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The Influence of the Annealing of Corn Starch on the Formation and Characteristics of Enzyme-resistant Starch

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Abstract

The Physical properties of corn starch were investigated by scanning electron microscopy, X-ray diffractometry and differential scanning calorimetry during the formation of enzyme-resistant starch (RS). Samples were studied in their native states and after annealing at 50, 55, 60 and 65°C in excess water (starch : water=1 : 3) for 48 hr. Starch granules became smaller and more rounded after annealing than in their native state. Annealing did not change the X-ray profile of native corn starch. After autoclaving-cooling cycles, native starch lost most of its crystallinity but annealed ones showed some of their crystallinity left as diffuse or poor B-type, which didn't relate to increasing RS yields. During formation of RS, however, both native and annealed starches changed their X-ray profile from A-type to poor B-type of retrograded amylose. Annealing caused an increase in gelatinization temperature and enthalpy, but a narrowing of gelatinization temperature range. Only starch annealed at 65°C, however, showed a decrease in enthalpy even though its gelatinization temperature increased, which appeared to be due to the partial gelatinization in the amorphous region during annealing. Peak height index (PHI), the ratio of ΔH to Tp-To, increased by annealing. PHI values, therefore, showed the possibility as an indicator to predict RS yield which cannot be differentiated by differential scanning calorimetry and X-ray diffraction data.

Key words: enzyme-resistant starch, annealing, corn starch, physical properties, yield, PHI

INTRODUCTION

The interest in enzyme-resistant starch (RS) results from its inclusion in the insoluble dietary fiber when applying the AOAC enzymatic-gravimetric method (1). *In vivo*, starch polymers are hydrolyzed by excess levels of pancreatic α-amylase. A portion of starch in starch-based foodstuffs, however, escapes digestion in the small intestine of man according to degree of gelatinization, granule size, amylose/amylopectin ratio, starch-protein interactions, amylose-lipid complexes, and percentage of retrograded starch (2).

Eerlingen and Delcour (3) classified RS into four different types. Type I RS represents physically inaccessible starches in partly milled grains and seeds and in legumes, which is locked in the plant cell. Type II RS is native granular starch with a B-type crystalline structure found in raw potatoes, bananas and high amylose starch. Type III RS is an indigestible starch fraction which is formed after certain heat-moisture treatments of the starch and may be present in products such as cooled, cooked starch foods (retrograded amylopectin and/or retrograded amylose). Finally, Type IV RS may be formed by chemically or thermally modifying the starch. Formation of glycosidic bonds other than α-(1,4) or α-(1,6) by heat treatment (e.g. caramelization and Maillard reactions) reduces the availability for amylolytic enzymes. Also, cross-linking or the presence of some substituents (e.g. hydroxy propyl) may reduce the digestibility of the starch. Physical and chemical characterizations have been carried out to explain the formation of RS (4-11). Accordingly, these investigations indicate that mainly retrograded and recrystallized amylose is involved in the formation of Type III RS. High levels of RS can be produced during the autoclaving and cooling of high-amylose starch (2,12).

Annealing, defined as the heating of starch in excess water at subgelatinization temperatures, was first shown by the birefringence studies of Gough and Pybus (13). They found that the physical properties of wheat starch are markedly altered by treatment for 72 hr in excess water at 50°C. Although up to 35% of the granules were damaged and lost their birefringence by this process, there was a significant increase in the gelatinization temperature and marked sharpening of the gelatinization range. Their findings were confirmed later by differential scanning calorimetry experiments (14-19). In addition to increased gelatinization temperatures and decreased gelatinization temperature ranges, increased gelatinization enthalpies were observed (16-18). However, Yost and Hoseney (15) and Stute (19) noted no change of the gelatinization enthalpy after annealing. Possible explanations for the phenomena include crystallite growth or perfection (15), the development of crystallinity in the amorphous regions of the granule (16,17), interactions between amylose and amylopectin (18), or the alterations of the coupling forces between crystallite and the amorphous matrix (19). By annealing, crystal perfection might be increased, giving rise to less amorphous material and altering the gelatinization characteristics.

Since high-amylose corn starches with higher transition

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temperatures and enthalpies than normal one showed the higher yield of enzyme-resistant starch in a previous study (20) of the formation of RS, physical treatment like annealing of normal starch may affect the formation of RS. The objective of this study, therefore, was to investigate whether this crystal perfection by annealing may affect the formation and physical properties of enzyme-resistant starch from normal corn starch by microscopic, x-ray crystallographic, and thermodynamic studies.

MATERIALS AND METHODS

Materials

Commercially available corn starch (Samseung Co., Korea), which was composed of 8.78% moisture, 0.28% protein, 0.18% ether extract extractable fat, 0.17% ash, and 25.9% amylose, were used to form RS. For the isolation of RS, heat-stable α-amylase (A-3306), protease (P-5380) and amyloglucosidase (A-9913), from Sigma Chemical Co., St. Louis, MO, were used.

Chemical analyses

The proximate composition of starch samples was determined according to the AACC method (21). The quantification of amylose was done from the blue value at 680 nm according to Gilbert and Spragg (22). All analyses were done at least in triplicate; average results of all analyses are given on a dry matter basis.

Annealing of starch

Starches were annealed by standing at various subgelatinization temperatures (50, 55, 60, 65°C) in excess water (starch : water=1 : 3) for 48 hours in the presence of 0.2% Na_2SO_3, respectively.

Formation and isolation of RS

RS was formed in an aqueous system (starch : water=1 : 3.5), after four autoclaving (121°C, 1 hr) and cooling (4°C, 22 hr) cycles, from starch samples according to the method adapted from Sievert and Pomeranz (2). A product containing RS was freeze-dried and ground in a mortar to pass a sieve with 500 μm diameter openings. The technique of RS isolation was based on the AOAC method for dietary fiber determination (1) as described by Eerlingen et al. (23). The isolated RS residues were freeze-dried and yields calculated as a percentage of the starch used for enzyme hydrolysis. The dried material was ground in a mortar for further use to pass a sieve with 500 μm diameter openings.

Scanning electron microscopy (SEM)

Morphological changes of starch samples during the formation of RS were observed with a Hitachi X-650 (Japan) SEM operated at 15 kV. Micrographs were analyzed to measure the size of starch granules using the Image-Pro Plus program (Media Cybernetics, USA).

X-ray diffractometry

X-ray powder diffraction analysis was performed with a MXP18 diffractometer (MAC Science, Japan). Operating conditions were as follows: target: Cu-K, scanning speed: 0.5°/min, voltage: 40 kV, current: 100 mA. Diffractograms of the samples were obtained from 2θ 5 to 40°.

Differential scanning calorimetry (DSC)

Measurements were performed at least in triplicate with a DSC-60 (Shimadzu, Japan). About 3 mg starch sample was accurately weighed into a hermetic aluminum pan and two times the sample weight of water was added and then allowed to equilibrate for an hour at ambient temperature. An empty pan served as the reference. The DSC was heated from 30 to 200°C at a heating rate of 10°C/min. For each endotherm, the onset (To), peak (Tp), completion (Tc) transition temperatures and enthalpy (ΔH) were determined.

RESULTS AND DISCUSSION

Morphological characteristics by SEM

Native corn starch granules were polygonal and/or spherical in shape, and annealed ones were more rounded in general; a concavity can be clearly observed at the center of a starch granule annealed at 65°C (Fig. 1). This seemed to be caused by rapid drying of the starch granules which had been previously swollen by water absorption and heating during the annealing treatment. The sizes of granules became smaller from an average diameter of 13.9 to 11.8 μm after the annealing treatment regardless of annealing temperatures (Table 1). It may be due to leaching some of the soluble components out during the treatments.

After autoclaving-cooling cycles, the granular structure of corn starch disappeared and a continuous spongy-like porous network with irregularly shaped swollen particles was visible. After the autoclaving-cooling cycles, untreated native starches were more fully gelatinized than annealed ones and there was not much difference found among samples with different annealing temperatures (Fig. 2). In all RS residues isolated after the enzyme digestion, the porous structures were no longer visible and similar compact and dense formations predominated regardless of annealing temperatures (Fig. 3). Similar morphological changes were observed during the RS formations from different amylose contents by Sievert and Pomeranz (2) and Yoon and Lee (20).

Crystalline characteristics by X-ray diffractometry

Both native and annealed starches showed A-type X-ray patterns as indicated by four strong peaks between 2θ 15.0 and 23.5° typical in a cereal starch and showed no changes in their degree of crystallinity except for one annealed at 65°C (Fig. 4). The degree of crystallinity in an annealed starch at 65°C decreased a little because of the partial gelatinization of the starch granule in the amorphous region. Annealing results in alterations within the crystallites of the granules, but since the starch granule is a complex system consisting of amorphus and crystalline regions, various alterations are possible. The number or the shape of the crystals can increase and the direction of the crystal growth can also change.

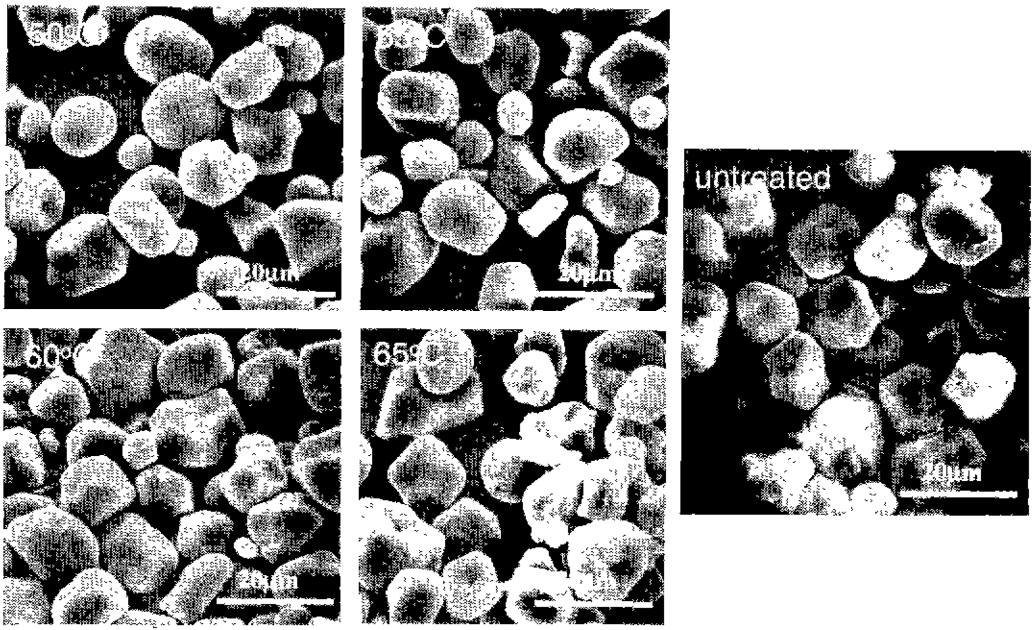

Fig. 1. Scanning electron micrographs of untreated and annealed corn starches at 50, 55, 60, and 65°C.

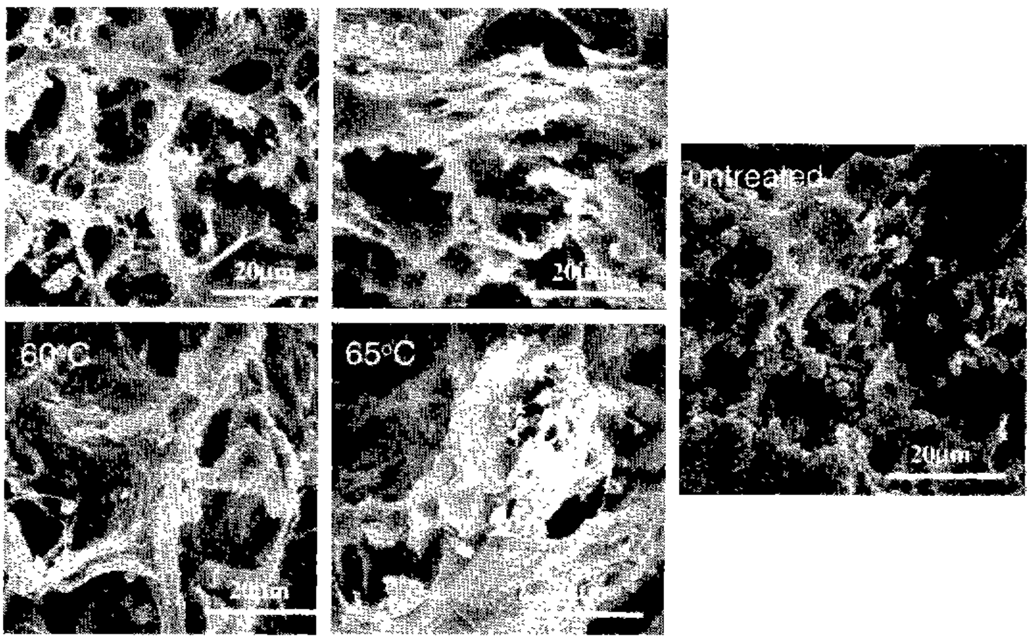

Fig. 2 Scanning electron micrographs of untreated and annealed corn starches at 50, 55, 60, and 65°C after 4 autoclaving-cooling cycles.

Table 1. Sizes and shapes of untreated and annealed corn starches

Treatment	Size range (μm)	Size average (μm)	Shapes
Untreated	8.0~21.2	13.9	polygonal, spherical
Annealed	4.0~19.5	11.8	
at 50°C	5.5~19.0	11.8	
55°C	4.0~19.5	11.8	more rounded
60°C	5.0~19.0	11 8	
65°C	5.0~18.5	11.9	

However, alterations caused by the annealing treatment does not necessarily correlate with an increase in crystallinity, as it expected, e.g. from other polymers or from alloys (19).

After 4 autoclaving-cooling cycles, native starch lost most of its crystallinity but annealed ones showed some of their crystallinity remained as diffuse or poor B-type (Fig. 4). The unique small peak at 2θ 5.6°, a strong reflections at 2θ 17.2° and a doublet around 22.0~24.0° are typical diffraction lines for a B-type structure. A poorly resolved doublet peak around

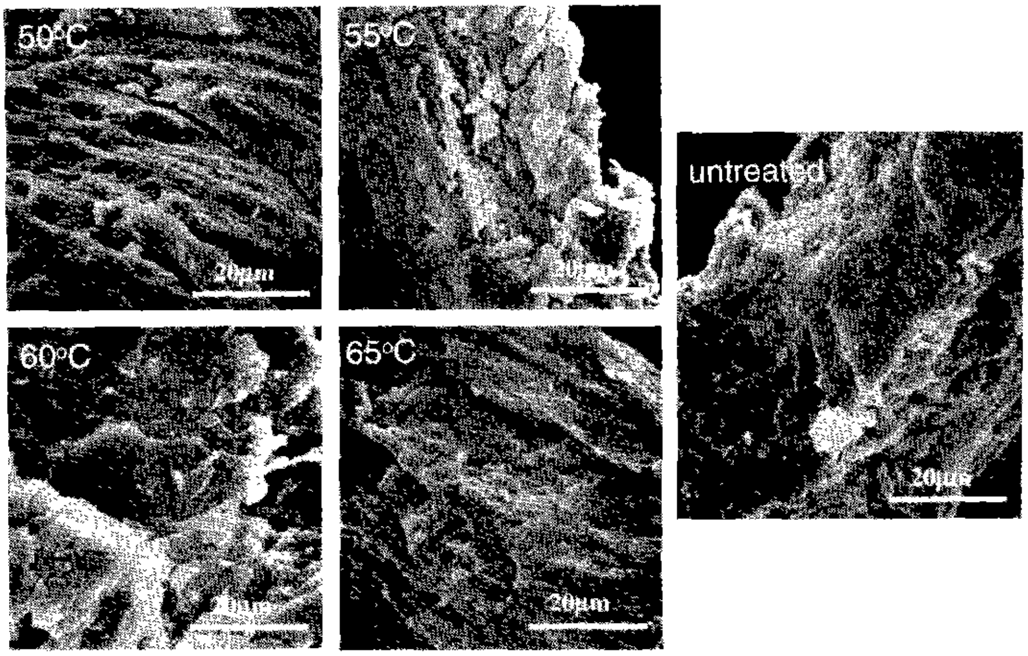

Fig. 3. Scanning electron micrographs of untreated and annealed corn starches at 50, 55, 60, and 65°C after enzyme digestion.

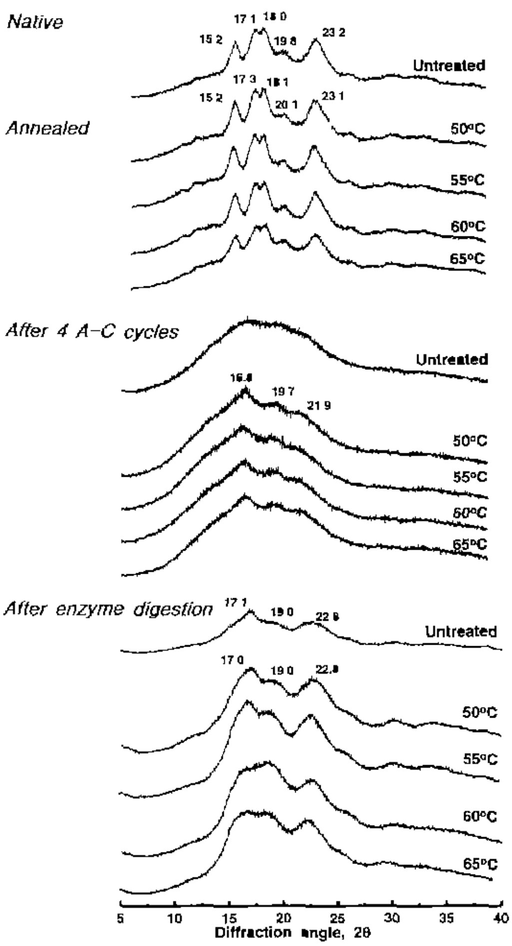

Fig. 4. X-ray diffractograms of corn starches in their native state and after annealing at 50, 55, 60 and 65°C, and untreated and annealed ones after 4 autoclaving-cooling cycles and enzyme digestion.

22.0~24.0° was emerged into a broad single peak around 22.8° after repeating the heating and cooling process. After the enzyme digestion, the crystalline fraction is concentrated, so a more pronounced B-type pattern was obtained for the isolated RS. Similar poor B-types, which are generally found for RS and retrograded starch (6,23), were obtained in all RS residues (Fig. 4). A structural transformation of native corn starch might be due to the increasing recrystallization of amylose chain double helices. During the retrogradation process of amylose solution with a concentration higher than 1.5%, phase separation occurs in a first step resulting in a continuous network of the polymer-rich phase; in a second step, double helices are formed in the polymer-rich phase and these aggregate to form the three-dimensional crystalline structue of the B-type (2). The broad diffraction lines after enzyme digestion strongly suggested that smaller and/or less perfect crystallites were present in RS than in native starch, whereas the sharp, well resolved pattern (Fig. 4) reflected a higher degree of crystallite perfection than in RS.

Thermodynamic characteristics by DSC

Annealing caused an increase in gelatinization temperatures (To, Tp) up to 10°C and enthalpy, but a decrease in gelatinization temperature range (Table 2 and Fig. 5). Only starch annealed at 65°C, however, showed a decrease in enthalpy even though its gelatinization temperature increased. This indicates that the sample has been partially gelatinized during annealing for 48 hr at 65°C which is too close to the gelatinization temperature of native starch (67°C). Regardless of annealing treatment, all RS residues exhibited endothermic transitions over a similar temperature range (136~169°C), with a mean peak temperature of ~156°C, which is generally found for retrograded amylose crystallites (Table 3). The RS

Table 2. The thermodynamic characteristics of untreated and annealed corn starches

Treatment	Transition temperatures (°C)			Enthalpy (J/g)
	To	Tp	Tc	⊿H
Untreated	67.15±0.32[1)]	71.15±0.58	80.94±0.20	11.22±0.04
Annealed at 50°C	72.99±0.09	75.66±0.23	83.09±0.50	11.56±0.73
55°C	74.19±0.09	76.90±0.09	84.16±0.06	12.06±0.51
60°C	76.10±0.02	78.64±0.11	86.41±1.04	12.11±0.69
65°C	79.79±0.14	81.66±0.13	88.13±0.50	8.20±0.15

[1)]Values are expressed as mean±standard error of at least three determinations.
To, Tp and Tc mean onset, peak and completion transition temperatures, respectively.
⊿H means transition enthalpy.

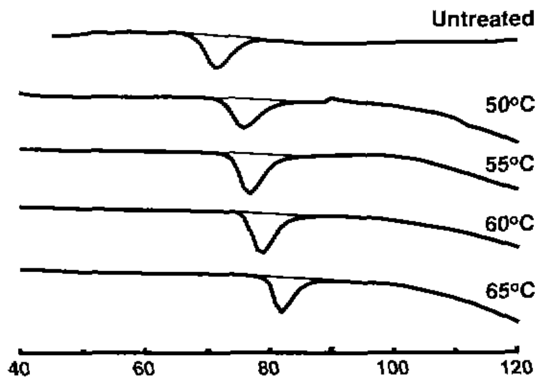

Fig. 5. DSC thermogram of untreated and annealed corn starches at 50, 55, 60, and 65°C.

Table 3. The thermodynamic characteristics of enzyme-resistant starches from untreated and annealed corn starches

Treatment	Transition temperatures (°C)			Enthalpy (J/g)
	To	Tp	Tc	⊿H
Untreated	143.82	156.23	168.70	27.42
Annealed at 50°C	145.83	159.71	165.43	14.74
55°C	144.22	160.91	168.24	14.22
60°C	146.23	149.14	165.12	15.23
65°C	136.42	156.51	163.89	16.73

To, Tp and Tc mean onset, peak and completion transition temperatures, respectively.
⊿H means transition enthalpy.

type Ⅲ is thermally very stable. When isolated RS is heated in the presence of water, an endotherm is revealed in the 120~165°C temperature range with a peak transition around 155°C and with enthalpy values varying from 8 to 30 J/g (2,3,12). In addition to increased gelatinization temperatures and decreased gelatinization temperatures ranges, a small increase of gelatinization enthalpies were noted except in starch annealed at 65°C (Table 2). Similar thermodynamic characteristics were observed by Krueger et al. (16,17) and Knutson (18).

Even though annealing caused a more crystalline configuration in the granule according to X-ray diffraction (Fig. 4) and DSC (Table 2) data, the changes did not seem to relate to the increase of RS yield (Table 4). Annealing appeared to permit partial melting of some crystallites and a general realignment of starch chains to a smaller and more regular order in the amorphous region. Therefore, annealing caused a shift of the endotherm to a higher temperature but a narrowing of the range. However, these structural changes were not sufficiently extensive enough to increase the crystallization of amylose resistant to amylolytic enzymes.

Table 4. The relationship between PHI values and enzyme-resistant starch yield from untreated and annealed corn starches

Treatment	Transition temperatures (°C)			Enthalpy (J/g)	PHI	RS yield (%)
	To	Tp	Tp-To	⊿H		
Untreated	67.15	71.15	4.00	11.22	2.81	14.5
Annealed at 50°C	72.99	75.66	2.67	11.56	4.33	11.7
55°C	74.19	76.90	2.71	12.06	4.45	11.2
60°C	76.10	78.64	2.54	12.11	4.77	10.7
65°C	79.79	81.66	1.87	8.20	4.39	10.4

PHI: Peak height index ⊿H/(Tp-To)

Since heat-stable α-amylase, protease and amyloglucosidase were used to isolate the RS as explained in the previous study (20), RS residues in this study can be considered as crystallization of amylose (Type Ⅲ RS) in a partially crystalline polymer system, which was confirmed by the B-type crystalline structure (Fig. 4) and single endotherm around 156°C (Table 3).

Peak height index (PHI), the ratio of ⊿H to Tp-To, provides a numerical value that is descriptive of the relative shape of the endotherm, e.g., a tall narrow endotherm has a higher PHI than does a short broad one, even if the energy involved in the transformation is the same (16). PHI, which was inversely related to RS yield, increased by annealing (Table 4). PHI values, especially, showed a good possibility as an indicator to predict RS yield which cannot be differentiated only by DSC and X-ray diffraction data, as well as a strong negative correlation with RS yield ($r=-0.932$, $p=0.118$, $n=3$) in the previous study (20).

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REFERENCES

1. AOAC : *Official methods of analysis*. 15th ed., Association of Official Analytical Chemists, Arlington, VA. (1990)
2. Sievert, D. and Pomeranz, Y. : Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chem.*, **66**, 342 (1989)
3. Eerlingen, R. C. and Delcour, J. A. : Formation, analysis, structure and properties of Type III enzyme resistant starch. *J. Cereal Sci.*, **22**, 129 (1995)
4. Englyst, H. N. and Cummings, J. H. : Digestion of the polysaccharides of some cereal foods in the human small intestine. *Am. J. Clin. Nutr.*, **42**, 778 (1985)
5. Berry, C. S. : Resistant strach. Formation and measurement of starch that survives exhaustive digestion with amylolytic enzyme during the determination of dietary fibre *J. Cereal Sci.*, **4**, 301 (1986)
6. Berry, C. S., I'anson, K., Miles, M. J., Morris. V. J. and Russel, P. L. : Physical and chemical characterization of resistant starch from wheat. *J. Cereal Sci.*, **8**, 203 (1988)
7. Engylst, H. N. and MacFarlane, G. F. : Breakdown of resistant and readily digestible starch by human gut bacteria. *J. Sci. Food Agric.*, **37**, 699 (1986)
8. Ring, S. G., Gee, J. M., Whittam, M., Orford, P. D. and Johnson, I. T. : Resistant starch : Its chemical form in foodstuffs and effect on digestibility *in vitro*. *Food Chem.*, **28**, 97 (1988)
9. Sievert, D. and Pomeranz, Y. : Enzyme-resistant starch. II. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chem.*, **67**, 217 (1990)
10. Russell, P. L., Berry, C. S and Greenwell, P. : Characterisation of resistant starch from wheat and maize. *J. Cereal. Sci.*, **9**, 1 (1989)
11. Gruchala, L. and Pomeranz, Y. : Enzyme-resistant starch : Studies using differential scanning calorimetry. *Cereal Chem.*, **70**, 163 (1993)
12. Szczodrak, J. and Pomeranz, Y. : Starch and enzyme-resistant starch from high-amylose barely. *Cereal Chem.*, **68**, 589 (1991)
13. Gough, B. M. and Pybus, J. N. : Effect on the gelatinization temperatures of starch granules of prolonged treatment with water at 50°C. *Starch*, **23**, 210 (1971)
14. Donovan, J. W., Lorenz, K. and Kulp, K. : Differential scanning calorimetry of heat-moisture treated wheat and potato starches. *Cereal Chem.*, **60**, 381 (1983)
15. Yost, D. A and Hoseney, R. C. : Annealing and glass transition of starch. *Starch*, **38**, 289 (1986)
16. Krueger, B. R., Knutson, C. A., Inglett, G. E. and Walker, C. E. : A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *J. Food Sci.*, **52**, 715 (1987)
17. Krueger, B. R., Walker, C. E., Knutson, C. A. and Inglett, G. E. : Differential scanning calorimetry of raw and annealed starch isolated from normal and mutant maize genotypes *Cereal Chem.*, **64**, 187 (1987)
18. Knutson, C. A. *Annealing of maize starches at elevated temperatures*. *Cereal Chem.*, **67**, 376 (1990)
19. Stute, R. : Hydrothermal modification of starches: the difference between annealing and heat/moisture-treatment. *Starch*, **44**, 205 (1992)
20. Yoon, J-Y. and Lee, Y-E. : Influence of amylose content on Formation and characteristics of enzyme-resistant starch. *J. Food Sci. Nutr.*, **3**, 303 (1998)
21. AACC : *Approved Methods of the American Association of Cereal Chemists*. 9th ed , American Association of Cereal Chemists, St. Paul, MN. (1995)
22. Gilbert, G. A. and Spragg, S. P. : Iodometric determination of amylose. In "*Methods in carbohydrate chemistry*" Whistler, R. L , Smith. R. J., BeMiller, J. N. and Wolfrom, M. L. (eds.), Academic Press, New York, Vol. 4, p.168 (1964)
23. Eerlingen, R. C., Crombez, M. and Delcour, J. A. : Enzyme-resistant starch I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation *Cereal Chem.*, **70**, 339 (1993)

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