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Annealing properties of potato starches with different degrees of phosphorylation

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Changes in the gelatinization temperature interval and gelatinization enthalpy with annealing time at 50°C were followed for a number of potato starch samples, with different degrees of phosphorylation, using differential scanning calorimetry. The gelatinization temperature increased with the length of the annealing time up to the maximum time of 1280 min and a clear relation to the degree of phosphorylation was observed. The gelatinization enthalpy changed very slowly during the initial period of annealing, but faster in the later stages of the process. The increase in enthalpy was largest for the samples with the highest degree of phosphorylation. The phosphate level remained almost unaffected during the entire process. Therefore, the effects observed are not caused by hydrolysis of the phosphate esters, but rather by their reorientation toward positions causing less interference of molecular and crystalline structure of amylopectin helices. © 1997 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

One characteristic feature of potato starch is the presence of phosphate groups linked to the glucosidic chains of amylopectin (Swinkels, 1985). Neither the biosynthetic pathway of the phosphorylation, nor the role of the phosphate grops in the plant, are yet fully known. However, it seems clear that phosphorylation is an integrated part of the starch synthesis (Nielsen *et al.*, 1994). In a recent study (Jacobsen, 1995; Nielsen, personal communication) of potato plants more or less deprived of phosphorus, it was shown that the amount of available phosphorus has an effect on the yield of starch as well as on the phosphorylation level.

The phosphate groups are covalently bound to the amylopectin molecules (Schoch & Maywald, 1956), about one third to the C-3 positions and the rest to the C-6 positions (Hizukuri et al., 1970; Tabata & Hizukuri, 1971). Physical and biochemical methods indicate that the C-3 phosphorylation level is almost constant, whereas the phosphorylation at the C-6 position differs between different potato varieties (Muhrbeck & Tellier, 1991; Bay-Smidt *et al.*, 1994). The C-6 phosphorylation can thus be used as an appropriate measure of the total degree of phosphorylation (Bay-Smidt *et al.*, 1994).

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The total amount of phosphate groups is very small, only one per 200–300 glucosidyl monomers (Posternak, 1935; Schoch *et al.*, 1956; Palasinski, 1980). Around 12% of the phosphate groups are bound to the A-chains and the remaining 88% to the B-chains (Takeda & Hizukuri, 1982). About one-third of them are bound to the inner sections of the B-chains and the other two-thirds either to the A-chains or to the outer sections of the B-chains. The phosphorylation is more pronounced in longer chains than in shorter ones and the phosphates seem to be evenly distributed over the whole chains, but not closer than nine glucosidic units from the branching points (Takeda *et al.*, 1982). Our earlier observations indicate that the degree of crystallinity is reduced by a high phosphate level (Muhrbeck *et al.*, 1991). A possible explanation is that dislocations in the amylopectin clusters are induced by the bulky phosphate groups interfering with the building up of the structure during the biosynthesis.

The phosphorylation level also affects the functional properties of the starch. The phosphate groups give weak ion exchanger properties to potato starch (Palasinski, 1980; Marsh & Waight, 1982; Oosten, 1990). For a representative comparison of different potato starches, it is thus essential that the ionic environment of the phosphate groups is the same in all samples. This can be achieved through complete ion exchange of the starch (Yamada *et al.*, 1987).

A gel or paste made from a high phosphorylated starch has a high transparency, a high viscosity and a short texture (Whistler & Daniel, 1984). The functional properties of native starch do not always fulfil the requirements for a specific application and it is, thus, often artificially modified. Modifications, such as various methods of chemical substitution or cross-linking and chemical or enzymatic degradation have been used for a long time (Koch & Röper, 1988). A number of methods of physical modification have also been investigated, e.g. high-pressure treatment (Kudla & Tomasik, 1992), radiation (Zylema *et al.*, 1985) and coating with other compounds (Eliasson *et al.*, 1981).

Thermal treatment at various temperatures and amounts of available water can be used to alter some of the functional properties of starch. One way is the so-called heat–moisture treatment at low water conditions (18–27%), temperatures above the gelatinization temperature (100°C) and for long time periods (> 16 h) (Hoover & Vasanthan, 1994). This treatment can alter the structure of the potato starch from the B-type of X-ray pattern towards the A-type pattern. The latter is normally only found in native cereal starches.

A second way to change the thermal properties of starch is by annealing, i.e. a pre-treatment in excessive amounts of water at temperatures below the gelatinization temperatures. This treatment results in perfection of the crystalline properties that narrows the gelatinization temperature interval by shifting the temperatures upwards (Knutson, 1990), without any reported additional changes to the structural properties of the potato starch. Annealing can thus be considered as a useful tool to gain a better control of the functional properties of the starch and its interactions with other components affecting the texture of various complex food systems.

The qualitative effects of the heat–moisture treatment or the annealing are readily monitored by X-ray techniques (WAXS). However, owing to an unfavourable signal-to-noise ratio, small quantitative changes are difficult to determine by this method. Most often, indirect methods are used, in particular differential scanning calorimetry (DSC). As the enthalpy change at the starch gelatinization is almost entirely related to the melting of the crystalline domains, it can be used as a good approximation of the degree of crystallinity, whereas the gelatinization temperature can be regarded as a measure of the perfection of the crystallites (Krueger *et al.*, 1987).

In this study, DSC was used to follow the changes in the crystalline domains of a number of potato starch samples during a prolonged period of annealing at 50°C, with special attention to the effects of the phosphorylation level of the starch.

MATERIALS AND METHODS

Four potato starch samples, Fecuva, Oleva, 88-AQC-43 and 89-BHZ-5, with two levels of phosphorylation and two typical sizes, but with similar amylose content (Table 1), were obtained from Danisco Biotechnology (Copenhagen, Denmark). The chemicals used for the ion exchange were all of analytical grade and distilled water was used in all preparation steps and for all experiments.

Ion exchange

Complete ion exchange of the samples was achieved by the method of Yamada *et al.* (1987). To completely exchange all counter-ions, the starches were first protonized with HCl and in a second step, the protons were replaced with potassium ions.

Size distribution

The size distribution of the starch samples after ion exchange was established, at Danisco Biotechnology, Copenhagen, Denmark, by the use of a Leica Quantimet image analysing system. A drop of starch suspension in Congo Red solution was observed under the light microscope (Reichert Polyvar 2). The damaged starch granules were stained by dye, so that they could be excluded from the analysis. The field image was transferred to an image processing system: the Quantimet 570 (version V01.02A) with the aid of a JVC 3-CCD colour camera.

Phosphorus determination

The C-6 phosphorylation was determined, at the Plant Biology Laboratory of the Royal Agricultural and Veterinary University of Denmark, according to the method previously described (Bay-Smidt *et al.*, 1994). Measurements were taken after 1280 min of annealing, as well as before the treatment.

Table 1. Granular area, amylose content, and degree of phosphorylation before and after annealing of the different starch varieties

Starch variety	Granular area (μm^2)	Amylose content (wt%)	Phosphate content (nmol G-6-P/mg starch) $t = 0$ min	Phosphate content (nmol G-6-P/mg starch) $t = 1280$ min
Fecuva	1387	30.9	12.87	12.36
Oleva	1831	30.9	13.34	12.87
88-AQC-43	1258	30.0	20.65	19.94
89-BHZ-5	1307	31.7	23.90	23.81

Annealing

Portions of starch of approximately 3 mg were weighed into coated aluminium DSC pans (TA Instruments Inc., USA) and mixed with water added by a syringe to a final moisture content around 75%, taking into account the water bound to the starch. Duplicates were made of all samples. A pilot study indicated that an annealing temperature of 50°C would be appropriate, as the gelatinization onset temperatures of the starch samples were between 55 and 58°C. After sealing, the pans were immersed in a water bath at 50°C for 2, 5, 10, 20, 40, 80, 160, 320, 840 and 1280 min. Prior to the DSC measurements, the samples were stored for 48 h at room temperature to confirm the stability of the effects of the treatment. The native reference samples were treated just like the annealed samples, except for the immersion in the water bath.

Differential scanning calorimetry

The thermal properties of the pre-treated samples were investigated on a Perkin-Elmer DSC-2C calorimeter (Perkin-Elmer Inc., USA) in the temperature range 20–100°C with a scanning rate of $10°C\,min^{-1}$. An empty pan was used as a reference. After the measurement, the pans were punctured and dried in a heating cabinet for 16 h at 105°C, cooled in a desiccator and re-weighed to determine the dry matter content. The enthalpy calculations are based on the dry matter content and given as mean values of two measurements.

RESULTS

A slightly decreased phosphorylation level was observed as a result of the annealing, but considered as negligible (Table 1). The following discussion refers to values before annealing.

The annealing caused the entire temperature range of the gelatinization to shift upward (Fig. 1). The range of the endotherm was narrowed as the shift was higher for the start (T_o) and peak (T_m) temperatures than for the completion (T_c) temperature. The peak temperature (T_m) was considered most reliable for the comparison of the effects of the phosphorylation level. Before annealing, all samples showed virtually the same gelatinization enthalpy, ΔH. The annealing process caused ΔH to increase (Fig. 2). The increase was higher for those samples with the higher phosphorylation. Even after 1280 min of annealing the gelatinization enthalpy was still increasing.

The rate of the shifts in ΔH and T_m both increased with the annealing time, being faster for T_m and more pronounced for the high phosphorylated starches. The starches with the lowest degree of phosphorylation, Fecuva and Oleva, had the lowest T_m values throughout the annealing process, but the shift in T_m over the whole interval was higher for these two starches (Fig. 1). The ΔH shift, however, was higher for the high phosphorylated starches (Fig. 3).

DISCUSSION

The observations, in the present study, support the view that part of the phosphate groups are located within the crystalline domains. Native potato starch yields a B-pattern, characteristic of most tuber starches. By X-ray (WAXS) investigations the B-pattern can be referred to a hexagonal unit cell with the cell dimensions $a = b = 18.5$ Å, c (fibre repeat) = 10.4 Å and $g = 120°$ (Wu & Sarko, 1978; Imberty & Peréz, 1988). The crystallinity of starch is strongly influenced by the distribution of water, and in the hexagonal B-type structure the water may have the function of an additional structural element, filling the cavity of the "missing" amylopectin double coil with approximately 36 molecules. Pilot

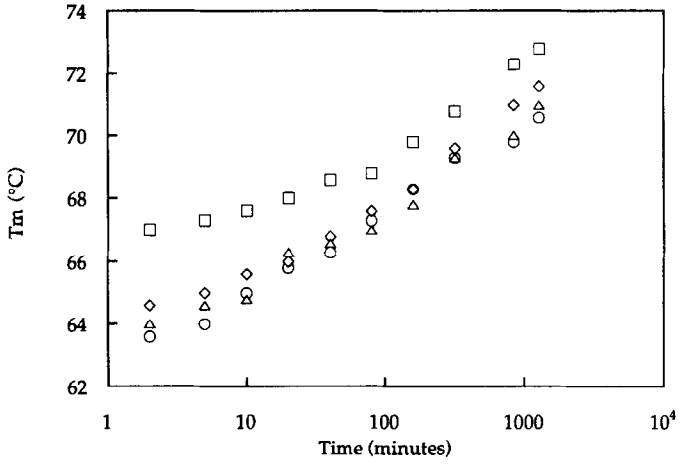

Fig. 1. Temperature of endotherm maximum (T_m) vs annealing time for (△) Fecuva; (○) Oleva; (◇) 88-AQC-43; and (□) 89-BHZ-5.

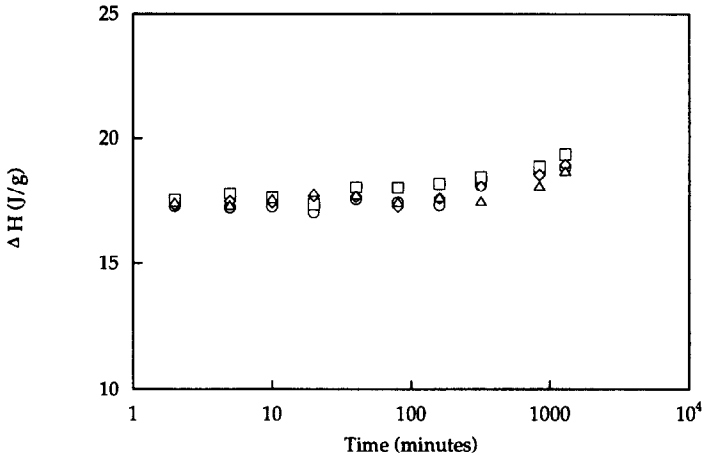

Fig. 2. Gelatinization enthalpy vs annealing time for (△) Fecuva; (○) Oleva; (◇) 88-AQC-43; and (□) 89-BHZ-5.

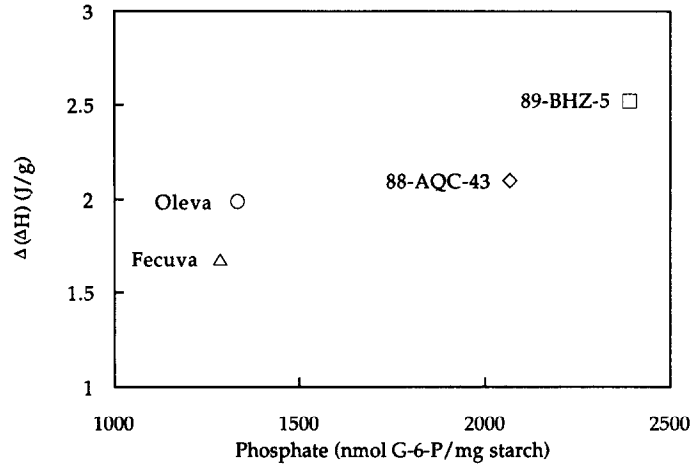

Fig. 3. Total shift in gelatinization enthalpy vs phosphate content for (△) Fecuva; (○) Oleva; (◇) 88-AQC-43; and (□) 89-BHZ-5.

studies (data not presented herein) show that the number of d-spacings of the B-pattern remains the same during the annealing period, but that a slight increase in the intensities of the patterns can be observed. The unchanged positions of the d-spacings show that the structure of the potato starch remains the same during the entire annealing process.

However, the present study shows that the phosphate groups have a retarding effect on the gelatinization properties. Generally, a shift in T_m must be considered as a qualitative phenomenon, related to rearrangements of double helices heading towards a higher perfection (Fig. 1), whereas the enthalpy rise is a quantitative effect, owing to the increase in the amount of crystalline material (Fig. 2). A combination of the temperature and enthalpy data in the present study shows that the high degree of phosphorylation has an influence, initially, on the annealing properties of potato starch, as both 88-AQC-43 and 89-BHZ-5 have a lower shift in T_m than Oleva and Fecuva, but that the influence of the phosphate on the crystallites gradually declines with the annealing time.

These observations can be explained by the rearrangement of the amylopectin double helices. The T_m shift is larger for low phosphorylated starches as the number of phosphate groups causing dislocations is smaller.

The phosphate esters of potato starch have an autohydrolytic effect on the starch (Palasinski & Schierbaum, 1971; Yamada *et al.*, 1987). As the reaction rate of this autohydrolysis might be increased by the high annealing temperature, the phosphorylation level was determined before and after the annealing process.

The degree of phosphorylation remains almost constant during the entire annealing process, indicating that the decreasing influence of the phosphate groups is not an effect of hydrolysis, but rather of their reorientation toward positions where they cause fewer dislocations in the amylopectin double helices. The rearrangement of the double helices will give some free space around the phosphate groups to be reorientated, and allow more amylopectin chains to associate, thus adding to the crystalline structure. This view is further supported by the observed delay in the increase in enthalpies and by the fact that the total shift in enthal-

pies is actually largest for the high phosphorylated starches. The larger enthalpy shift also indicates the potential to use annealing as a tool to reduce the dislocations around the phosphate groups. The present investigation thus demonstrates that annealing can be a convenient way to gain a better control the gelatinization properties of phosphorylated potato starches.

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