High-Pressure Gelatinization of Wheat Starch and Properties of Pressure-Induced Gels

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Wheat starch suspensions of 5% dry matter were treated at 86 °C, 15 min, or with pressure at 600 MPa, 25 °C, 15 min. Both treatments were found to induce no further melting peak when differential scanning calorimetry was used. Under these previous conditions, starch suspensions subjected to pressure gave original products in terms of swelling index (water binding), amylose release and specific gravity. Pressure induced starch gelatinization with preservation of the granular structure. As a consequence, peculiar properties were expected for pressure-induced gels of 30% dry matter obtained at 600 MPa, 25 °C, 15 min. By using Young modulus measurements, calorimetry, X-ray diffraction, and drying kinetics experiments, results showed a limited retrogradation for gels obtained under pressure.

INTRODUCTION

Starch granules in suspension in water melt when temperature reaches the temperature of gelatinization (T_m). Under appropriate conditions, gelation occurs on cooling with amylose generating a gel matrix containing starch granules essentially composed of amylopectin (Alloncle and Doublier, 1991; Leloup et al., 1992; Myoshi et al., 1992; Teo and Seow, 1992; Inaba et al., 1994; Kainuma, 1994). Depending on storage conditions, a semicrystalline state is formed more or less rapidly (Miles et al., 1985; Morris, 1990; Genin and René, 1995).

High-pressure treatments induce irreversible modifications of rice starch suspensions of 5% dry matter (DM) treated at 500 MPa and 45 °C for 1 h (Ezaki and Hayashi, 1992). Starch kernels were found to retain their granular form. Using high-pressure differential thermal analysis on 16% DM wheat suspensions, Muhr and Blanshard (1982a) showed that starch gelatinization under pressure (329 MPa, 62 °C) induced a reduction of the total suspension volume. Muhr et al. (1982b) also showed that cereal starches were more sensitive to pressure at room temperature than tuber starch. In situ microscopic observations of starch granules (about 0.1% DM, room temperature) during a high-pressure treatment showed that about one-third of wheat granules were swollen and iodine discolored above 300 MPa (Douzals et al., 1996). Granule swelling was observed during compression and pressure release phases. In addition, measurements of the compressibility of starch suspensions (β_t) under pressure showed a reduction of

the total volume (starch and bulk water) while gelatinization occurred under pressure. Differential scanning calorimetry results, obtained upon 16% DM wheat starch suspensions, showed that starch gelatinization began above 300 MPa and was completed at 600 MPa. Lower viscosity of pressure-treated suspensions (550 MPa, 15 min, 25 °C) was observed in comparison with a thermal treatment (Stute et al., 1996). These latter results were related to lower amylose solubilization and weaker granules swelling due to pressure.

Since pressure is known to modify biological macromolecules such as proteins (Hawley, 1971; Kunugi, 1993; Dumay et al., 1994) one expects peculiar properties of high-pressure-treated products. The aim of this paper was to point out effects of pressure on low starch content suspensions of 5% DM. Wheat starch gelatinization following pressure treatment was characterized through measurements of swelling index (grams of water absorbed per gram of DM) and amylose release. Furthermore, thermal and high-pressure treatments of 30% DM suspensions were compared using mechanical, calorimetric, and structural methods of aging evaluation.

MATERIALS AND METHODS

Starch. All samples were prepared with commercial wheat starch (PROLABO, France). Dry matter and amylose content of wheat starch powder were respectively 12% and 23% (on a DM basis). Dry matter content was verified in a dry air oven at 105 °C, 48 h, and amylose content was checked using iodine titration described afterward.

Starch Gels Preparation and Storage Conditions. Starch suspensions were prepared at different concentrations and sealed in rubber bags (medical finger bags of 15 mL, 18-mm diameter, and 60-mm length). Starch suspensions of 30% DM were either treated by heat in a bath at 86 °C (15 min) or pressurized at 600 MPa (15 min, 25 °C). Heated starch suspensions were cooled in water at 20 °C for 1 h before measurements corresponding to "fresh gels" at time 0. High-pressure treatment was realized by using a 150-mL high-

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pressure cell connected to a high-pressure circuit described elsewhere (Douzals et al., 1996). Pressure was generated with a hydropneumatic pump, (NOVA SWISS, Switzerland). Compression and release rates were about 20 MPa s⁻¹. The high-pressure circuit included fitting, $^1/_4$ in. high-pressure tubing, valves, and a pressure sensor, (NOVA SWISS, Switzerland). Pressure was measured with a precision of 7% of the total scale. Starch gels were stored at 4 or 25 °C after both thermal or pressure treatments.

Swelling Index Measurements. Gelatinization was characterized using swelling index (s.i.) measurements described by Doublier (1995) and corresponded to the quantity of water absorbed by 1 g of starch DM in 5% DM starch suspensions. Thermal and pressure treatments were realized without stirring. Different ranges of temperature or pressure were applied from 30 to 96 °C or from 0.1 to 600 MPa. After return at normal conditions of pressure and temperature (0.1 MPa and 25 °C), samples were removed from rubber bags and centrifuged at 2000 rpm, during 10 min. Pellets were weighed and then dried at 105 °C during 48 h in order to quantify DM content. Results are given with a precision of ± 0.5 g of water per gram of DM.

Iodine Titration of Gel-Soluble Fraction. In the above protocol, supernatants were titrated with iodine M/20000. With the use of a precise injection pump (0.01 mL per amount, Schott Geräte, Germany), small quantities of iodine were added to the solution and potential measurements were plotted using a calomel−platinum single electrode, with an accuracy of 1 mV. Equilibrium points were found using the first derivative method (i.e., maximum slope of the curve). Amylose equivalent contents were calculated considering that 100 mg of pure amylose and pure amylopectin can respectively bind 20 and 2 mg of pure iodine (0.5 M) (Duprat et al., 1980). Iodine titration calibration was verified using pure amylose and amylopectin in suspension with water as well as with native wheat starch suspensions.

Specific Gravity Measurements. Specific gravity was measured at 25 °C upon 30% DM treated suspensions, using a 100-mL water pycnometer containing 15-g samples. Specific gravity of native and treated products was then calculated using the following:

$$SG = \frac{M_S}{V_S} = d_W \frac{M_S}{[M_S - (M_T - M_W)]} \qquad (1)$$

where SG is the specific gravity in grams per cubic centimeter; M_S the mass of the sample; V_S, the sample volume; d_W, the specific density of pure water (found to be 0.999 at 20 °C); M_T, the total mass of a constant volume in the pycnometer including the sample; and M_w, the mass of the same volume in the pycnometer full of water. Experiments were repeated 3 times and the precision of the measurement was about ±7 mg cm⁻³.

Drying Kinetics Measurements. Starch gels of about 15 mL (25% DM) were removed from rubber bags, placed in aluminum pans (7-cm diameter) and individually dried in a dry air oven at 105 °C. Experiments were realized for different storage times (0−10 days) and temperatures (4 and 25 °C). Samples were weighed every hour during 7 h and at 24 h with a precision of ±1 mg for samples of about 15 mL (∼15 g).

Young Modulus (E). Starch gels of 30% DM were preserved in rubber bags and the elastic modulus in compression was measured using a texturometer−rheometer (RHEO, France) fitted with a 10-mm-diameter piston. The compression speed was 0.12 mm s⁻¹, and the maximum force (F) was 5 N. The elastic modulus in compression, i.e., Young modulus (E) of the gel, was determined from the slope of the linear part of the force/displacement curve using

$$\sigma = \frac{f}{S} = E\frac{dl}{l} \qquad (2)$$

where σ is the strain in Newtons per squared centimeter; f, the force applied in Newton; s, the piston area in squared
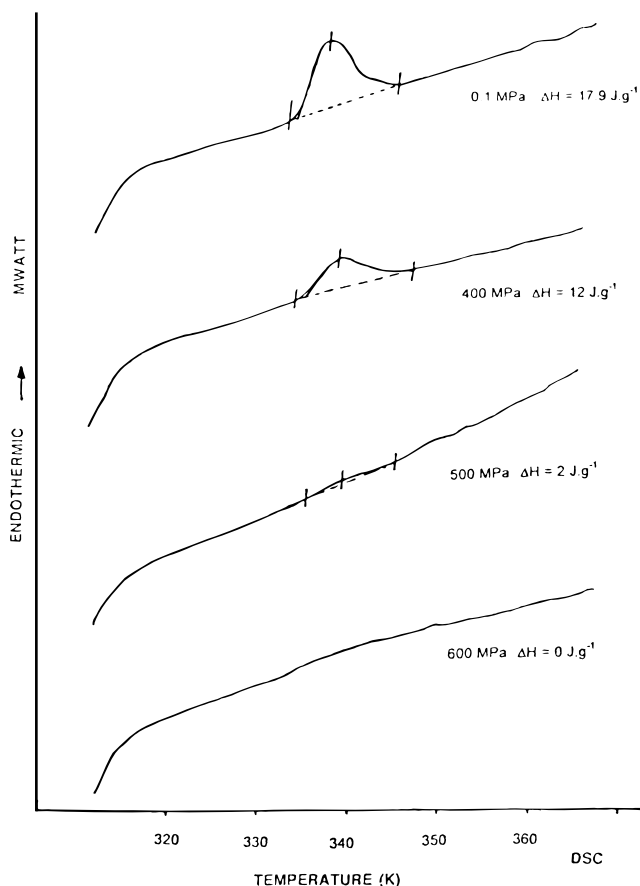

Figure 1. DSC pattern of native wheat starch suspension and wheat starch suspensions (30% DM) treated at 400−600 MPa.

centimeters; and dl/l, the relative displacement. Precision in E measurement was found to be 5%.

Gel Powder Preparation. Starch gels were removed from rubber bags and rinsed with acetone as described elsewhere (Roulet et al., 1988). The gels' initial DM was 30%. After several washings, powders of about 95% DM were obtained and stored at 20 °C.

Differential Scanning Calorimetry. Gel powders were dispersed in water to obtain samples of 30% DM before calorimetric measurements. Calorimetric analysis was conducted using a Perkin-Elmer DSC II apparatus with a heating rate of 10 °C min⁻¹ from 300 to 430 K with about 40-mg samples sealed in 7-mm-diameter pans. Two heating−cooling cycles were realized but the relevant results were observed during the first heating. Apparatus calibration was realized with indium.

X-ray Diffraction Measurements. Gels powder samples (90% DM) were analyzed with a Siemens apparatus (Germany) using Cu Kβ wavelength (1.3954 nm) via a monochromatic filter. Samples were placed in a Plexiglas plate and analyzed from 3° to 30° (2θ angle) with a step size of 0.03° and 20 s exposure. Correspondence between diffraction angle (θ) and wavelength is given by the Braggs equation:

$$2d \sin \theta = n\lambda \qquad (3)$$

where d is the reticular distance between crystal planes in nanometers; θ, the diffraction angle in degrees; n, a numeric factor; and λ, the wavelength in nanometers.

RESULTS AND DISCUSSION

Calorimetric Comparison of Gelatinization Conditions. Calorimetric results of starch suspensions previously treated under pressure (400−600 MPa, 15 min, 25 °C) are shown in Figure 1. Wheat starch

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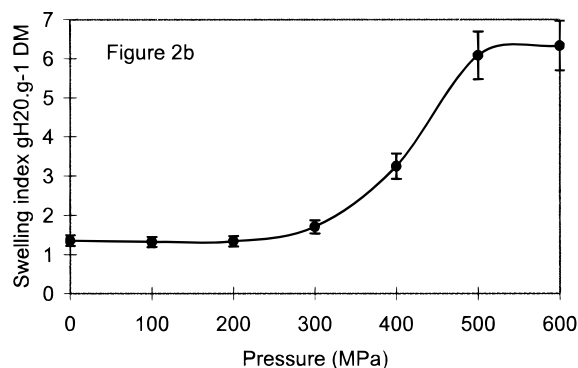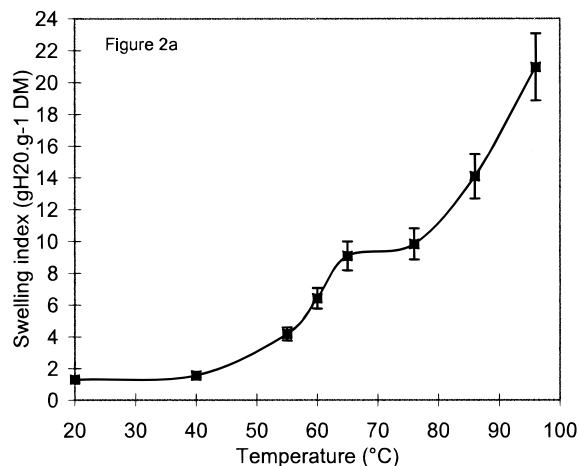
Douzals et al.

Figure 2. Swelling index vs temperature (a) or pressure (b) of 5% DM wheat starch suspensions. The swelling index was measured after treatment at normal conditions (25 °C, 0.1 MPa). Both treatments were applied for 15 min without stirring in 15-mL rubber bags. Bars indicates the confidence interval of the mean at 95%.

suspensions treated at 500 MPa were completely melted as no further melting peak appeared. When considering the DSC pattern of a sample treated at 0.1 MPa (Figure 1), the melting peak began at 60 °C (333 K) and ended at 72 °C (345 K). As a first result, high pressure induced starch gelatinization much more gradually (400−600 MPa) in comparison with a thermal treatment (10−15 °C). Indeed, under isostatic conditions, pressure was immediately applied without pressure gradient. Partial gelatinization of starch was easier under pressure than in the case of a thermal treatment.

Starch Gelatinization Related to Swelling Index. Swelling index measurements after a thermal treatment at 0.1 MPa, 15 min (20 °C to 96 °C), and high pressure treatment at 25 °C, 15 min (0.1 MPa to 600 MPa) are respectively shown in parts a and b of Figure 2. Melting temperature (T_m) of wheat starch corresponded to 65 °C as shown in Figure 1 with a corresponding swelling index of about 8 g of water per gram of insoluble DM. Maximum swelling index of 21 g of H_2O (g of DM)$^{-1}$ was obtained at 96 °C (Figure 2a). These results were in agreement with literature (Doublier, 1995). With reference to the DSC curves of at 0.1 MPa, 25 °C, shown in Figure 1, the melting peak was located between 60 and 72 °C. Moreover, gelatinization under pressure occurred between 400 and 600 MPa. The swelling indexes for these gelatinization intervals were respectively 6.42−9.40 g of water per gram of insoluble DM (60−72 °C) as shown in Figure 2a and 3.24−6.32 g of water per gram of insoluble DM (400−600 MPa) as

Table 1. Iodine Titration of Pure Amylose, Amylopectin, Native Wheat Starch Suspension, and Soluble Fraction after a High-Pressure or Heating Treatment upon Wheat Starch Suspensions of 5 % DM

sample	DM content, %	iodine, mg, for 100 mg of sample[a]	amylose[b]/ amylopectin[c] equivalent in %
amylose[d]	nd	20 ± 0.3	100[b] ± 1.5
amylopectin[d]	nd	2 ± 0.03	100[b] ± 1.5
native suspension of wheat starch	10%	5.08	25.4[b]
soluble fraction after 86 °C, 15 min	nd	1.195	5.97[b]
soluble fraction after 600 MPa, 25 °C, 15 min	nd	0.68	3.4[b]

[a] Precision in iodine volume measurements was ± 0.01 mL. [b,c] The soluble fraction is supposed to contain only amylose (*b*) or amylopectin (*c*). [d] From Duprat et al., 1980.

shown in Figure 2b. Water binding was different when starch melted by heat or pressure at the degree of melting shown by DSC. These results were in agreement with the in situ microscopic observation of wheat starch granules (Douzals et al., 1996). Granule swelling was observed under pressure during gelatinization, suggesting that water binding involved a reduction of the total volume of the suspension.

Iodine Titration of Starch Gel Soluble Fractions. The results of iodine titration of soluble fraction extracted from low starch content of 5% DM gelatinized products are shown in Table 1. Only two gelatinization conditions (86 °C and 600 MPa) were studied, assuming that these conditions allowed a complete gelatinization of wheat starch as determined by DSC. As shown in Table 1, wheat starch suspensions previously treated at 86 °C bound more iodine than in the case of a pressure treatment at 600 MPa. Assuming that amylose can bind much more iodine than amylopectin (Duprat et al., 1980; Miles et al., 1985; Alloncle and Doublier, 1991), the quantity of released amylose was found to be about 6% at 86 °C and 3.4% at 600 MPa. These first results confirmed microscopic visualization, under pressure, showing granules structure preservation during gelatinization under pressure (Douzals et al., 1996). It can be suggested that pressure involved starch granule gelatinization with a lower release of amylose probably related to a lower water binding. These two phenomena would preserve starch granules entity when gelatinization occurred under pressure.

Specific Gravity Measurements. Specific gravity of untreated suspensions and gels in rubber bags were measured, and results are shown in Figure 3 for different dry matter contents and gelatinization conditions (600 MPa, 15 min, 25 °C) and heating (86 °C, 15 min). It was found that above an initial 25% DM, both treatments led to complete gelation, as no water release (syneresis) occurred immediately after treatment. As shown in Figure 3, starch suspensions treated under pressure were more condensed than the initial suspensions meaning a reduction of the total volume of the starch suspension after treatment in comparison with the initial suspension volume. These results were in accordance with previous measurements of the macroscopic compressibility of water starch suspensions during a high-pressure treatment (Douzals et al., 1996). This previous study indicated a shift in the macroscopic compressibility of starch suspensions during a high-pressure treatment due to irreversible volume modifications induced by pressure. For starch products obtained
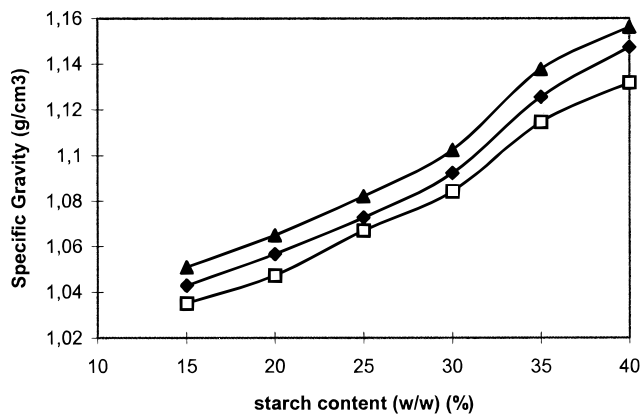
Figure 3. Specific gravity of wheat native suspensions: (◆), 86 °C, 15 min; and (□) 600 MPa, 25 °C, 15 min. Triangles (▲) represent induced gels as a function of the initial dry matter content. Precision in specific gravity measurement was ±7 mg cm⁻³.
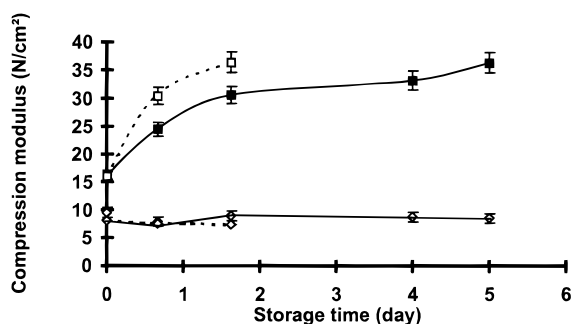

Figure 4. Young modulus of wheat starch gels (30% DM) obtained thermally (—■—, 86 °C, 15 min) or with high pressure (—◇—, 600 MPa, 25 °C, 15 min) stored at 25 °C and (···□···, 86 °C) and (···◇···, 600 MPa) stored at 4 °C vs storage time. Bars indicate the confidence interval of the mean at 95% level.

by heating, points were always situated below the initial suspension dots for the same DM content. These last results were in agreement with previous work using high pressure differential thermal analysis (Muhr and Blanshard, 1982a). These authors suggested that starch gelatinization at atmospheric pressure would involve a slight increase in the total volume of 0.2 mm³ per gram of DM with initial suspensions of 30% DM. As pressure is known to modify low-energy linkages, such as hydrogen bonds, specific gravity results could be explained by particular conformations of water−starch linkages under pressure where water molecules would occupy a lower volume than in bulk water at the same pressure level (Douzals et al., 1996). From a macroscopic point of view, pressure treatment would also restrain starch granules, radial expansion as the intermolecular space was limited in this case, as opposed to the expansion observed on heating.

In conclusion, specific gravity measurements confirmed previous results concerning swelling index and iodine titration. On the basis of an equivalent melting degree given by DSC, pressure and temperature effects on starch gelatinization were different in terms of water binding, suspension volume variations, and iodine release.

Young's Modulus. The compression modulus measurements of high starch content gels (30% DM) are shown in Figure 4 for gels obtained under pressure or heat at two storage temperatures as a function of time. Young's modulus was significantly higher for thermally
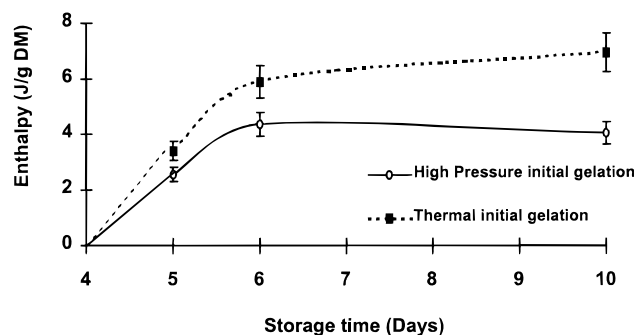
induced gel than pressure-induced gels for the same initial starch content of 30% DM. These results were in accordance with previous measurements of amylose release at lower starch contents (Table 1) and with other experimental information (Roulet et al., 1988; Alloncle et Doublier, 1991). As the quantity of amylose surrounding starch granules was lower in this case (Table 1), starch gels obtained under pressure had a weaker matrix. When considering results shown in Figures 3 and 4, pressure generated softer but denser gels than those obtained by a thermal treatment. Since specific gravity and gel strength has not yet been simultaneously studied, there is no obvious relationship between these two parameters, except for the role of amylose. Stute et al. (1996) indicated gel strength values (with equal piston area) of 1.1 and 6.9 N for wheat starch suspensions (8.6% DM content) after respective treatments at 600 MPa, 25 °C, 15 min, and at 90 °C, 15 min.

The main factors that influence starch gels elasticity are dry matter content, heating temperature, and shear stress on stirring. As gels have a behavior intermediate between that of solids and liquids, no direct relationship can be described between Young's modulus which characterizes the elasticity (deformity) of starch gels and density as expected for an ideal solid. Moreover, thermal and pressure treatments gave quite different Young's modulus for low storage temperature. Thermally induced gels were more sensitive to low storage temperatures as shown by a significant increase in Young's modulus as a function of time (Figure 4). These last results would indicate that starch gels obtained under pressure would be less sensitive to aging. However the slight evolution of Young's modulus for pressure-induced gels could possibly mean that the method used might have not been adapted. Hence, further analyses were carried out in order to characterize starch gels aging using differential scanning calorimetry.

Differential Scanning Calorimetry. Starch gel evolution was blocked using several acetone washings with a subsequent drying at 30 °C to obtain starch gels powders. These samples were rehydrated at 30% DM before DSC measurements. Results are shown in Figure 5 for heat- and pressure-induced gels stored at 4 and 25 °C as a function of storage time before acetone dehydration. This graph shows that the two treatments gave different melting peaks corresponding to recrystallized compounds. A melting enthalpy of 4 J g⁻¹ DM was found for pressure-induced gels (600 MPa, 15 min, 25 °C) and 7 J g⁻¹ DM for thermal-induced gels (86 °C, 15 min) after 10 days of storage.

Figure 5. Evolution of the melting enthalpy of retrograded starch gels previously obtained with pressure (—○—, 600 MPa, 25 °C, 15 min) or heating (···■···, 86 °C, 15 min). Bars indicate the confidence interval of the mean at 95% level.
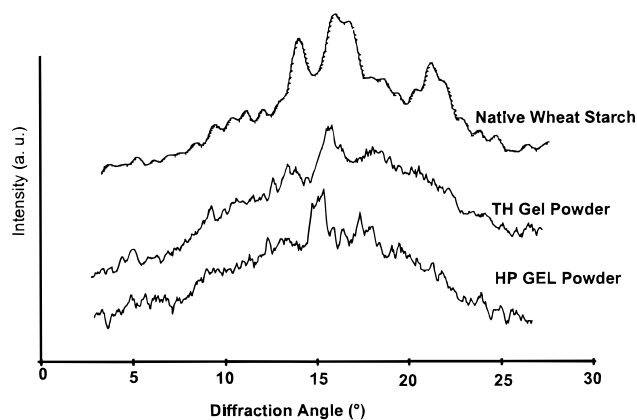
Figure 6. X-ray diffractograms of native wheat starch and dehydrated gels previously obtained under pressure (HP Gel Powder) or heat (TH Gel Powder) at 6 days storage. Ordinates correspond to X photons intensity given in arbitrary units.

Figure 7. Drying kinetics of 30% DM starch gels obtained at 600 MPa, 25 °C, 15 min (—◇—) or 86 °C, 15 min (—■—). The dotted curves correspond to gels stored during 48 h at 4 °C for high pressure (600 MPa, 25 °C, 15 min., ···◇···) and temperature (86 °C, 15 min, ···□···). Precision in DM measurements was 1%.

These results showed that starch retrogradation was possible after gelatinization under pressure in accord with literature that indicated a lower retrogradation for rice starch treated under pressure at 450 MPa, 1 h, 45 °C (Ezaki and Hayashi, 1992). Nevertheless, Figure 2a,b and Table 1 showed that starch gelatinization at 600 MPa, 25 °C, 15 min, would preserve granule integrity with a limited hydration as well as a lower release of amylose than in the case of a treatment at 86 °C, 0.1 MPa. Starch retrogradation intensity (or kinetics) could then be related to the presence of amylose inside or outside starch granules as gelatinization under pressure restrained amylose release from starch granules. Hence, further experiments were necessary to characterize the structural conformation of retrograded compounds for heat and pressure treatments. This was performed using X-ray diffraction method.

X-ray Diffractograms. As starch retrogradation was found to be possible after a pressure treatment, the structural form of recombined crystallites was studied using X-ray diffraction. Starch gel retrogradation was blocked using dehydration with acetone at different storage times. X-ray diffraction results are presented in Figure 6 for wheat starch for the native and the retrograded samples from the two initial treatments (gel reduced into powders). As shown in Figure 6, the native crystalline conformation of wheat starch (A-type) was different from retrograded forms, analogous to B-type starch, regardless of the previous melting process. B-type crystalline structure was characterized by specific diffraction peaks located at angles of 5°, 16°, and 18°, as opposed to the diffraction peaks of the native form situated at 14°, 16°, and 22°. It is commonly accepted that A-type cereal starch retrogrades into a B-type crystalline structure after a heating treatment (Miles et al., 1985; Roulet et al., 1988; Teo and Seow, 1992).

These results indicated that starch retrogradation of heat- and pressure-induced gels led to an analogous crystalline form according to Ezaki and Hayashi (1992). Their studies were on rice starch previously melt at 450 MPa, 45 °C, 1 h, so that the intensity of retrogradation was not comparable.

Drying Kinetics. Dry matter of 30% DM starch gels is shown in Figure 7 as a function of drying time at 105 °C. Previous high-pressure and thermal treatments were compared at two different storage times, 0 (fresh
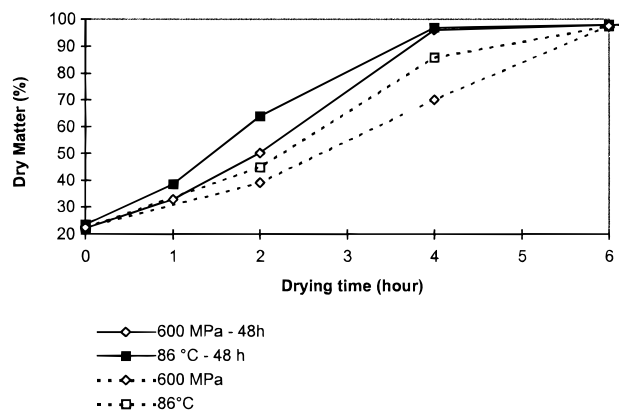
gels) and 48 h at 4 °C. As shown in Figure 7, fresh gels obtained after thermal treatment dried more rapidly than pressure-induced gels. Furthermore, after 48 h at 4 °C, this phenomenon still persisted even if both gels reached the plateau at 100% of dry matter earlier than fresh gels. Starch gels obtained under pressure also seemed less sensitive to storage conditions (time and temperature) which are known to favor starch retrogradation. These last results can be related to previous presumptions of the role of amylose and hydration during gelatinization under pressure or heat.

CONCLUSION

A comparison between thermal and pressure gelatinization process was based on the achievement of wheat starch gelatinization given by DSC. The results concerning 5% DM starch suspensions revealed peculiar properties of pressurized products in terms of swelling index and amylose release from starch granules. When considering 30% DM gels, pressure-induced gels were characterized by a weaker matrix related to the lower release of amylose from starch granules observed upon low starch content suspensions. However, specific gravity measurements showed residual effects of pressure or temperature on starch suspensions. High pressure at 600 MPa, 25 °C, 15 min, generated denser gels than a thermal treatment at 86 °C, 15 min, for the same starch content. These observations were related to previous results with microscopy, assuming that starch granules were preserved during gelatinization under pressure. Thus, combination of the limited granule expansion (water binding) and low amylose release would mostly explain the specific behavior of starch gels obtained at 600 MPa, 25 °C, 15 min. Drying kinetics and Young's modulus experiments showed a significant susceptibility to aging for gels obtained at 86 °C, 0.1 MPa, 15 min, in comparison with a pressure treatment at 600 MPa. DSC and X-ray diffraction upon starch gels indicated starch retrogradation for both treatments, but quantitatively greater in the case of heat-induced gels. Nevertheless, structural conformations of retrograded compounds were similar regardless of the initial gelatinization treatment. Further experiments will be focused on deeper investigations of the relationships between specific gravity, Young's modulus, and other functional properties of starch gels. Industrial interest

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would concern the limited expansion of melted granules under pressure as well as rheological properties of pressure-induced gels.

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