starch granules under high pressure with macroscopic measurements of energy or volume variation of starch suspensions. Microscopic observations of starch granules were performed by an original visualization system working under pressure. These two scales of observation would allow one to understand the mechanism of starch gelatinization under pressure and especially the contribution of water–starch interactions.

MATERIALS AND METHODS

Starch. Wheat starch (ref E123359, Merck, France) consisted of 23% amylose and 75% amylopectin (w/w). Moisture content was measured after 24 h of drying at 105 °C and was 16.4 ± 0.5% on a dry matter basis. Specific density measurements using the picnometric method gave a value of 1.29 ± 0.1 g cm⁻³.

Potato starch (ref 21146290, Prolabo, France) consisted of 26% amylose and 73% amylopectin (w/w). Moisture content

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Table 1. Diameter Distribution for Wheat and Potato Starch Observed by Microscopy in Reference to Glass Balls of 10 μm Diameter^a

starch	mean diameter (μm)	extremes (μm)
wheat		
1 ^b	10	3–17
2 ^b	29	18–58
potato	37.5	10–110

^a Precision of the diameter measurement was about 5%. ^b Distribution was bimodal for wheats 1 and 2 and unimodal for potato.

was $14.8 \pm 0.5\%$ on a dry matter (DM) basis. Specific density measured in the same conditions was $1.31 \pm 0.1 \text{ g cm}^{-3}$. For each experiment, water–starch suspensions were prepared 1 h before pressure treatment and were continuously stirred at 20°C .

Microscopic observations were dependent on the large scale of starch granule diameter. Table 1 indicates mean diameters, extreme diameters, and distribution mode for 768 wheat granules and 652 potato granules colored with iodine.

High-Pressure Visualization Cell. Microscopic observations were obtained by a unique high-pressure cell (25 μL , 700 MPa) manufactured by LIMHP, CNRS, Villetaneuse, France. The copper beryllium body cell included optical ports (sapphire windows) of $10 \pm 0.1 \text{ mm}$ diameter, 5 mm thick for the lower one, 10 mm thick for the upper one, through which light can pass.

The high-pressure chamber (0.8 mm thick) was situated between the two sapphires and connected to a high-pressure system. To prevent leaks, different kinds of gold joints and gaskets were used.

Starch suspensions were 0.1% starch content (starch weight/suspension weight on a dry matter basis) and prepared with distilled water at room temperature. The high density of the starch granules prevented them from moving in the high-pressure cell during the time of pressure application.

Microscopy and Image Analysis System. The high-pressure cell was placed on an inverted light microscope using an objective lens $\times 20$ (Nachet, France). Images were observed directly through a binocular and recorded via a charge coupled device (CCD) camera. This system allowed image analysis using Visilog Software (Noesis, France). Each image consisted of a 256×256 pixel matrix of 256 gray levels and contained about 30 granules. Granules were individually analyzed to find their projected area (S) in comparison with glass balls of $10 \mu\text{m}$ diameter. Starch granules were assumed to be spherical.

Then, volume (V) was calculated using the following equation:

$$V = 4S^{3/2}/3\sqrt{\pi} \quad (2)$$

Special care was taken with focus and lightness adjustment. Image thresholding conditions were kept constant during all of the experimentation.

The error in calculation of diameter and volume did not exceed 4%. Furthermore, starch granules were iodine colored because of the excellent contrast needed for image analysis.

Starch granule visualization was achieved under pressure during two cycles of pressurization at room temperature. Image analysis allowed the following of individual granules and their specific change every 100 MPa.

High-Pressure System for Microscopic Measurements. As shown by Figure 2, pressure was generated with a 400 MPa hydropneumatic piston pump (Haskel, 903 C, Great Britain). Distilled water was used as compression fluid. Pressure was measured with a 700 MPa pressure gauge (G515/7000, Sedeme, France) with a precision of 0.5%. The pressure was transmitted to the visualization cell via $1/4$ in. tubing. This high-pressure system included valves, fitting, and tubing designed for high-pressure work. The compression rate was about 5 MPa s^{-1} and was the same for each experiment. Two cycles of pressurization were carried out at the same pressure level.

High-Pressure System for Compressibility Measurements. Pressure was generated with a 700 MPa manual screw piston pump of 3 mL (Nova Swiss, Switzerland). Suspensions contained 16% starch (DM basis) and were compressed into the body of the pump. Volume changes (ΔV) were measured through the variation of the initial position of the screw with the pressure variation. Pressure ranges were about 10 MPa per point of measure. Compression and release were controlled at a rate of 50 MPa s^{-1} but can be slowed down if necessary. Two cycles of pressurization were achieved upon the same suspension.

The parameter "compressibility", which is usually employed when a pure liquid is considered, is not exactly appropriate for this work. The initial biphasic suspension was transformed to a dispersion during the high-pressure treatment. Then, macroscopic compressibility of liquids is given by the equation:

$$\beta_t = -\Delta V/V(\Delta P) \quad (3)$$

where β_t is the macroscopic compressibility of the suspension in bar^{-1} , ΔV is the volume change of the suspension in milliliters, V is the initial volume in milliliters, and ΔP is the pressure variation in bar. Precision of the compressibility measurement was about 1%.

The initial volume was determined with pure water using Tait's equation (Hayward, 1967) and given by

$$\Delta V/V = P/[P(m) + K_0] \quad (4)$$

where K_0 is the bulk modulus at zero pressure in bars and m the slope of the bulk modulus pressure curve as described by Hayward (1967). P and V are given in bars and milliliters, respectively. The values of coefficients $m = 3.306$ and $K_0 = 22421$ were estimated from pure water compressibility measurements. Initial volume V was found to be equal to $4.78 \pm 0.01 \text{ mL}$.

Initial volume determination was dependent on a complete fitting of the high-pressure system. So a primary vacuum was applied.

Energetical Measurements after a Pressure Treatment. Using the high-pressure system described for compressibility measurements, starch suspensions were compressed from 100 to 600 MPa. The enthalpy of gelatinization (ΔH_f) and the temperature of maximum peak (T_m) were subsequently determined using differential scanning calorimetry (DSC) with three repetitions. The DSC apparatus was a Perkin-Elmer DSC2 with a temperature precision measurement of 0.1 K and 0.5 J g^{-1} dry matter for enthalpy of transition, which was manually determined.

RESULTS AND DISCUSSION

Microscopic Observations of Starch Granule Gelatinization under High Pressure. Figure 1a represents the iodine colored wheat starch granules in suspension with water at atmospheric pressure. Figure 1b represents the same granules at 420 MPa. Some wheat granules lost their iodine coloration, and significant swelling was observed above 300 MPa. No noticeable effect on the diameter was shown: gelatinization seemed to affect small or large granules equally.

The evolution of the volume of about 90 wheat granules was represented on compression and release in Figure 3. The maximum pressure applied was 420 MPa. Two populations were identified, with regard to their subsequent behavior, and the discrimination was observed above 300 MPa on compression. About 25% of wheat granules were swollen with a simultaneous iodine discoloration, which indicated starch melting.

This increase in granule volume was particularly relevant between 300 MPa on compression and 300 MPa on release. At the end of the release, melted wheat granule swelling reached 78% for the mean. On the

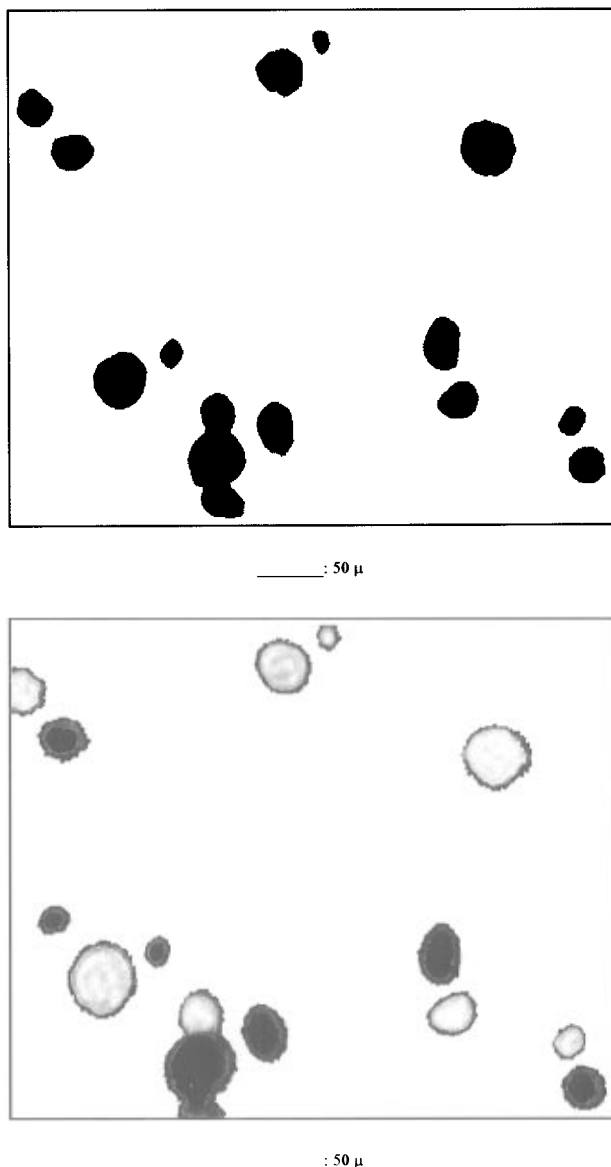


Figure 1. (a, top) Suspension of wheat starch granules in water at room temperature. Starch granules have been iodine colored. (b, bottom) Suspension of wheat starch granules at 420 MPa. Gelatinized granules have lost their iodine coloration. Undamaged granules are still iodine colored.

other hand, undamaged granules were only modified at the end of the release when a slight swelling of 25% was observed without iodine discoloration.

The same granules were submitted to another pressure cycle at the same level as shown in Figure 4 in which the evolution of wheat granule volume is plotted versus pressure during the compression and release phases. Unlike previous results, the volume of wheat starch granules was found to decrease during the compression phase and increase during the release, showing a reversible behavior under pressure. No further swelling or iodine discoloration occurred during the second cycle.

Change in the volume of potato starch under pressure is shown in Figure 5 for the first and second cycles. Maximum pressure was 300 MPa.

First compression induced an irreversible swelling of 15% observed at the end of the release of the first cycle, but no discoloration occurred. A second compression at the same level involved a reduction of granule volume

under pressure. No further modifications were observed in terms of swelling or iodine discoloration.

These primary results showed that wheat and potato starch granules did not reduce their volume when pressure increased. Moreover, a significant number of wheat starch granules swelled and discolored during the compression phase and looked gelatinized. Potato and undamaged wheat granules were only slightly swollen at the end of the first cycle. Those granules remained iodine colored, so only hydration can be suggested to explain such an increase in granules volume.

Whatever modifications were induced by pressure, a second pressurization gave reversible results for wheat as well as for potato granules.

Calorimetric Analysis of Water–Starch Suspensions Treated by High Pressure. Macroscopic measurements of energy were carried out on starch suspensions to confirm microscopic results. DSC gave a quantitative indication of starch gelatinization on suspensions previously treated under pressure.

DSC results were provided by thermograms (Figure 6), on which melting enthalpy (ΔH_f) was given versus temperature for wheat starch. Table 2 indicates the melting enthalpy and the percentage of melted mass versus pressure referring to initial sample mass of wheat starch. DSC results at atmospheric pressure were according to previous work (Eliasson, 1980; Lund, 1984) which indicated ΔH_f of 13.5 and 13.2 J g⁻¹ of wheat starch DM, respectively.

Potato starch gave no relevant modifications of gelatinization enthalpy, and results are not presented there. As shown in Figure 6, a significant decrease in peak area was observed after a 300 MPa treatment, and wheat starch thermograms indicated no more melting enthalpy after a 600 MPa treatment. It can be concluded that, in this case, starch had already melted under pressure.

Hence wheat starch gelatinization can occur between 300 and 500 MPa with, respectively, 15–88% of melted starch mass in reference to initial sample mass. In comparison with a high-pressure treatment, starch gelatinization under atmospheric conditions is completely achieved at about the melting temperature ($\pm 10^\circ\text{C}$). A high-pressure treatment would allow graduated levels of gelatinization.

Evolution of the Compressibility of a Water Starch Suspension under Pressure. Wheat and potato starch suspensions were compressed up to 600 MPa using a manual high-pressure pump. Using the convenient representation of a pure liquid compressibility given by eq 3, starch suspension compressibility was calculated versus pressure.

The evolution of a wheat starch suspension during the compression and release phases is represented in Figure 7. During the compression below 300 MPa, the compressibility of a starch suspension was close to that of pure water. Over 300 MPa, the reduction of the volume was higher for the starch suspension than for pure water at the same pressure. At 600 MPa, volume reduction was 14.1% for pure water and 15.7% for the starch suspension. During the release, as shown in Figure 7, the starch suspension compressibility was always higher than that of water. Furthermore, the starch suspension was of higher compressibility than water when measured during the compression phase for the same pressure value. A volume reduction remained significant after return to atmospheric pressure. When starch suspensions were submitted to another pressure

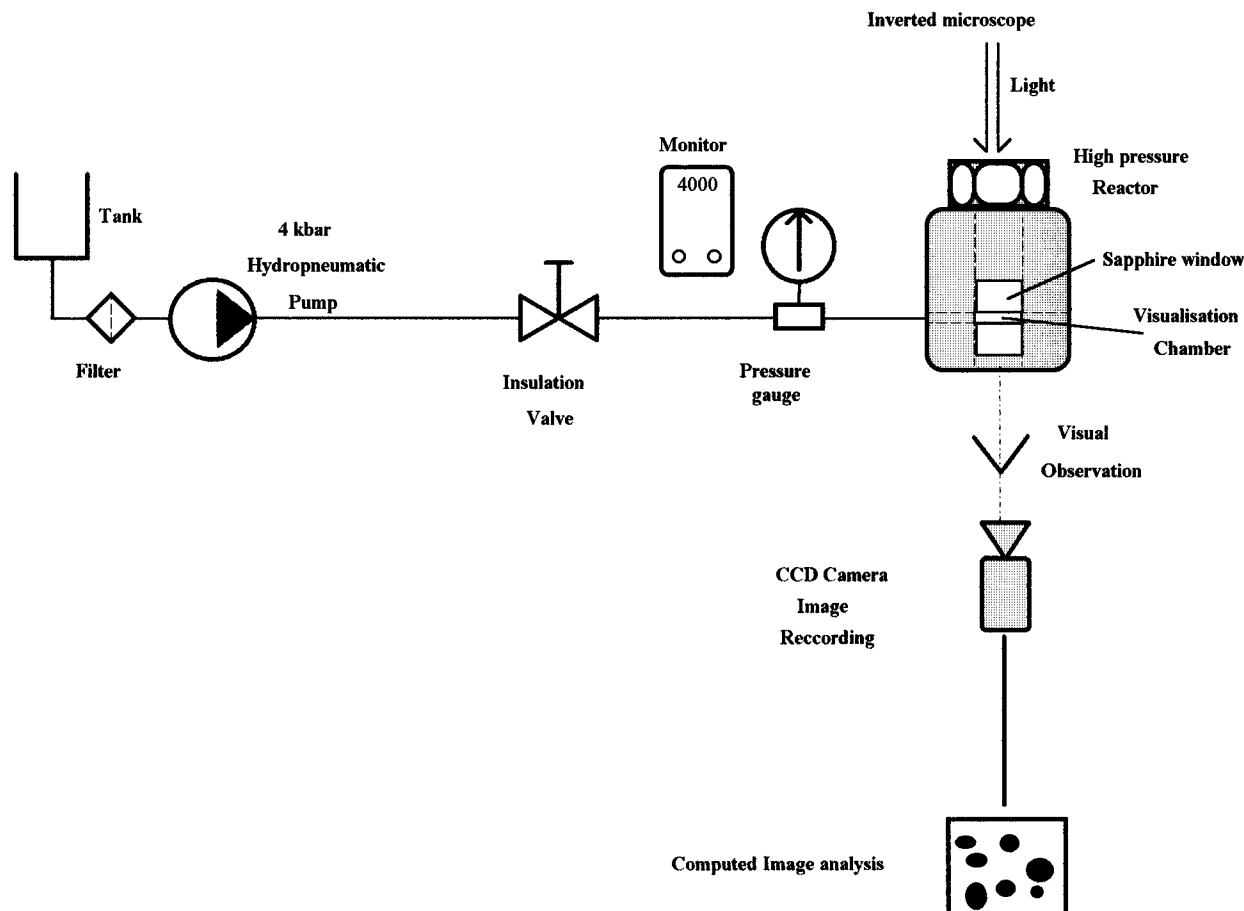


Figure 2. High-pressure circuit, visualization cell, and image analysis equipment.

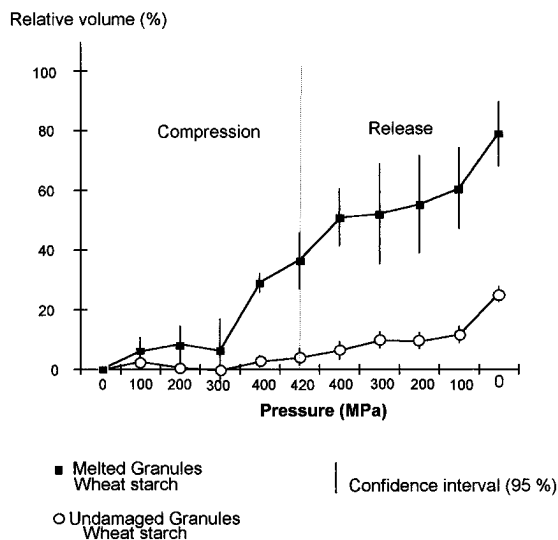


Figure 3. Volume increase in wheat starch granules during the compression and release phases for melted (30) and undamaged (60) granules in reference to initial volume. Bars represent the confidence interval of the mean (95% level).

cycle at the same level, the compressibility of the starch suspensions was found to be equal during the compression and release phases.

Potato and wheat starches gave quite similar results except for the amplitude of the lags which was lower for potato. Therefore, only wheat starch results are represented.

Microscopic results showed that wheat starch gelatinization under pressure was followed by significant granule swelling and discoloring. Hydration can be

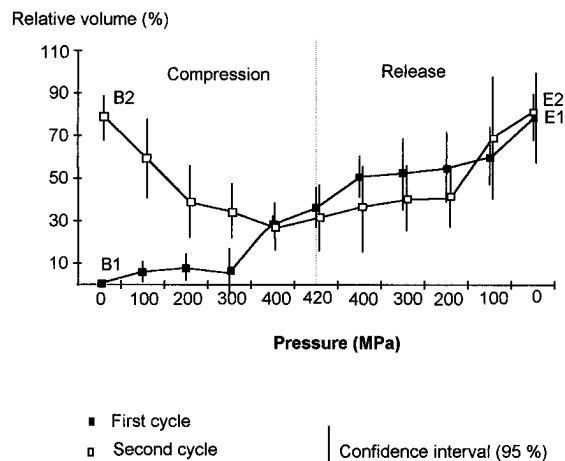


Figure 4. Volume evolution of melted wheat granules during the compression and release phases for two cycles of pressurization in reference to initial volume. Bars represent the confidence interval of the mean (95% level). B, beginning of the cycle; E, end of the cycle.

suggested for undamaged wheat and potato granules that swelled. Compressibility measurements indicated a reduction of the suspension volume while wheat starch was melting.

This apparently paradoxical behavior indicated that water–starch interactions under pressure were only possible with a reduction of the total volume, according to Le Chatelier's principle. A schematic representation of wheat starch granule behavior under pressure is given in Figure 8. During the compression phase, the starch granule volume did not decrease as predicted by starch compressibility under pressure (Kudla and To-

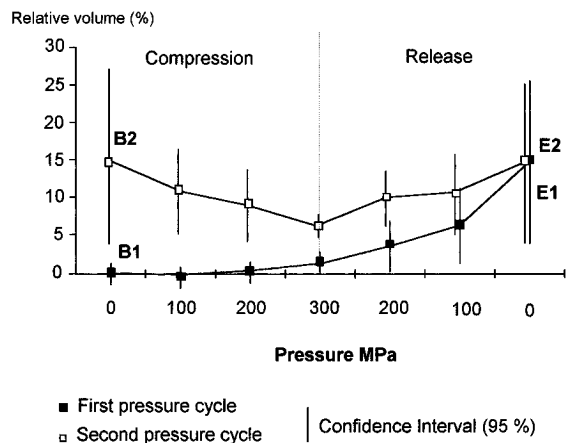


Figure 5. Volume evolution of 60 potato granules during the compression and release phases for two cycles of pressurization in reference to initial volume. Bars represent the confidence interval of the mean (95% level). B, beginning of the cycle; E, end of the cycle.

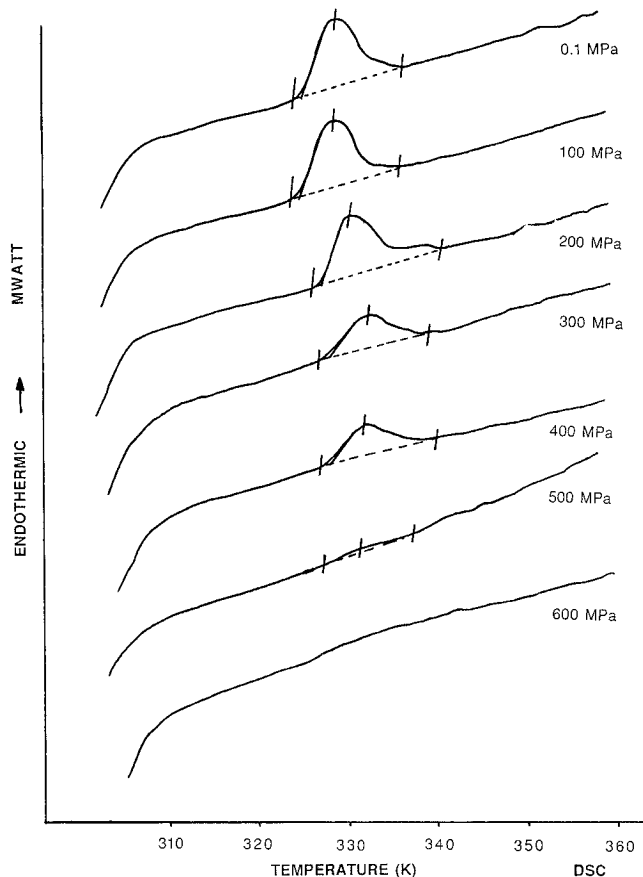


Figure 6. DSC thermograms of wheat starch suspensions (16% w/w DM basis) after different levels of pressurization.

masik, 1983). So, it could be suggested that hydration occurred in the case of damaged wheat granules. Granule hydration would allow preservation of the initial granule volume and so would counteract the compression effect. The macroscopic consequence of starch hydration was a decrease in the bulk suspension volume. Such a behavior was verified for undamaged wheat and potato granules.

As shown in Figure 8, some wheat granules were swollen and discolored above 300 MPa, while the suspension volume was more restricted than that of pure water. This discoloration was confirmed by DSC measurements and was found to correspond to starch

Table 2. Evolution of the Enthalpy of Gelatinization (ΔH_f) and of the Gelatinized Mass (in Reference to Initial Mass of Dry Matter) with Pressure Increase for Wheat Starch-Water Suspensions^a

pressure (MPa)	ΔH_f (J g ⁻¹)	gelatinized mass (%)
0	17.9	0
100	17.8	0
200	17.9	0
300	15.2	15
400	12	33
500	2	88
600	0	100

^a 16% w/w DM basis. The precision in ΔH_f measurement is ± 0.5 J g⁻¹ of DM and $\pm 2.8\%$ for starch gelatinized mass.

Compressibility (MPa⁻¹)

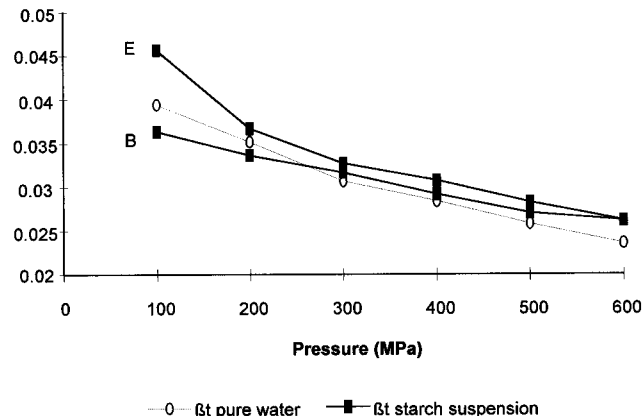


Figure 7. Evolution of the compressibility (β_t) of a wheat starch suspension (16% starch content w/w DM basis) and pure water during a cycle of pressurisation. B, beginning of the cycle; E, end of the cycle.

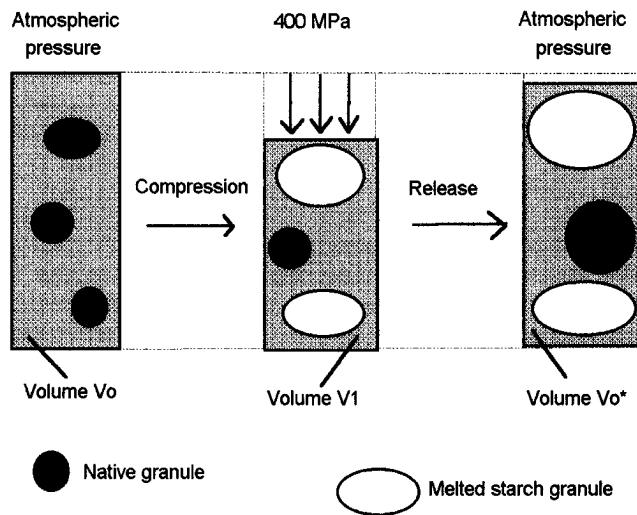


Figure 8. Schematic representation of wheat starch granule behavior in suspension with water during a 400 MPa treatment.

granule gelatinization. This phase transition involved a massive water entry into starch granules and the creation of hydrogen bonds between water and starch enhanced by the high-pressure level. This granule swelling was accompanied by a reduction of the bulk suspension volume.

To explain the previous observations, the water molecules linked with starch must then occupy a smaller volume than the molecules in pure water. Such an explanation validates the principle of Le Chatelier and appears to be consistent with previous works

implicating hydrogen bonds under pressure (Suzuki and Tanigushi, 1972; Gekko and Fukamizu, 1991a,b).

Concerning potato starch, our results were in agreement with previous work indicating a total gelatinization of potato starch at only 800 MPa at room temperature (Kudla and Tomasik, 1992). These authors suggested that the particular behavior of potato starch under pressure was related to specific crystalline conformation.

CONCLUSION

Comparison of microscopic and macroscopic measurements allowed to more precise understanding of starch gelatinization under pressure. Microscopic observations showed that wheat starch gelatinization began above a pressure of 300 MPa without previous heating. This phenomenon occurred during the compression phase for wheat starch granules as confirmed by DSC results. At 420 MPa, 33% of the starch mass had melted. From a macroscopic point of view, pressure induced wheat starch hydration and a complete gelatinization at 600 MPa. These two phenomena involved a reduction of the bulk suspension volume as well as an increase in granule volume.

High pressure enhanced irreversible transformations during the first pressurization cycle. A second cycle at the same pressure level gave no further modifications.

As shown with calorimetric analysis, wheat starch gelatinization was found to be graduated as a function of the pressure level. This behavior, which did not occur for a thermal treatment, could have technological applications.

Indeed, such graduated gelatinization allows preservation of starch granule integrity and so would prevent amylose solubilization, which is responsible for large variations of viscosity.

It has been shown that starch gelatinization under pressure led to specific water–starch linkages. Further works would allow verification if such a specific structure would correspond to particular functional properties, such as rheological properties and retrogradation mechanisms.

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