Resistant Starch Formation Following Autoclaving of Buckwheat (*Fagopyrum esculentum* Moench) Groats. An In Vitro Study

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Buckwheat groats (cv. Darja) were autoclaved at 120 °C for 1 h and cooled to room temperature (the temperature decreased to 25 °C in 150 min). A portion was treated further with two additional autoclaving/cooling cycles. A higher number of autoclaving/cooling cycles did not affect the proportion of slowly digested starch (SDS) or resistant starch (RS) fraction found in treated buckwheat but gave a significant ($P < 0.001$) rise in the retrograded starch (RS_3) amount. In native groat starch, 24.6% of apparent amylose and 28.3% of true amylose was determined. In samples treated with three autoclaving/cooling cycles there was significantly ($P < 0.001$) more apparent and true amylose in the starch in comparison to the native sample or to samples autoclaved once. This may be due to gradual cleavage of some glycosidic bonds of starch, resulting in shorter linear chains, which could be then more predisposed to forming both amylose-like helices and more complex aggregates.

Keywords: *Fagopyrum esculentum; autoclaving; amylose; starch digestibility; retrograded starch*

INTRODUCTION

Some early investigations on the physicochemical properties of buckwheat starch were carried out by Kim et al. (1977). The values for amylose were 25% in isolated starch (i.e., 28.4% of amylose in dry matter of starch). Further, amylose in buckwheat samples was studied by Soral-Smietana et al. (1984), and a relatively high amylose content (42−52% of starch) was reported in their samples.

Inherent characteristics of starch, industrial processing and storage conditions are important for the digestibility and the nutritional value of starch based products. At present, special emphasis is placed on resistant starch, i.e., a fraction of starch that escapes enzymatic hydrolysis in the small intestine. It exists as physically inaccessible starch (RS_1), native granular starch (RS_2), and retrograded starch (RS_3) (Englyst et al., 1992). In total RS, chemically and thermally modified starch may be included as well (Asp and Björck, 1992).

The botanical structure and physical form of the food are of great importance to the rate and extent of starch digestion. It is evident that processing conditions, such as the amount of water and different cooking techniques, influence the resistant starch formation (Siljeström et al., 1986; Björck et al., 1987; Sievert and Pomeranz, 1989; Xue et al., 1996). During storage, development of the retrograded amylose in starches may also result in limited accessibility to amylases (Berry, 1986). Several components may interact with starch or have an inhibitory effect on the starch degrading enzymes and thus reduce the total starch digestibility (Thompson and Gabon, 1987; Thompson et al., 1987). The amount of starch consumed, the viscosity of the whole meal, the extent of chewing, and the transit time through the colon may have some influence on the bioavailability of ingested starchy foods as well (Tovar, 1992). The undigested starch may exhibit nutritionally advantageous effects similar to those of dietary fiber.

Buckwheat groats are dehusked seeds of plant *Fagopyrum esculentum* Moench, which is not cereal but has grains with cereallike starchy endosperm. Traditionally, the dehusking of buckwheat seeds is performed after the hydrothermal treatment of seeds in conditions resembling an autoclaving process. In the food preparation process—from groats to ready-to-eat food—additional cooking and cooling cycles can be applied, since the groats are first cooked, cooled, and mixed with other ingredients and then baked or cooked again.

The purpose of our study was largely threefold. The first was to estimate to what extent changes in starch and starch components can occur under hydrothermal treatment. The second was to suggest an explanation for the differences in in vitro bioavailability of starch found after different treatments of buckwheat groats by studying the relationships between the observed parameters (i.e., amylose content, cooling/heating cycles, storage time, retrogradation, rate of hydrolysis). Finally, the intention of the present work was also to reevaluate the nutritional value of buckwheat with special emphasis on the rate of glucose release from buckwheat starch, a factor which can be positively utilized by diabetic patients.

MATERIALS AND METHODS

Materials. *Samples.* The buckwheat groats (cv. Darja), which were exceptionally dehusked in the raw state without earlier hydrothermal treatment (prepared by the dehusking machine), were purchased at a local mill in Slovenia and prepared for analysis. The groat grains with a shape resembling a triangular pyramid (with height 3.5 mm and an average weight of 1000 dehusked seeds of 17.4 g), as starting material, were composed of 73.5% starch (dmb), 15.7% proteins (dmb), and 3.2% fats (dmb). Only whole, undamaged groat grains were used.

Enzymes. For determination of starch digestibility fractions, pancreatin from porcine stomach mucosa (Sigma), amyloglucosidase (AMG) from *Aspergillus niger* (300 AGU/mL (AGU =

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Table 1. Percentage of Total Starch, Rapidly Digested Starch, and Slowly Digested Starch (dmb) in Raw and Treated Groat Samples[a]

		TS		RDS		SDS	
no. of days	raw: 73.5 ± 0.51 treated: 1 cycle		raw: 73.5 ± 0.51 treated: 3 cycles	7.3 ± 0.51 1 cycle	7.3 ± 0.51 3 cycles	32.8 ± 1.96 1 cycle	32.8 ± 1.96 3 cycles
0		72.2 ± 1.48	72.2 ± 1.65	63.3 ± 1.34	58.0 ± 1.88	1.4 ± 1.26	1.5 ± 0.71
5		74.0 ± 1.32	72.3 ± 2.60	59.4 ± 1.66	57.3 ± 1.73	3.5 ± 1.31	3.7 ± 1.83
10		74.1 ± 1.28	72.9 ± 2.20	58.1 ± 1.09	56.1 ± 0.93	4.6 ± 1.41	3.0 ± 0.93
20		77.5 ± 1.22	74.0 ± 1.40	61.8 ± 1.76	56.6 ± 1.48	2.8 ± 2.84	3.6 ± 1.38
Significance[b]							
between days		ns	ns	ns	ns	ns	ns
between 1 and 3 cycles		ns		*c*		ns	
interaction days × cycles		ns		ns		ns	

[a] Mean values and standard error of measurements. [b] ns = nonsignificant. [c] Significant at $P < 0.01$.

Table 2. Percentage of Resistant Starch, Retrograded Starch, and Apparent and True Amylose (dmb) in Raw and Treated Groat Samples[a]

	RS		RS_3		apparent amylose		true amylose	
	raw: 33.5 ± 1.92 treated: 1 cycle	raw: 33.5 ± 1.92 3 cycle	1.0 ± 0.06 1 cycle	1.0 ± 0.06 3 cycle	18.1 ± 0.19 1 cycle	18.1 ± 0.19 3 cycle	20.8 ± 1.00 1 cycle	20.8 ± 1.00 3 cycle
0	7.5 ± 1.87	12.7 ± 2.48	4.7 ± 0.19	7.0 ± 0.15	17.8 ± 0.44	20.2 ± 0.02	21.0 ± 1.51	24.8 ± 0.26
5	11.2 ± 2.21	11.3 ± 2.50	4.5 ± 0.21	6.7 ± 0.14	17.0 ± 0.26	19.7 ± 0.51	21.2 ± 0.62	23.4 ± 0.23
10	11.6 ± 1.59	13.8 ± 2.43	4.4 ± 0.10	6.6 ± 0.16	16.6 ± 0.44	19.3 ± 0.06	20.6 ± 0.27	22.9 ± 0.16
20	12.9 ± 2.36	13.7 ± 1.62	4.5 ± 0.08	6.6 ± 0.09	16.7 ± 0.38	18.5 ± 0.60	21.0 ± 0.77	23.8 ± 0.53
Significance[b]								
between days	ns	ns	ns	ns	ns	*c*	ns	ns
between 1 and 3 cycles	ns		*e*		*e*		*d*	
interaction days × cycles	ns		ns		ns		ns	

[a] Mean values and standard error of measurements. [b] ns = nonsignificant. [c] Significant at $P < 0.05$. [d] Significant at $P < 0.01$. [e] Significant at $P < 0.001$.

anhydroglucose units), Novo Nordisk), and Promozyme from *Bacillus acidopullulyticus* (200 PUN/g, Novo Nordisk) were used. Enzyme solutions were prepared following the procedures of Englyst et al. (1992) and Eerlingen et al. (1994).

Reagents. An enzymatic kit D-Glucose Test-Combination (Boehringer, Mannheim, Germany) for determination of released glucose in starch fractions was used. The reagents for apparent and true amylose analyses were prepared according to Morrison and Laignelet (1983).

All reagents used in this work were of analytic grade.

Methods. *Sample Preparation.* Raw grains of buckwheat groats (20 g per sample) were mixed with water (groats:water = 1:3.4 (w/w)). Samples were sealed in baby food jars (internal diameter, 55 mm; height, 66 mm), autoclaved at 120 °C for 1 h, cooled to room temperature (the temperatures were 98, 56, 28, and 25 °C after 30, 60, 120, and 150 min, respectively) and separately further treated. Sample 1 was immediately lyophilized; samples 2−4 were put into a refrigerator (+4 °C) for 5, 10, and 20 days storage, respectively, and then lyophilized. The other four samples were exposed to two additional autoclaving/cooling cycles. Sample 5 was then immediately freeze-dried; samples 6−8 were lyophilized after removal from the refrigerator after 5, 10, and 20 days, respectively. The obtained freeze-dried materials were milled to pass a 0.3 mm screen and utilized in the in vitro study. Two preparations for each sample were carried out independently.

Measurement of TS, RDS, SDS, and RS. Total starch (TS), rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS) contents in samples of buckwheat groats were determined according to the procedure of Englyst et al. (1992). The criteria for this classification is the amount of glucose released after incubation with enzymes, i.e., 20 min for RDS and 120 min for SDS. Results for total starch are obtained by treatment of samples using 7 M KOH (the final concentration of KOH in buffer solution is 2 M). RS content is calculated from the part of glucose that remained bound in the starch after 120 min of enzyme incubation. Incubations were performed in quadruples for each of two repeated sets of samples, and glucose was determined after collecting the particular starch fraction.

Measurement of RS_3. The retrograded starch (RS_3) was determined according to the method of Englyst et al. (1992). Each milled sample was suspended in the acetate buffer and boiled for 1 h. After cooling the suspension to 40 °C, the mixture of pancreatin and pullulanase (Promozyme) was added and incubation was carried out at the same temperature overnight. After precipitation with absolute ethanol, the steps of centrifugation, discarding the supernatant, and rinsing the residue were repeated with 85% ethanol, absolute ethanol, and acetone, respectively. The residue was treated with 4 M KOH, neutralized with glacial acetic acid, and hydrolyzed by amyloglucosidase at 60 °C. After cooling to room temperature 4 M KOH was added, followed by centrifugation and glucose measurement. The amount of glucose was measured using the above-mentioned D-glucose kit. Triplicate RS_3 determinations were performed.

Determination of Apparent and True Amylose. A colorimetric procedure (in triplicate) for the separate determination of apparent and true amylose in autoclaved buckwheat groats followed the method of Morrison and Laignelet (1983).

Statistical Analysis. The data were statistically analyzed by multifactorial analysis of variance to determine significant differences among samples using Statgraphics 5.0.

RESULTS AND DISCUSSION

The results for TS, RDS, SDS, RS, and RS_3 are presented in Tables 1 and 2. Buckwheat groats contain 73.5% starch in the dry matter of the raw material. The levels of TS do not change statistically significantly during the treatments applied in these studies. A relatively high proportion, that is about half of the total starch of native buckwheat groats, is not degradable by amylolytic enzymes (Table 2).

Thermal processing followed by storage resulted in the improved availability of buckwheat starch (Table 1). In processed samples the amount of RDS is noticeably higher than in the native one, although it does not increase further with the repeating of heat treatment. In samples autoclaved once we found significantly more RDS ($P < 0.01$) than in samples which were autoclaved and cooled three times. The number of autoclaving/cooling cycles did not affect the proportion of SDS or RS fraction in treated buckwheat; however, it did lead to significant differences ($P < 0.001$) in the RS_3 amount (Tables 1 and 2).

With the colorimetric procedure we found 18.1% (dmb) of apparent and 20.8% (dmb) of true amylose in cv. Darja. Compared to the native buckwheat groats, in treated samples the difference between true and apparent amylose slightly rose with the number of treatment cycles. A possible explanation is the formation of amylose−lipid complexes, but in this case relatively constant true (i.e., total) amylose levels would also be expected. In our case, samples which were autoclaved and cooled three times had significantly higher values for both apparent and true amylose, in comparison with samples autoclaved once. There is a similar pattern for RS_3. Also, there is a high positive correlation between the content of RS_3 and the apparent amylose in studied samples ($r = 0.94$; $P < 0.001$). Some studies dealing with the structural changes of macromolecules during starch processing suggest that a depolymerization may occur during milling or some thermal treatments, drastic processes being more effective (Colonna et al., 1992). According to Davidson et al. (1984), in extruded wheat samples branch points of amylopectin are the most susceptible to rupture; however, the degradation products are also macromolecules. In buckwheat groats samples the autoclaving process presumably results in gradual cleavage of some glycosidic bonds; the resulting products may then be more predisposed both to forming amylose-like helices and to forming more complex aggregates, i.e., retrograded amylose and/or amylose−lipid complexes.

We analyzed only RS_3 as we had expected it could be the main fraction of total RS in autoclaved, lyophylized, and milled samples of buckwheat groats. In fact, from the difference between values of RS and RS_3 it follows that some amount of RS is in a form other than RS_3. As reported by Englyst et al. (1992), in pearl barley, boiled for 1 h, in the sum of RS_1, RS_2, and RS_3 (8.6% of dry matter) 6.9% was present as RS_1 and 1.7% as RS_3. In buckwheat groats the content of RS_3 obtained by the described method was higher than those for boiled pearl barley. After three repeated heating and cooling cycles RS_3 raised even up to 7% (dmb), which is comparable with the values obtained for the boiled, stored, and reheated black bean (Velasco et al., 1997).

The storage temperature of gelatinized starch is important in the process of its retrogradation. Several studies (Berry, 1986; Eerlingen et al., 1993) were carried out to estimate the amount of RS formed at different temperatures. In this experiment +4 °C was chosen as a value close to the temperature at which products from buckwheat groats or flour are usually kept to extend their shelf life. The differences between samples stored for a different period of time were not significant for the observed parameters. According to Eerlingen et al. (1993), storage of autoclaved wheat starch at low temperature (0 °C) resulted in a low yield of RS. Although the low temperature may accelerate the nucleation rate, high viscosity limits the propagation step in resistant starch formation (Eerlingen et al., 1993). In our case, the retrogradation of starch may also be affected by other components (mainly lipids and proteins) coexisting in buckwheat groats. Considering the storage time of the samples, no significant differences in true amylose were found, but there was a slight decrease of apparent amylose (statistically significant only within three cycles of treatment) during storage (Table 2). Presumably, a part of amylose has formed complexes with lipids, which can retard the retrogradation process (Gudmundsson, 1994). Thus, no effects of storage time on starch availability in buckwheat groats can be observed.

Compared to some commonly used starch sources and products, hydrothermally treated buckwheat is grouped among those products, having the major part of starch degradable within 20 min as RDS. Two types of procedures were studied; repeated hydrothermal treatment appears to affect the course of in vitro starch digestion more than storage at +4 °C for different periods studied. From the nutritional point of view, groats prepared by a single treatment do not have the advantageous features of slowly digestible starch; but three cycles of heating and cooling retard the course of buckwheat starch hydrolysis to some extent.

ABBREVIATIONS USED

TS, total starch; RDS, rapidly digested starch; SDS, slowly digested starch; RS, resistant starch; RS_3, retrograded starch.

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LITERATURE CITED

Asp, N. G.; Björck, I. Resistant starch. *Trends Food Sci. Technol.* **1992**, *3*, 111−114.

Berry, C. S. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *J. Cereal Sci.* **1986**, *4*, 301−314.

Björck, I.; Nyman, M.; Pedersen, B.; Siljeström, M.; Asp, N. G.; Eggum, B. O. Formation of enzyme resistant starch during autoclaving of wheat starch: Studies *in vitro* and *in vivo*. *J. Cereal Sci.* **1987**, *6*, 159−172.

Colonna, P.; Leloup, V.; Buléon, A. Limiting factors of starch hydrolysis. *Eur. J. Clin. Nutr.* **1992**, *46* (Suppl. 2), S17−S32.

Davidson, V. J.; Paton, D.; Diosady, L. L.; Larocque, G. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. *J. Food Sci.* **1984**, *49*, 453−458.

Eerlingen, R.; Crombez, M.; 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.* **1993**, *70*, 339−344.

Eerlingen, R.; Haesendonck, van, I. P.; Paepe, de G.; Delcour, J. A. Enzyme-resistant starch. III. The quality of straight-dough bread containing varying levels of enzyme resistant starch. *Cereal Chem.* **1994**, *71*, 165−170.

Englyst, H. N.; Kingman, S. M.; Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **1992**, *46* (Suppl. 2), S33−S50.

Gudmundsson, M. Retrogradation of starch and the role of its components. *Thermochim. Acta* **1994**, *246*, 329−341.

Kim, S. K.; Hahn, T. R.; Kwon, T. W.; D′Appolonia, B. L. Physicochemical properties of buckwheat starch. *Korean J. Food Sci. Technol.* **1977**, *9*, 138−143.

Morrison, W. R.; Laignelet, B. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci.* **1983**, *1*, 9−20.

Sievert, D.; Pomeranz, Y. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chem.* **1989**, *66*, 342−347.

Siljeström, M.; Westerlund, E.; Björck, I.; Holm, J.; Asp, N.-G.; Theander, O. The effects of various thermal processes on dietary fibre and starch content of whole grain wheat and white flour. *J. Cereal Sci.* **1986**, *4*, 315−323.

Soral-Smietana, M.; Fornal, L.; Fornal, J. Characteristics of buckwheat grain starch and the effect of hydrothermal processing upon its chemical composition, properties and structure. *Starch* **1984**, *36*, 153−158.

Thompson, L. U.; Gabon, J. E. Effect of lectins on salivary and pancreatic amylase activities and the rate of starch digestion. *J. Food Sci.* **1987**, *52*, 1050−1053, 1058.

Thompson, L. U.; Button, C. L.; Jenkins, D. J. A. Phytic acid and calcium affect the in vitro rate of navy bean starch digestion and blood glucose response in humans. *Am. J. Clin. Nutr.* **1987**, *46*, 467−473.

Tovar, J. Bioavailability of starch in processed legumes. Doctoral thesis, University of Lund, Lund, Sweden, 1992.

Velasco, Z. I.; Rascon, A.; Tovar, J. Enzymic availability of starch in cooked black beans (*Phaseolus vulgaris* L) and cowpeas (*Vigna* sp.). *J. Agric. Food Chem.* **1997**, *45*, 1548−1551.

Xue, Q.; Newman, R. K.; Newman, C. W. Effects of heat treatment of barley starches on in vitro digestibility and glucose responses in rats. *Cereal Chem.* **1996**, *73*, 588−592.

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