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PREPARATION OF WHEAT RESISTANT STARCH
Treatment of gels and DSC characterization

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A suitable thermal treatment of gels of various starch varieties was assessed to achieve the formation of resistant starch (i.e. amylose crystals). On the basis of DSC data, the yield of amylose crystals and their thermal stability did not seem correlated with the amylose content of the starch. This last parameter may not therefore be referred to as the only factor that defines a resistant starch promising starch variety.

Keywords: *calorimetry, DSC, resistant starch, RS III, starch gels*

Introduction

Starch and starch-containing products are one of the energy source in the human diet. It is well known that starch contains mainly linear (amylose) and branched (amylopectin) glucose polymers.

Various study have shown that part of the dietary starch, called resistant starch (RS), escapes enzymatic digestion in the small intestine and can undergo bacterial fermentation in the large intestine [1]. RS has been recently introduced as a functional food ingredient to human nutrition and is currently referred to as pre-biotic, i.e. a substance that can regulate intestinal micro-flora [2, 3].

According to the specific mechanism of preventing enzymatic digestion RS are classified into four types [4, 5]: (a) RS I, physically inaccessible being entrapped in a cellular matrix; (b) RS II, native granular starch, such as raw potato or banana starches; (c) RS III, retrograded or crystalline starch, which may be formed during different food processing; (d) RS IV, chemically modified starch.

RS III seems of practical interest since it preserves its nutritional characteristics during cooking processes [1, 3, 6]. To form RS III two steps are required: starch gelatinization, namely, thermal disruption of the granular structure in excess of water [7], starch retrogradation, namely, partial re-crystallization of amylose and amylopectin [8]. It has been reported that an increase of amylose content in native starches promotes formation of resistant starches [9–12] and, consequently, RS III is usually prepared from gelatinized starches with more than 40% amylose. RS III from high amylose maize, barley and pea have all been thoroughly studied; nonetheless, only high amylose maize starches are currently used in the industrial production of resistant starch [2, 6, 13].

The role of debranching enzyme on gelatinized starches coupled with drying, extrusion or crystallization by addition of salts [14, 15] have been reported in literature, while data about the formation of resistant starches with annealing treatment of gel are still scarce.

The present work aims at defining an annealing treatment that allows comparison between various wheat starches with different amylose content in order to highlight the factors that make a given starch variety more suitable to the production of RS III.

Experimental

Materials and methods

Winter wheat cultivars of Russian selection, 'Beseda', 'imeni (im) Rapoporta' and 'Bulava', cultivar was selected by means of chemical mutagenesis, using ethylene-imine as a mutagen, while of 'Moskovskaja-39's wheat was selected by traditional breeding. These wheats were grown in Central Russia (Moscow's regions) in the 2000 season.

Spring wheat cultivars of Italian selection, 'Nobel' and 'Valle d'oro', derived from different breed-

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Table 1 Amylose content (amylose/amylopectin mass% ratio) in investigated native starches

Wheat variety	Amylose content
Leona	2.1
Beseda	11.2
Moskovskaya-39	21.0
Valle d'oro	21.8
Nobel	25.0
Rapoporta	26.0
Bulava	39.6

ing programmes by public and private companies. Cultivars were selected more than twenty years ago.

Native starches were isolated according to Ritcher *et al.* [16]. The starch from 'Leona' wheat variety considered in this study was kindly supplied by Dr. P. Seib.

The amylose content in starch samples of Russian selection 'Beseda', 'imeni (im) Rapoporta', 'Bulava', 'Moskovskaya-39'; was determined using a method described previously [16]. The amylose content of starches from the Italian selection ('Nobel', Valle d'oro') was determined by using the enzymatic kit supplied by Megazyme International Ireland Ltd. (Bray Business Park, Co Wicklow, Ireland). The Megazyme method was adapted for the determination of amylose content also in the case of the amylopectin wheat 'Leona' cultivar. The relevant values of amylose content in wheat starches are shown in Table 1.

Resistant starch preparation

The preparation of RS III was based on heat-moisture treatment and subsequent cooling of the gels:

- the starch was suspended (50 mass/mass%) in a phosphate buffer (pH 6.0, 55.6 mmol L^{-1}) and heated in a wide glass tube under constant agitation to prevent sedimentation of the starch granules. When the viscosity of the suspension increased, indicating gelatinization, the agitation was stopped;
- The tubes were kept at 95°C for 30 min and then cooled to 200°C.

Four different thermal treatments of starch gels were exploited in order to assess the one suitable for all the starch varieties to achieve the highest RS III yield.

1 The gel was reheated in an oven at 121°C for 2 h and then cooled down to 4°C and kept at this temperature for 2 h; it finally was stored at 20°C overnight;
2 The gel was reheated in an oven at 121°C for 2 h and then cooled down to 4°C and kept at this temperature for 2 h; the gel was re-heated up and kept to 121°C for 2 h and then again cooled down to 4°C and finally stored at 20°C overnight;
3 The gel was reheated in an oven at 121°C, kept at this temperature for 2 h and then cooled down to 95°C and kept at this temperature for 19 h; it was finally cooled down at 20°C and stored overnight;
4 The gel was reheated in an oven at 121°C for 2 h and then cooled down to 4°C and kept at this temperature for 2 h; the gel was again re-heated up and kept at 121°C for 2 h and then cooled down to 95°C and kept at this temperature for 48 h; it finally was stored to 20°C by overnight, 3, 6 or 11 days.

Differential scanning calorimetry (DSC)

The measurements were performed with a Perkin Elmer DSC6. An indium standard was used for calibration. The samples were placed in PerkinElmer stainless steel pressure-resistant 60 μL pans which were sealed and allowed to equilibrate overnight at ambient temperature. The DSC runs were performed from 20 to 170°C at 2°C min^{-1} scanning rate. The mass ratio of solid material to water was 1:1. The raw data were worked out with the dedicated software IFESTOS which was assembled by the authors for handling raw calorimetric data according to the suggestions by Barone *et al.* [17] to obtain the trend of the excess heat capacity, $C_P^{ex}(T)$, which allowed evaluation of the enthalpy drop ΔH by a straightforward integration of the corresponding trace. Details (baseline choice, etc.) are reported elsewhere [18]. Errors were evaluated on the basis of at least two replicas.

Results and discussion

Figure 1 shows the DSC traces obtained from starch samples with 50% moisture content. The first peak (at about 65°C) is related to the starch gelatinization, while the second peak (at about 105°C) is related to the dissociation of amylose-lipid complexes. It is quite apparent that both the temperature and the area of the first signal change with the starch variety. The relevant details are out of the scopes of the present work, since they would require extra investigations to be carried out with a more sensitive instrument (e.g. a micro-calorimeter) and in the presence of excess water [19, 20]. Our study instead aims at defining the appropriate conditions to sustain the growth of amylose crystals (resistant starch) so as to attain the highest possible level in each starch variety and allow the comparison between different starches.

In view of this, the dissociation of amylose-lipid complexes (higher temperature peak in the thermogram) deserved a major attention. It is, in fact, well known that amylose can form complexes with fatty

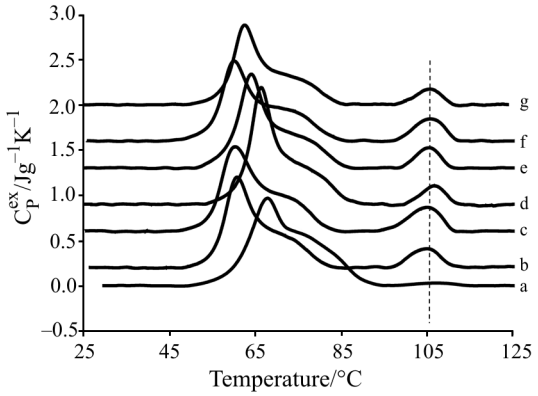

Fig. 1 DSC traces from starches of different origin at 50% moisture content. Leona variety is a waxy wheat that contains only 2% amylose (Table 1). Curves lettering: a – Leona, b – Beseda, c – Moskovskaja-39, d – Valle d'oro, e – Nobel, f – Rapoporta, g – Bulava

acids, mono- and di-glycerides and that such complexes may give rise to V-type crystal phases that undergo fusion around 100–120°C. The fusion of amylose-lipid complexes is indeed a dissociation process: the lipid molecule settled along the axis of the amylose helix slips out. Since the exterior environment is much less hydrophobic than the helix core, the lipid molecules are actually squeezed away allowing the amylose helixes to come closer to one another and therefore form nuclei of A-type crystals.

The dissociation temperature of amylose-lipid complexes could be referred to as a threshold above which the formation of amylose helixes precursors of crystals is supposed to take place.

The temperature range of this dissociation strongly depends on the moisture content of the sample [18], becomes crucial for low moisture levels. In the experiments performed in this work, the dissociation of the amylose-lipid complexes occurred at about 105°C (peak maximum) for every starch variety considered (Fig. 1). The relevant enthalpy drop was almost the same (1–2 J $K^{-1}g^{-1}$) for all the starch varieties (except for Leona starch that contains almost no amylose) and could not be correlated with the amylose content of the starch variety (such finding is not surprising since either the molecular mass of the amylose or the kind of lipids are not necessarily the same for starches of different origin).

Once defined this temperature reference threshold, a physically suitable annealing treatment could be designed and exploited. The success of the annealing treatment and/or the compliance of a given starch variety to form amylose crystals was therefore judged after inspection of the after-treatment DSC trace, where an endothermic peak with onset well above 105°C was expected first.

The complex annealing protocol was designed taking into account that the growth of a crystal phase requires a previous nucleation step (that prevails at low temperatures), which and a growth step (that prevails just below the melting point). Both steps take place within the starch gel and are hindered by the high viscosity of the medium. Expectations about the behavior of the system therefore are rather uncertain, since even the literature information does not match the peculiarities of the systems investigated in this work. Taking into account these considerations several preliminary attempts were exploited and finally four procedures (see material and methods) were designed, all of which implied dissociation of amylose-lipid complexes (rest at 121°C) as the fist step.

Figure 2 shows the DSC traces recorded after various thermal treatments 1, 2, 3; ('Experimental') of Bulava and Rapoporta starches. The comparison between these traces support the conclusion that the formation of amylose crystal phases was not fully accomplished in either starch. Treatment 1 did not allowed a sufficient growth, because it did not imply sufficiently long rest periods at high temperature (close to the expected melting point). The low temperature (namely, 4°C) has a totally different effect on the formation of amylopectin crystals ('retrograda-

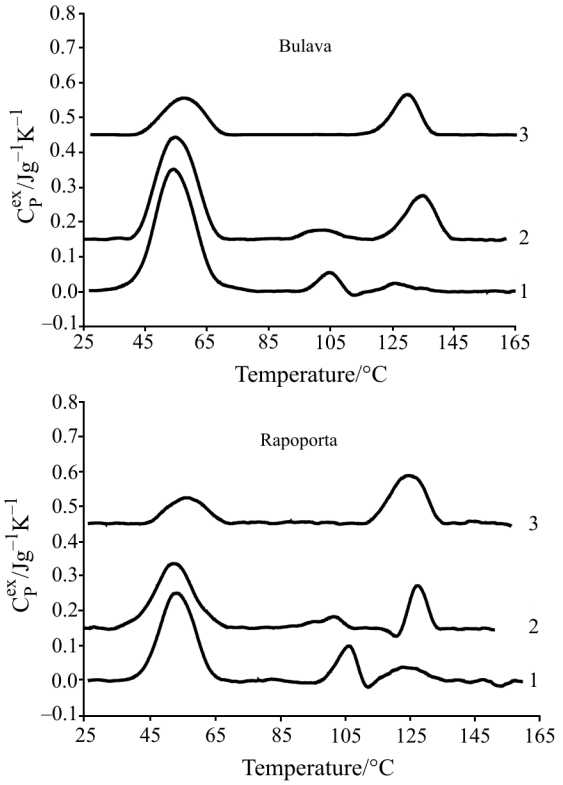

Fig. 2 DSC traces of annealed starch gels from wheat cultivars Bulava and Rapoporta according to the treatment protocols 1, 2 and 3, described in 'Experimental' (lower curves, middle curves, upper curves respectively)

tion') that undergo 'fusion' (more properly, they dissolve in the available water) at 35–40°C. Treatment 2 (Fig. 2, middle curves) implied cycling back from 4 up to 121°C with rest periods at either temperatures. This annealing protocol produced a good yield of amylose crystals in Bulava starch, but was once again unsatisfactory for the Rapoporta variety. In treatment 3 (upper curves), after a step at 121°C, where the amylose-lipid complexes were supposed to undergo dissociation, a rest period at 95°C was designed to enhance growth. This protocols produced improved yield of amylose crystals in Rapoporta starch, but brought no advantage for Bulava starch, which instead showed a smaller endothermic signal respect the treatment 2.

These findings suggested a further modification of the annealing protocol, namely treatment 4, that combined the cycling between 121 and 4°C (treatment 2) in order to enhance nucleation with a long rest at 95°C in order to enhance growth and a final storage at 20°C for various days, with the aim of achieving a steady level of the crystallization process for each starch variety. This condition was attained after a 6-day storage. The relevant DSC traces of these samples are shown in Fig. 3.

The first peak (lower temperature) corresponds to the melting of the retrograded amylopectin. The second peak corresponds to the melting of the RS III crystals. The data reported in Table 2 show the fusion enthalpy and the temperature of the maximum of this second peak related to RS III melting. Taking into account the experimental error, these data do not indicate a neat correlation between amylose content and yield of amylose crystals (fusion enthalpy). As for the melting temperature, here referred to as an index of thermal stability, Russian varieties can indeed indicate some regular trend (the higher the amylose content, the higher the melting point), but this is not the case of the Italian varieties, which seem poorly inclined to allow formation of RS III.

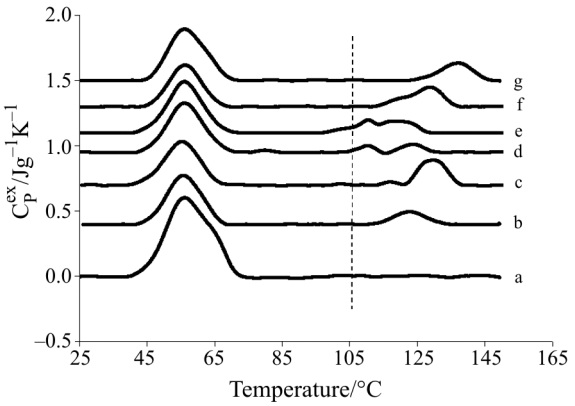

Fig. 3 DSC traces relevant to starch samples that had undergone treatment 4 and were left at rest at 20°C for 6 days ('Experimental'). Curves lettering: a – Leona, b – Beseda, c – Moskovskaja-39, d – Valle d'oro, e – Nobel, f – Rapoporta, g – Bulava

Table 2 Melting temperature (peak maximum) and enthalpy of the RS III crystals evaluated from the relevant DSC traces shown in Fig. 3

Wheat variety	T_{max}/°C±0.5	ΔH/J g^{-1}±0.2
Leona	–	0.0
Beseda	123.5	1.1
Moskovskaya-39	130.5	2.0
Valle d'oro	120.0*	1.6**
Nobel	124.5*	1.8**
Rapoporta	130.0	1.8
Bulava	138.0	1.6

*second part of the peak
**overall area of a complex signal

Other factors therefore have to be also taken into account, like, for example, the molecular mass of the amylose and the local environment (namely, the amylopectin gel) that can affect the growth rate of RS III and its extent.

To check these peculiarities of the systems investigated, some preliminary light scattering data were collected to roughly estimate the distribution of the amylose molecular mass. Large differences were indeed observed which still deserve further investigations (to be presented in a next paper).

Conclusions

A suitable thermal treatment allowed to achieve the formation of RS III from various starch varieties and compare their behaviour. On the basis of DSC data, the yield of RS III and its thermal stability (fusion temperature range) did not seem correlated with the amylose content of the starch.

This parameter may not therefore be referred to as the only factor that defines a RS III promising starch variety.

Other factors may be at work, such as the distribution of the amylose molecular mass, the local environment, like the amylopectin gel, pH and ionic strength, etc. To shed light on these aspects further researches are in progress.

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