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Partially-gelatinised starches by high hydrostatic pressure as oligoelement carriers

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Abstract

Zinc deficiency is a world wide problem, centred on the lower income population, though not restricted to it. Food supplementation with magnesium is also considered of interest for correcting deficiencies in this oligoelement. High-pressure partially gelatinised starch is proposed as a vehicle for inclusion of these metals in food products. While high-pressure levels (and/or temperature) give rise to total gelatinisation, lower pressures, at temperatures close to ambient, yield partially-gelatinised products, where only a portion of the intramolecular bonds in the amylopectin double helix has been substituted by intermolecular bonds between the unfolded amylopectin and water, as it can be deduced by differential scanning calorimetry, while the resultant granular microstructure is strongly altered (observed by low temperature scanning electron microscopy). Production of this modified starch implies lower energy expense, compared to thermal gelatinisation, and it guarantees higher homogeneity in the final degree of gelatinisation. The effect of moderated high-pressure treatments (400 MPa, 25°C, 35 min) on potato and corn starches has been studied. The effect of pressure treatment, after alkaline treatment or the addition of another hydrocolloid (guar gum) has also been considered. The observed preferential binding of zinc and magnesium salts to these modified starches (after determination of metal content by atomic absorption spectrophotometry) is favourable to the use of these products as metal carriers.

Additional key words: differential scanning calorimetry, magnesium, metal binding, micronutrients, scanning electron microscopy, supplementation, zinc.

Resumen

Almidones parcialmente gelatinizados mediante altas presiones como transportadores de oligoelementos

La deficiencia de cinc es una cuestión de alcance mundial, centrada en la población de menores recursos, aunque no restringido a ella. La suplementación de alimentos con magnesio también se considera de interés para corregir las carencias de este oligoelemento. Como vehículo para la inclusión de estos metales en productos alimenticios se propone el almidón parcialmente gelatinizado por alta presión. Mientras que altos niveles de presión (y/o temperatura) causan gelatinización total, menores presiones a temperaturas cercanas a la ambiente rinden productos parcialmente gelatinizados, en los que sólo una proporción de uniones intramoleculares de la doble hélice de amilopectina han sido sustituidas por enlaces intermoleculares entre la amilopectina desenrollada y agua, según se deriva de datos de calorimetría diferencial de barrido, mientras que la microestructura del gránulo resulta muy alterada (observada por microscopía electrónica de barrido de baja temperatura). La producción de este almidón modificado conlleva menor gasto energético, en comparación con la gelatinización térmica, así como garantiza mayor homogeneidad en el grado de gelatinización final. Se ha estudiado el efecto de tratamientos moderados de alta presión (400 MPa, 25°C, 35 min) sobre almidones de patata y de maíz. También se ha considerado el efecto de la presurización después de un tratamiento alcalino o de adición de otros hidrocoloides (goma guar). La observación de la unión preferencial de sales de cinc y de magnesio a estos almidones modificados (tras determinación del contenido en metales mediante espectrofotometría de absorción atómica) es favorable al empleo de estos productos como portadores de metales.

Palabras clave adicionales: calorimetría diferencial de barrido, cinc, magnesio, micronutrientes, microscopía electrónica de barrido, suplementación, unión de metales.

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Introduction[1]

Starch, a major food component, is widely employed as an ingredient in many processed foods. Starch granules are complex structures with different proportions and spatial arrangements of two glucose polymers, linear amylose (with α1-4 bonds) and branched amylopectin (with α1-6 bonds). Energy is stored in starch as immobilised sugar units within these two polymers. Starch granules are osmotically inactive and not easily accessible to degradation. Specific plant enzyme systems can process the complex and compact granule structure as required during seed germination, fruit ripening and other physiological plant events. On the other hand, human enzymes cannot properly degrade starch. It must be previously thermally gelatinised in an irreversible physico-chemical reaction with water, at temperatures between 50 and 90°C depending on its botanical source.

Gelatinisation involves several sequential physical, chemical and mechanical processes (Hoover, 2001). The highly ordered granule structure, evidenced by the presence of a characteristic feature (Maltese cross) observed through polarizers under optical microscopy is lost during gelatinisation. Additionally, its component molecules escape from the granules, firstly the linear amylose and then the branched amylopectin. This leads to granular binding and a large increase in the suspension viscosity, due to formation of long-range interactions. This explains the frequent use of starch as a food thickening and binding agent. One of the main tools to investigate starch gelatinisation is differential scanning calorimetry (DSC). The thermograms obtained in heating scans show a wide transition curve, reflecting the many associated processes and the heterogeneous nature of starch granules. It shows a maximum in endothermic heat exchange between 50 and 90°C, but because gelatinisation is an irreversible process and, so, scan rate-dependent, the onset temperature of the process is most often reported. The associated enthalpy roughly corresponds to the number of sugar monomer-sugar monomer hydrogen bonds (within the amylopectin helix) that have been replaced by water-sugar monomer interactions. Chemical, enzymatic or physical modifications of starch structure are reflected both in gelatinisation temperature and enthalpy.

Food formulation requires the introduction of additives as colorants, flavours, aromas and also molecules of nutritional value, including minerals, vitamins, and antioxidants. These substances are often unstable, reactive and difficult to bind or to assimilate from supplemented food. Pharmaceutically active compounds can also be introduced into food in this way. The uses of starch as a food formulations stabilizer reflects the interaction of water with the polymer network formed on gelatinisation. Its binding and gelling properties can be modulated by modification of the native granules or the gelatinised free-chains. High hydrostatic pressure processing (HHPP) of food is a novel technology with an increased presence in food processing. It is most often employed to reduce food microbial load with minimal associated chemical composition change and without resorting to the addition of chemical preservatives. Other HHPP applications include modification of food enzymes, proteins and other macromolecular components. The equilibrium structure of macromolecules (including proteins and starch) is altered by high-pressure in a similar way. In both cases equilibria are displaced towards the smallest volume species (Le Chatelier), breaking secondary and tertiary structures but leaving covalent bonds intact. Covalent bonds require much higher energy than that supplied by hydrostatic pressure. When there is no pressure-transmitting fluid medium, hydrostatic conditions do not apply and local pressure can be much higher. The shear forces acting under these conditions can induce covalent bond splitting. The pressure-denatured state of proteins has been reported to be different from thermally or chemically induced ones (Smeller, 2002; Molina-García, 2003). Kinetically driven processes are favoured, in a similar way, when the intermediate state volume is smaller than that of the reactant species.

Starch treated at sufficiently high-pressure becomes gelatinised, as its hydrated state has a lower specific volume (Katopo *et al*., 2002). Pressure- and thermally-gelatinised starches are reported to be not completely equivalent (Knorr *et al*., 2006). The former process is still under study. The lower thermal agitation (a function of temperature) could be the cause of differences in molecular drifting: amylose and amylopectin movement away from their position in the granule could be reduced compared with thermally gelatinised starch, and this could be reflected in rheological behaviour of the resulting product. At pressures lower than those required for complete gelatinisation, about 600 MPa at room

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[1] Abbreviations used: AA (atomic absorption spectroscopy), cryo-SEM (low temperature scanning electron microcopy), DSC (differential scanning calorimetry), HHPP (high hydrostatic pressure processing).

temperature (Smeller, 2002; Knorr *et al*., 2006), starch becomes partially gelatinised. Many of the amylose chains protrude from the granules, so that interactions among them and with other molecules becomes possible. However, swelling, amylose solubilisation and granule dispersion are restricted in pressure (as compared to heat) gelatinisation. Starch gelatinisation under pressure also leads to altered gel retrogradation behaviour (Douzals *et al*., 1996). Additionally, some intact granule properties are maintained, and thus the digestibility of partially-pressure-denatured starch would be lower or slower than that of completely gelatinised starch. This property can be exploited to carry and deliver bound molecules further into the digestive track. HHPP acts almost instantaneously and independently from sample geometry and size, not giving rise to pressure gradients. Thus, pressure partially gelatinised starch would be more homogeneous and easier to obtain than its thermal equivalent.

Zinc and magnesium supplementation is, at present, of high interest, in the case of at risk populations. Modified starch is considered a suitable vehicle for oligoelement transport, due to its multiple uses as food ingredient, low-cost and the versatility of its interactions with foods. In this study, the preferential union of these two metals to partially pressure-gelatinised starch was investigated as a model for the vehiculisation of a wide range of food additive molecules, which may show food modification properties or have nutritional or medical interest. Starch alkaline treatment associated with pressure, to increase and modulate gelatinisation, was also considered, as well as the addition of gum guar, another hydrocolloid, that could form interaction bridges between starch and the molecules bound to it. DSC, low-temperature scanning electron microscopy (cryo-SEM) and atomic absorption spectroscopy (AA) were employed to investigate starch state and metal binding.

Material and Methods

Native, alkaline-treated or guar gum-added suspensions of potato or corn starch were treated with high-pressure (a single condition of pressure, temperature and treatment duration). Later, the treated starch was mixed with zinc or magnesium salts and washed. The DSC behaviour of the pressure-treated samples was investigated, as well as its aspect by cryo-SEM. The metal content in the final samples was determined by atomic absorption spectrometry. All treatments and determinations were performed, at least, in triplicate.

Sample preparation

Dry potato (analytical grade, Panreac, Castelar del Vallès, Spain) and corn (Roquette, Barcelona, Spain) starch, selected for comparison purposes, were obtained (but, with 12 and 15% residual water, respectively). Starch also contains a small amount of tightly bound phospholipids, less than 0.1% for potato and around 0.8% for corn starch (Buleon *et al.*, 1998; Vasanthan *et al.*, 1999; Debet and Gidley, 2006). The starches were used without further purification or defattening and suspended in deionised water at a concentration of 10% w/w. Although no stirring was available during the pressure treatments and starch granules quickly sedimented, the overlying water was considered to be in equilibrium with the gelatinisation process without the need to resort to the addition of an auxiliary hydrocolloid (as employed by, e.g., Stolt *et al.,* 2001; Kawai *et al.*, 2007). This procedure, successfully followed by many workers (e.g., Błaszczak *et al.*, 2005a; Oh *et al.*, 2008), was adopted after tests with different water contents showed that the overlying water was enough to guarantee the required water/starch ratio (data not shown). Milli-Q deionised water was used for all sample preparation (Millipore Inc Bedford, MA, US).

Guar gum (Sigma-Aldrich, San Louis, MO, USA) was solubilised in 50% of the final water volume by stirring under moderate heating (under 50°C). Then, starch previously suspended in the rest of the water, was added and mixed, stirred and left for starch hydration and equilibration for 12 hours before further treatment. Just before pressurisation, the suspension was stirred thoroughly. The gum:starch suspensions had a final concentration of 0.3% gum and 10% starch.

Alkaline-treated 10% w/w starch suspensions were prepared just before pressurisation by adding a 1% solution of NaOH (Panreac) to the starch suspensions, to a final concentration of 0.1% w/w, while the suspension was stirred thoroughly at room temperature. The alkaline solution was removed when the sample was decanted, after pressure treatment.

Pressure treatment

All samples were subjected to pressure treatment under the same conditions, i.e. 25°C initial vessel temperature, 400 MPa and 35 min treatment time. Briefly, the experimental HHPP set-up employed (previously described elsewhere: Otero e*t al.,* 2000,

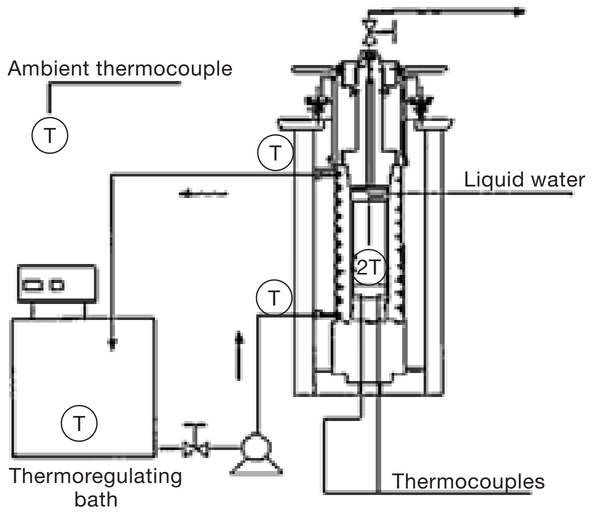

Figure 1. Diagram of the high-pressure vessel used for hydrostatic pressure treatments.

2002) was a pilot-scale unit from GEC Alsthom ACB (Nantes, France) with vessel temperature controlled by an ethylene glycol mixture circulating through a surrounding jacket (Fig. 1). Pressure was increased by pumping fluid in the vessel, at 100 MPa min^{-1}, and it was released by opening a valve to the atmosphere, which could be as quick as 100 MPa s^{-1}.

The internal vessel volume was 2.35 L. It was equipped with two flexible inner thermocouples type T and a pressure gauge (type PR811, ASCO Instruments, Chateaufort, France), which was located in the pressure, circuit, at 15 cm from the vessel. Both thermocouples and pressure gauge had been recently calibrated for use in this equipment and their data were collected by a quick data acquisition system (Fluke, Helios I, Fluke Precision Measurement Ltd, Norwich, United Kingdom) connected to a computer equipped with Labtech Notebook software (Laboratory Technology Corporation, Wilmington, MA, USA). Error in the temperature measurement systems was estimated to be below 0.5°C, while error in the pressure was under 5%. Both pressurisation and depressurisation induce changes in temperature in both sample and the pressure transmitting medium (Otero *et al.*, 2000). Therefore, the value of 25°C reported here must be considered an average over time.

Samples were treated in polypropylene flat 125 mL cylindrical containers (of 3 cm in height) immersed in water as the pressure-transmitting medium. After pressure treatment, starch was decanted and freeze-dried before further studies were carried out. Control DSC determinations showed no difference between freeze-dried and fresh samples (data not shown). Pressure treatments were performed in triplicate.

Metal addition

Metals were added as zinc sulphate and magnesium acetate (analytical grade, Panreac). Dry, treated and untreated starch samples (1 g) were suspended in 0.5 M zinc sulphate or magnesium acetate aqueous solutions (10 mL). After overnight agitation at room temperature, the suspensions were centrifuged at 5,000 g with a table centrifuge and the pellet was water-washed (resuspension plus stirring in 10 mL deionised water followed by centrifugation) and oven-dried. The metals were quantified by atomic absorption (AA) spectrophotometry, using a Model 5100 spectrophotometer, Perkin-Elmer, Norwalk, Connecticut, USA). AA analyses were performed in triplicate and are reported as averages. Cross-determinations (i.e., quantification of magnesium in samples added with zinc and vice versa) were performed, always yielding negative results.

Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) determinations were performed with a TA-1000 instrument (TA Instruments, New Castle, DE, USA), at a scanning rate of 10°C min^{-1}. To minimize hydration variability, starch samples were dispersed in water (10% w/w) 24 h before DSC analysis. Then, 15 µL of slurry were introduced in weighted aluminium pans, sealed and re-weighted. After analysis, pans were punctured and dried at 110°C to constant weight, to ensure accurate moisture and starch content determinations. Thermograms were analysed following standard procedures (Universal Analysis Program, TA Instruments). Analyses were performed in triplicate.

Scanning electron microscopy

Low-temperature scanning electron microscopy (SEM) was carried out with a Zeiss DSN-960 scanning microscope equipped with a Cryotrans CT-1500 cold plate (Oxford, UK). Cryo-SEM allows sample observation without chemical fixing or drying. The procedure consists of sample freezing by immersion in liquid nitrogen, breaking and etching. Sample holders were fitted into a special bracket on the microscope and placed in the pre-chamber cold plate (–180°C), where samples were broken to obtain a suitable observable surface. Samples were then inserted in the microscope and etching was performed for 3 min at 90°C. After etching, samples were gold coated and observed at –150/–160°C under secondary and backscattering electron modes. A large number of granules (over 1,000) corresponding to different regions of the sample on the stage were observed before representative electron micrographs were taken.

Results

Both potato and corn starch appeared destabilized after high-pressure treatment (400 MPa, 25°C and 35 min) in DSC thermograms. When compared with native potato starch (curve a), the gelatinisation curve for the HHPP-treated potato starch (curve b) was displaced towards lower temperatures (by 1-2°C) and the enthalpy of the process was partially reduced, by approximately 30% (Fig. 2). In potato samples there was an average enthalpy decrease from 20.1 ± 0.9 J g^{-1} to 16.3 ± 0.8 J g^{-1}. Thermograms for alkaline-treated (curve c) and guar gum added starch (curve d) show that, from a calorimetric point of view, these processes had no significant effect on starch gelatinisation. Similar results were obtained for corn starch (data not shown). On pressurisation gelatinisation enthalpy was reduced from 14.2 ± 1.0 J g^{-1} to 9.5 ± 1.0 J g^{-1}14 in corn starch.

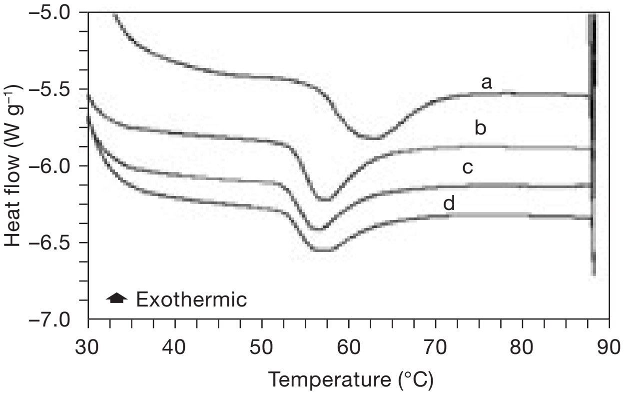

Figure 2. Typical DSC thermograms obtained with potato starch. Scanning speed was 10ºC min^{-1} and samples were 10% starch aqueous suspensions (thoroughly mixed and incubated for at least 1 hour) containing 1.5 µg of starch. a: native starch. b: high-pressure partially-gelatinised starch. c: high-pressure partially-gelatinised starch after alkaline treatment. d: high-pressure partially-gelatinised starch after addition of guar gum. Heat flow units are W/wet sample g.

Cryo-SEM observation of native and treated samples show that, in spite of minor changes to the gelatinisation thermograms caused by the pressure treatment (Fig. 2), starch granule microstructure was partially-disintegrated (Fig. 3). The granular integrity of native starch (Fig. 3A) was significantly reduced in HHPP-treated starch, showing a core «ghost» surrounded by a residual network of polymer chains (Fig. 3C and 3F). Figure 3C and 3F show chosen field areas where more semi-intact granules can be seen. Both alkaline treatment and guar gum addition resulted in the appearance of filaments interconnecting the remaining granules, probably due to amylose leaking from them and, in the case of Figure 3C, also to the gum. General microscopic observation showed that alkaline treatment and gum addition had little effect, other than the appearance of filaments, on the aspect of pressurised starch granules. The observed effects were similar in both corn and potato starch (data not shown).

Pressure-treated starch samples tended to bind metals preferentially compared to untreated starch (Fig. 4). Partially gelatinised potato starch, without alkaline treatment or gum addition only, bound significantly more magnesium. Alkaline and pressure-treated samples of both starch types bound more magnesium and zinc. Zinc binding by samples of both starch types containing guar gum was increased approximately four-fold. In all cases estimated error was less than 5%.

Discussion

Potato and corn starch samples treated at 400 MPa (at 25°C for 35 min), were studied by DSC and observed by cryo-SEM to evaluate structural changes caused by this treatment. The pressure and temperature levels used were lower than those reported giving complete starch gelatinisation (Smeller, 2002; Knorr e*t al.*, 2006; Kawai e*t al.*, 2007). In agreement with published reports, all thermograms presented a thermal gelatinisation curve, showing that in no case was gelatinisation complete. The enthalpy reduction observed reflected the fraction of amylopectin helix hydrogen bonds that were replaced by bonds with water during pressure treatment suggesting that pressure-treated starch samples retained approximately 70% of their original intramolecular bonds.

Cryo-SEM enables observation of starch with little extrinsic manipulation. The branched structure observed,

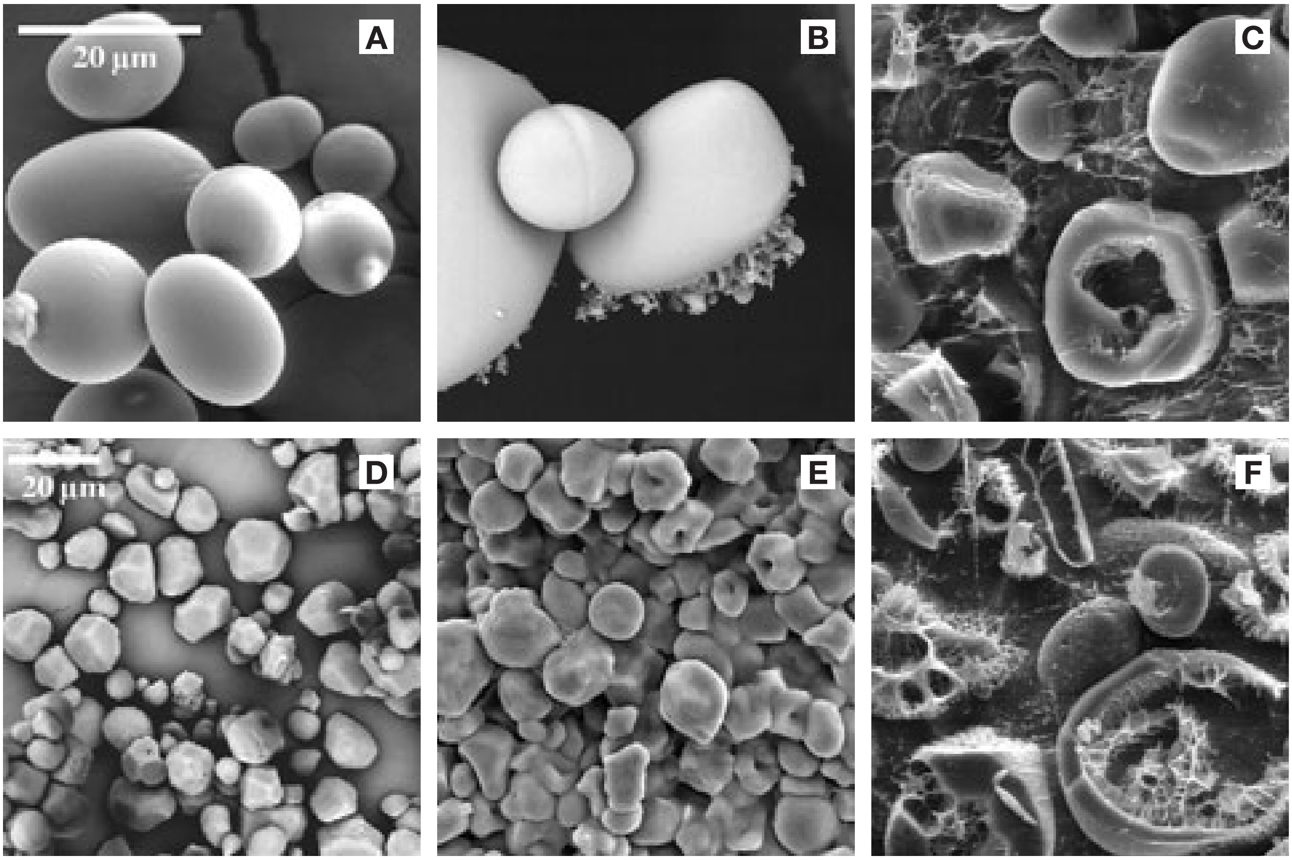

Figure 3. Cryo-SEM micrographs of starch granules after different treatments. A, B, C: potato starch granules; A: native, B: high-pressure partially-gelatinised, C: high-pressure partially-gelatinised with addition of guar gum. D, E, F: corn starch granules; D: native, E: high-pressure partially-gelatinised. F: high-pressure partially-gelatinised after alkaline treatment.

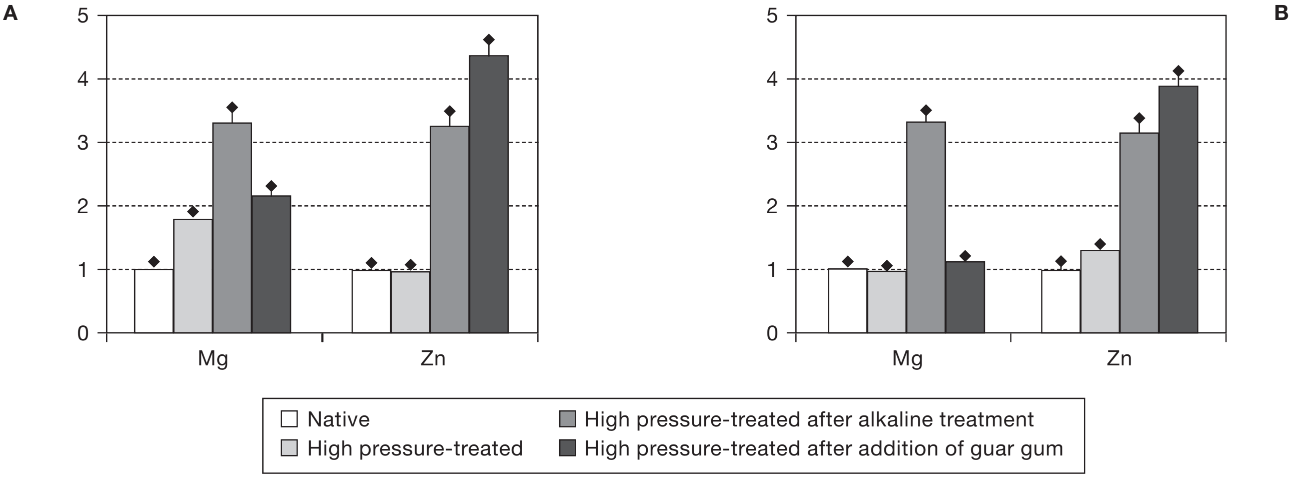

Figure 4. Metal mass bound to starch after different treatments, relative to that bound by native starch A) potato starch, B) corn starch. Bars above each column are the standard deviation.

down to the nanometric scale, is a promising substrate for food and medical applications. The numerous water hydrogen bonds could be replaced by appropriate polar molecules while the large carbohydrate chains could offer non-polar substances a harbouring place. Moreover, the gelatinisation of pressure-treated starch would be completed, as shown by the DSC thermogram, at lower temperatures than the corresponding native starch.

The degree of starch gelatinisation and the degradation observed in granules was roughly similar for both potato and corn starch. Nevertheless, the literature assigns a higher pressure resistance to potato starch (e.g., Douzals *et al.*, 1996; Katopo *et al.*, 2002; Bauer and Knorr, 2005). A possible explanation may be the higher surface resistance of potato starch granules to pressure degradation (Sevenou *et al.*, 2002; Błaszczak *et al.*, 2005b; Oh *et al.,* 2008). A higher superficial resistance may have the effect of showing intact or only slightly affected potato granules when studied by optical or room temperature-electron scanning microscopy, while internal granule degradation may have progressed to a further degree. It must also be considered that most studies have been performed on fully gelatinising pressure treatments, rather than in partial gelatinisation, as in this case. While complete starch gelatinisation is reported to take place under harsher conditions (higher pressure and/or temperature) (e.g., Knorr *et al.,* 2006), the same authors reported studies where gelatinisation behaviour of both corn and potato starch had been altered by treatments at 400 MPa (Thevelein *et al.*, 1981; Muhr *et al.,* 1982). Specifically for corn, an 18% swelling degree was found after pressurisation at 400 MPa (Oh *et al.*, 2008).

Though small, a decrease in the gelatinisation temperature of pressure-treated starch was found (Fig. 2). This behaviour is generally observed (Knorr *et al.*, 2006). A parallel with proteins suggests that the «granule stability» of pressurised samples was reduced. This could only be strictly true for equilibrium thermal processes and starch gelatinisation has a significant non-equilibrium aspect. Water access to the inner regions of starch granules may be an important initial step in the whole gelatinisation process. Pressurisation, at moderate pressure levels, could give better access of water molecules to these regions, so that subsequent thermal gelatinisation can start at lower temperatures.

Preferential metal binding, in spite of higher bound magnesium mass, for high-pressure treated potato starch, can be seen as especially high for the two other associated starch modifications: alkali treated and samples with added guar gum (Fig. 4). Alkali treated samples showed high but non-specific binding, which could be a result of actual akalinisation of the starch substrate. Metal species bound may, then, may be in a different state from ionised salts, forming hydroxides or metal oxides. This could have different consequences on the transport, binding stability and assimilation of the metals. Further research into the state of the metal atoms bound to starch is deemed necessary.

Samples with added guar gum showed a significant degree of binding of magnesium salts to potato starch but not to corn starch. The binding for zinc was higher (over 4-fold that of native starch) and was similar for both corn and potato starch. An effect based on structural factors (generation of binding sites of adequate size) or mediated by the role of gum in starch gelatinisation must be considered, as guar gum is non-ionic. From the current data, it is not possible to extract further conclusions on the differential behaviour of zinc and magnesium. Both form divalent salts have a similar ionic size. It may also be of interest to investigate the role of anions on metal binding, as they might be responsible for salt retention (e.g., Ciesielski and Tomasik, 2003).

With regard to the differences in binding between potato and corn starch, the degree of gelatinisation and microscopic aspects of the samples being similar, it is again difficult to achieve further insight into the binding process, as pressure-treated potato starch bound more magnesium salts while gum guar-added (pressure-treated) corn starch retained more zinc salts. This is not easy to explain from facts such as native ionic starch behaviour.

The granular structure, although weakened, could allow formation of chelate-type bonds in which large molecules or atoms could be attached by freely moving starch amylopectin branches. These molecules or atoms could be trapped or encapsulated in the semi-disintegrated granules, and they would be released on the digestion of the remaining starch core. The digestion of these granules would, consequently, be easier than that of native starch but slower than completely gelatinised granules. Therefore, partially pressure-gelatinised starch could be used to transport and deliver substances for the food and pharmaceutical industry including nutrients, oligoelements, vitamins, antioxidants, colorants, flavours, aromas and pharmaceuticals. The preferential binding of zinc and magnesium to high-pressure modified starch (potato and corn), alone or with a small percentage of added hydrocolloids (e.g., guar gum) and the use of alkaline treatment confirms the potential of these modified starches as metal carriers. The role of guar gum in zinc binding and the differences in metal union of the alkaline-treated samples still need to be investigated.

Thermal processes also yield partially gelatinised starch, but pressure appears to be an advantageous production procedure, as the lack of pressure gradients during treatment would give rise to more homogeneous partially gelatinised granules. Pressure is transmitted almost instantaneously and can homogeneously be increased, maintained or decreased at will, irrespective of sample size and dimensions. Additionally, production of modified starch by high hydrostatic pressure requires less energy expense than thermal gelatinisation.

A treatment duration of 35 min was chosen here as a laboratory-intended process, in order to ensure a more homogeneous gelatinization state and to minimise the influence of thermal factors in the process. It has been suggested that lengthy treatments will not be industrially viable, due to economical reasons, being the equipment the most expensive factor in the whole process. An industrial adaptation of the production of partially gelatinized starch would not require such long time, due to optimization of the process, as the minimum treatment duration required to obtain the desired gelatinization degree has still not been determined. Also, temperature could be moderately risen so that, in combination with pressure, the process duration would be substantially reduced. Another question to be taken into consideration is that, while industrial equipment currently in the market is optimized for short-time and higher-pressure treatments, an increasing number of processes propose the use of longer lower-pressure treatments. Such may be insect and other pests destruction in food and non-food material, fish parasites destruction (Molina and Sanz, 2002) and treatments involving ice I freezing (Fernández *et al.*, 2007; Urrutia *et al.,* 2007). In them, the use of equipment optimized for lower pressure, and consequently, significantly cheaper, would make economically feasible the application of longer treatment.

The «open» microstructure of these partially-gelatinised starch granules suggests many possibilities for molecular binding, while compatibility with other food components is ensured. Further studies should focus on the bioassimilation of bound metals, its stability, and the characteristics of binding and diversification of bound ligands.

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