Effects of Cooking on Starch and β-Glucan of Rolled Oats

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ABSTRACT

In this study of the effect of cooking on rolled oats, oatmeal was prepared according to manufacturers' recommendations by cooking rolled oats gradually from room temperature, or rapidly by addition to boiling water. The cooked mixture was then centrifuged, and three layers were separated and analyzed chemically and histochemically. Distinct differences were observed between the two cooking methods. The gradually cooked oatmeal had more solubilized (1→3)(1→4)-β-D-glucan and starch and had a more viscous supernatant. The solids of the gradually cooked sample were more hydrated and the middle starchy layer occupied a larger volume. Microscopic examination revealed differences in the appearance of both the cell walls and the starch of the two differently cooked samples. In general, the disruption of the endospermic cell walls of the gradually cooked sample was greater than for the rapidly cooked sample. The loss of starch granule integrity was greater in the gradually cooked sample, and the middle starchy layer showed a more open structure. Presumably all of these factors contribute to the well-recognized creamier texture of gradually cooked oatmeal. The possible relationship of these observations to the rate of digestion was examined in a model system using salivary α-amylase. Uncooked oatmeal was more slowly digested by salivary α-amylase than cooked oatmeal, but small differences observed between the gradual- and rapid-cooked samples are probably not physiologically significant.

One of the most popular hot breakfast cereals in North America and the United Kingdom is oatmeal porridge prepared from rolled oats. Because whole grain is used to make rolled oats, this cereal is a good source of protein, polyunsaturated fatty acids, minerals, and vitamins (Weaver et al. 1981, Lockhart and Hurt 1986). Rolled oats are also rich in complex carbohydrates (with starch as the major component), and the bran fraction obtained from the outer part of the groat (dehulled oats) is a good source of a soluble dietary fiber that significantly reduces serum cholesterol in hypercholesterolemic subjects (Anderson et al. 1984). The major active component is believed to be a (1→3)(1→4)-β-D-glucan (hereafter referred to as β-glucan), which, in histochemical studies, has been shown to be located in the endosperm and aleurone cell walls of the grain (Wood et al. 1983). This polysaccharide dissolves in water to give highly viscous solutions (Wood 1986) and is the major ingredient of oat gum.

Traditional oatmeal porridge is prepared by cooking rolled oats in water to give a thick and viscous product. However, the exact texture of the porridge varies depending on the preparation method. A creamy and smooth oatmeal results from cooking gradually, starting in water at room temperature, whereas a grainy and less sticky product comes from adding the rolled oats to boiling water. Although the gelatinization of starch clearly plays a dominant role in producing the thick nature of oatmeal porridge, it was hypothesized that solubilization of the β-glucan might also have a significant effect, particularly in relation to differences observed in the two cooking methods. Despite the identification of β-glucan as a soluble dietary fiber, little is known about its solubilization on cooking. Indeed, to our knowledge no work has been published on any of these processes during cooking of rolled oats.

The present study describes effects of two manufacturer-recommended cooking methods on the structural organization, viscosity development, and solubilization of starch and β-glucan in rolled oats. Because viscosity clearly differed between the two cooking methods and because viscosity of a food may influence the blood glucose response (Jenkins et al. 1982), the rate of maltose released from oatmeal digested by human salivary enzymes was also studied.

MATERIALS AND METHODS

Cooking of Rolled Oats

All cooking experiments were carried out at least in duplicate. Three different batches of "Quick" (3-min) rolled oats were purchased from the local supermarkets and mixed thoroughly before sampling. For the rapid-boiling method, which simulated preparation procedures recommended by the manufacturer, rolled oats (15 g) were added rapidly to 120 ml of boiling water in a beaker and stirred. When boiling resumed (about 1–2 min), the mixture was simmered for various times up to 15 min. For the gradual-cooking method, rolled oats of the same amount were added to 120 ml of water in a beaker at room temperature. The mixture was stirred until it was brought to the boil (about 6 min) and then simmered as for the rapid-cooking method. Evaporation losses were minimized by covering the beakers with watch glasses. Uncooked samples (15 g) were obtained by stirring rolled oats in 120 ml of water at room temperature. Immediately after cooking or soaking, samples were diluted with cold water (100 ml) and centrifuged at 33,000 × g for 90 min. Three distinct layers were separated and each was recovered and weighed. Duplicate aliquots (1 ml) of the uppermost supernatant layer were taken for starch and β-glucan analyses.

Solubilization of β-Glucan and Starch

To determine whether the amounts of solubilized β-glucan and starch varied according to the different volumes of water present in the samples, the following experiments were conducted. Rolled oats (2 g) were cooked in either 15 or 30 ml of water by the rapid- or gradual-cooking method. The samples were cooked for 3 min after they reached boiling. Samples cooked in 15 ml of water were diluted with an additional 10 ml of water. The mixtures were then centrifuged (33,000 × g, 1 hr, 8°C) to collect the supernatants, and the residues were washed with 10 ml of water. After further centrifugation, the washings and supernatant were combined and diluted to 25 ml, recentrifuged to clarify (33,000 × g, 2 hr, 8°C), and duplicate aliquots (1 ml) were analyzed for β-glucan and starch. The samples cooked in 30 ml of water were directly centrifuged without dilution. The supernatant was removed for β-glucan analysis. The residue was then washed twice with water (15 and 10 ml), and the washings were combined with the supernatant. Duplicate aliquots were analyzed to obtain values for β-glucan and starch solubilized.

To monitor β-glucan release into solution during cooking, rolled oats (105 g) were added to water (840 ml) in a Ronson Cook's Stir blender (MK II), then stirred and heated to the boil over a period of 15 min. Samples were removed by ladle at intervals, the temperature was recorded, and they were transferred to centrifuge tubes and cooled in an ice bath. The samples were then centrifuged

This publication is contribution 721 from the Food Research Centre, Agriculture Canada, Ottawa, ON.

Food Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6.

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Cereal Chem. 64(6):373–379
saliva and 5 ml of distilled water inside a dialysis tubing (Spectrator Memb., 32 mm, molecular weight cutoff; 6–8,000) and simultaneously dialyzed against 200 ml of distilled water with constant stirring. Three 1-ml aliquots were removed from the dialysate every 30 min and analyzed by HPLC using the previously described procedures, but with maltose as a standard.

Microscopy
Materials present in the centrifugally separated layers (the middle and bottom layers) were examined microscopically. All samples were fixed in 3% glutaraldehyde (in 0.01 M phosphate buffer, pH 7.2) for 24 hr. Frozen sections were prepared from the middle-layer materials. Fixed samples were embedded in Histocut (Fisher Scientific Co., Fair Lawn, NJ), a support medium for cryo-sectioning, mounted on cold object disks, and frozen immediately at −25°C. Sections were cut 6–8 μm thick using a cryo-microtome (Reichert-Jung Scientific Instruments, Belleveille, ON). Glycol methacrylate (GMA)-embedded sections were prepared from materials of the middle and bottom layers. Fixed samples were dehydrated through a series of changes of alcohols (Yiu 1986) and were embedded in GMA resin. Sections were cut 2–4 μm thick, using an ultramicrotome (Sorvall Inc., Newtown, CT) equipped with a glass knife. All sections were affixed to glass slides for subsequent staining and microscopic examination.

The above sections were stained in one of the following reagents: 1) 0.1% (w/v) Calcium in 50% ethanol for 1 min; 2) 0.12% (w/v) fluorescein Lactis alimentarius agglutinin (FLCA) (Cedarlane Laboratories Ltd., Hornby, ON) in 0.01 M sodium phosphate buffer, pH 7, for 2 min; or 3) 0.5% iodine (w/v) in 5% aqueous potassium iodide solution (IKI) for 2 min. Stained sections were rinsed in distilled water, air-dried, mounted in immersion oil, and examined by fluorescence or bright-field optics using a Zeiss Universal Research Photomicroscope (Carl Zeiss Ltd., Montreal, PQ). The microscope was equipped with both a conventional bright-field illuminating system and a III RS epi-illuminating condenser combined with an HBO 100 W mercury-arc burner for fluorescence analysis. The III RS condenser contained two fluorescence filter systems with a dichromatic beam splitter and an exciter barrier filter set for maximum transmission at 365 nm/418 nm (for examining Calcium fluorescence) and 450–490 nm/520 nm (for FLCA). Sections stained with IKI were viewed under bright-field optics. Starch birefringence was examined after polarized light (using polarizing filters). Micrographs were recorded on 35 mm Ektachrome 400 daylight film. Micrographs obtained during microscopic examination of the middle-layer materials were analyzed using an IBAS image analyzer (Kontron, West Germany). Field-specific measurements, measuring the field area occupied by IKI-stained objects within the field of view and the area percentage (area % = [field area/reference area] × 100), were used to assess and compare structural differences between materials resulting from the rapid- and the gradual-cooking methods.

RESULTS AND DISCUSSION
Effect of Cooking on Centrifugal Fractions
Rolled oats were prepared by gradual-cooking, rapid-cooking, or soaking, and the mixtures were analyzed first by centrifugal separation. The solids-to-liquid ratio of cooking recommended by the manufacturer produced no supernatant at the centrifugal force of 33,000 × g. To obtain a supernatant for analysis, the cooked samples were diluted with water. Upon centrifugation, the diluted cooked oatmeal was separated into three distinct layers. The top layer was a clear, viscous fluid covered with a thin film of turbid, buoyant materials (probably lipid). The middle layer was a white paste, and the bottom layer contained the cooked solids. The proportions of the three layers differed depending on the treatment (Fig. 1a). Not unexpectedly, in the soaked sample the top aqueous layer occupied the largest portion of the mixture. Cooking, resulting in starch gelatinization and protein denaturation, decreased the volume of this portion. The middle layer of the soaked sample was not much different in texture from the bulk of
the solids and occupied only 0.4% (wet weight) of the total mixture. However, in the cooked samples, the middle layers were distinctive features as they represented 17.6 and 34.4% (wet weight) of the rapid-cooked and gradual-cooked samples, respectively. The bottom layers contained mostly soaked or cooked oat flakes, which differed in proportion depending on the preparation method (Fig. 1a). Furthermore, gradual cooking resulted in solids that retained more water than those obtained from rapid cooking or soaking (Fig. 1a).

Effect of Cooking on Solubilization of Starch and β-Glucan

Viscosities, starch, and β-glucan contents of the top, essential clear layers of soaked, gradual-cooked, and rapid-cooked samples (15 g) were determined. In all experiments the gradual-cooking method gave supernatants with higher concentrations of β-glucan and starch than the rapid-cooking method. The average from duplicate killings on two separate days is shown in Figure 1b along with measured viscosities. The increase in starch and β-glucan concentrations presumably accounted for the increased viscosity of the gradual-cooked samples, which had values that were 67% higher than those of the rapid-cooked samples. The

<table>
<thead>
<tr>
<th>Cooking Volume (ml)</th>
<th>Cooking Method</th>
<th>β-Glucan Solubilized (mg/ml)</th>
<th>Starch Solubilized (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Gradual</td>
<td>nd⁴</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Rapid</td>
<td>nd</td>
<td>9.0</td>
</tr>
<tr>
<td>30</td>
<td>Gradual</td>
<td>1.08</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td>Rapid</td>
<td>0.44</td>
<td>17.8</td>
</tr>
</tbody>
</table>

²g Samples were used.
⁴Total = supernatant and washings combined (see Materials and Methods for details).
nd = Not determined.

Fig. 1. Top, Relative distribution of oatmeal layers separated from oatmeals (15 g samples) prepared by 3-min soaking (S), rapid cooking (H), or gradual cooking (G).

Water-retention capacity =

\[
\frac{\text{total volume of water (220 ml) - volume of supernatant}}{\text{total solids (15 g)}}.
\]

Bottom, Starch and β-glucan contents and viscosity of the top layers separated from the same oatmeal samples.

Fig. 2. Solubilization of β-glucan during cooking.

Fig. 3. Effect of different preparation methods on β-glucan release from rolled oats. Soaking (○—○), rapid cooking (■—■), and gradual cooking (●—●). Cooking time = simmering time.

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Fig. 4. Glycol-methacrylate-embedded sections of the middle layers separated from (a) gradual-cooked and (b) rapid-cooked oatmeals by centrifugation and stained with potassium iodide to show differences in starch aggregation (*).

Fig. 5. Areas occupied by aggregated starch in the middle layer materials centrifugally separated from rapid-cooked (H) and gradual-cooked (G) oatmeals. Results were based on digital image analysis on light micrographs (see Materials and Methods for details).

A twofold average increase in β-glucan concentration between the gradual- and rapid-cooked samples was higher than the average increase in the starch concentration, although the absolute concentration of solubilized starch was higher in all experiments. This suggests that solubilized β-glucan was the major factor which controlled the viscosity variation between the supernatants from the two cooking methods. α-Amylase treatment of the supernatant from the gradual-cooked sample resulted in a 28% loss in viscosity, confirming β-glucan as the major contributor to supernatant viscosity.

As shown in Figure 1a, the different cooking methods resulted in a different distribution of water between hydrated solids and the supernatant. The decreased volume and increased concentration of soluble components in the free water (water that separated on centrifuging) could contribute to the textural difference between the two cooked oatmeals. To determine whether competition by cooked starch and protein for limited water might influence β-glucan hydration and solubilization, cooking experiments were conducted using 2-g samples in either 15 or 30 ml of water. The results are summarized in Table 1. It was found that the total amounts of solubilized β-glucan and starch in the supernatant and washings were two to three times higher in the gradual-cooked sample than the rapid-cooked sample, regardless of the solids-to-liquid ratio. The supernatants of the samples cooked in 30 ml of water could be analyzed directly without dilution, and the β-glucan concentration in the gradual-cooked sample was similarly 2.5 times higher than that of the rapid-cooked sample.

Results of these experiments indicate that the solubility differences related to different cooking methods are not a function of changes in free (i.e., supernatant) water volume or competition by rapidly gelatinizing starch and denaturing protein for a restricted water supply. This was further supported by the finding that the rate of β-glucan released during cooking (Fig. 2) did not decrease, but continued to increase even after the temperature at which starch gelatinized.

Furthermore, both gradual- and rapid-cooked samples continued to release more β-glucan during extended cooking periods (up to 15 min), but the gradual-cooked samples maintained a higher level of solubilized β-glucan (Fig. 3). The amount of β-glucan released by the gradual-cooking method for any one cooking period was generally about twice as large as that induced by the rapid method. Therefore, total cooking time alone was not responsible for increasing β-glucan release. The method of preparation was the most significant factor to be considered for increasing the release of this soluble fiber in cooked oatmeal.

Effect of Cooking on the Microstructure of Oatmeal

Light microscopy was used to examine materials present in the middle and bottom layers of oatmeal samples after centrifugal separation. The texture of the middle layer varied from dry, floury, and slightly sticky in the uncooked sample to wet and pastelike in the two cooked samples. Furthermore, the middle layer of the gradual-cooked sample appeared to be smoother in texture than that of the rapid-cooked one. Microscopy revealed that the uncooked material consisted of mostly intact starch granules, many of which remained birefringent when frozen sections of the sample were examined under polarized light. The two cooked samples contained materials that were stained by IKI and FLCA to form colored and fluorescent structures, respectively, but were no longer recognizable as individual starch granules. Instead, aggregates of swollen and folded structures, typical of the appearance of gelatinized oat starch (Yiu 1986), were observed. Furthermore, many aggregates of the gelatinized starch were interspersed with void spaces where water was trapped during centrifugation. The gradual-cooked material contained larger void spaces and more expanded structures (Fig. 4a) than the rapid-cooked sample (Fig. 4b). Results of digital image analysis of micrographs revealed that the mid-layers of the rapid-cooked samples had 57% ± 6 of the total area occupied by gelatinized starch, whereas the value for the gradual-cooked samples was 41% ± 6 (Fig. 5). Relatively small numbers of micrographs (18 of
the rapid-cooked materials and 19 of the gradual-cooked samples) were analyzed, and hence, the results may not be completely representative. Nevertheless, quantifiable differences existed in the organization of solid and aqueous phases. This finding, together with data on the water-binding capacity of the samples (Fig. 1a), indicated that the gradual-cooked oatmeal was relatively more hydrated than the rapid-cooked sample. The difference in hydration apparently contributed to the textural variation in the cooked oatmeal.

Oat flakes, which sedimented in the bottom layer by centrifugation, were analyzed by fluorescence microscopy. The investigation focused mainly on the effect of cooking on the

Figs. 6-8. Glycol-methacrylate-embedded sections of 6, oatmeal prepared by (a) soaking, (b) rapid cooking, and (c) gradual cooking and stained with FLCA to show differences in the structural organization of starch. 7, Glycol-methacrylate-embedded sections of oatmeal samples prepared by (a) gradual cooking and (b) rapid cooking and stained with Calcofluor to reveal differences in cell wall breakdown in the inner oat endosperm. 8, (a) Gradual-cooked and (b) rapid-cooked oatmeal stained with Calcofluor to show the different degree of cell wall breakdown in the sub-aleurone layer of oats (arrows).
structural composition of starch and cell walls where β-glucan is located. Most starch present in the uncooked oatmeal appeared structurally intact, having its characteristic polyhedral appearance, but many compound starch grains were broken into individual granules (Fig. 6a). Disrupted starch granules were formed as the result of oat processing (Lookhart et al. 1986, Yiu 1986). Cooking further changed the structural organization of the starch. Most rapid-cooked samples contained swollen starch granules. Many of them appeared as convoluted coils that remained confined to the original domain of the compound starch grain (Fig. 6b). By comparison, starch gelatinization was more extensive in the gradual-cooked samples, judging from the presence of additional, distorted structures of starch and the absence of recognizable compound starch grains (Fig. 6c).

There was some cell wall breakage in the starchy endosperm of the uncooked oatmeal but cooking produced considerably more severe structural changes. The degree of the cell wall breakdown was more extensive in the inner endosperm of the gradual-cooked oatmeal (Fig. 7a) than in that of the rapid-cooked sample (Fig. 7b), as reflected by the decrease in the number of intact cell walls. The aleurone and subaleurone layers, which have thicker cell walls, were less affected by cooking. However, partial disruption of the subaleurone layer rich in β-glucan was more noticeable in the gradual-cooked samples (Fig. 8a) than in the hot-cooked oatmeal (Fig. 8b). The results clearly demonstrated the effect of gradual cooking on solubilization of β-glucan in rolled oats. The outer endosperm layers are reported to be relatively more resistant to mechanical processing and heat than the rest of the oat endosperm (Yiu 1986), and the greater thickness of the outer endosperm cell walls results in a more concentrated β-glucan content in this area of the grain (Wood et al. 1983). This may explain, at least in part, why only a fraction of the total β-glucan reserve in the whole grain was released by cooking. However, intrinsic physicochemical differences between solubilized and unsolubilized β-glucan may play a role, as seems to be the case for barley β-glucan (Woodward and Fincher 1983).

Cooking Methods and Oatmeal Digestibility

It was of interest to establish whether the two cooking methods would influence the digestibility of oatmeal. Hence, prepared oatmeals were subjected to in vitro digestion by enzymes present in the human saliva. HPLC analysis of the dialyzed hydrolysates revealed that cooking considerably increased the amount of readily digestible starch in rolled oats (Fig. 9), a finding similar to that reported by Snow and O’Dea (1981). The present study shows that dialyzed hydrolysates of the rapid-cooked samples contained slightly more maltose (from digested starch) than those of the gradual-cooked oatmeal. However, because of the described differences in the microstructure of the two cooked types, identical sampling was not assured. Cooked solids in the rapid-cooked oatmeal tended to have slightly more tissue-bound starch (Fig. 6b) than those in the gradual-cooked sample, which produced more solubilized starch (Fig. 1b). It is therefore difficult to assess differences (if any) in the availability of oat starch for enzymic digestion. More work is required to elucidate the effect of different cooking methods on the rate of starch digestion in model systems and to determine if this has any physiological significance. The present study also confirmed that raw oat starchy is highly digestible by human salivary enzymes. The finding is based on the microscopic observation which showed a decrease in the number of FLCA- and IK1-reacting starch structures in the digested sample of uncooked oatmeal (Fig. 10a) and on the increased amount of maltose detected during the digestion of soaked oatmeal by α-amylase (Fig. 9).

In samples treated with salivary α-amylase, a decrease in the number of detectable cell wall structures in the inner endosperm was more noticeable in the gradual-cooked samples than in the rapid-cooked oatmeal (results are not shown). The fluorescence intensity of the Calcofluor-stained cell walls was much reduced in

![Image](image-url)
samples derived from either cooked product as compared to the uncooked oatmeal. However, the structurally sturdy aleurone layer with its detectable β-glucan content remained largely intact, whereas the subaleurone layer had partially eroded cell walls of diminished β-glucan content (Fig. 11). The above observation was made in both digested samples, and little difference was detected between the two, although the subaleurone layer of the hot-cooked oatmeal seemed to be slightly less disrupted.

Conclusions
The present study shows that the cooking method plays a significant role in determining the nature of starch gelatinization and the amounts of β-glucan and starch solubilized in oatmeal. The amounts of these constituents directly affect the viscosity and the texture of the cooked oatmeal. In comparison with the rapid-cooking method, the gradual-cooking method induced more structural disruption of the cell walls, which consequently led to an increased release of β-glucan from the cooked oatmeal. The textural, or mouthfeel, differences between the rapid- and gradual-cooked samples arise from both increased polysaccharide (especially β-glucan) solubilization and from the different characteristics of the gelatinized starch.

Cooking and gelatinization greatly enhanced the rate of enzymic hydrolysis of oat starch by human saliva, although raw oat starch was also readily digestible by the enzymes. Cereal cell walls are not digested by human digestive enzymes, but mechanical disruption of these structures might increase availability of nutrients within the cell. Hence, both cooking methods may, at least in part, improve the digestibility of the oat flakes by inducing cell wall breakdown. However, solubilization of the cell wall β-glucan, with attendant increase in viscosity, might further modify the rate of nutrient absorption. Although in vitro measurements revealed only small differences between rapid- and gradual-cooked samples, preliminary results suggest greater differences in other oat products (Yiu et al., unpublished). These aspects are under further investigation.

ACKNOWLEDGMENTS
The authors thank E. Bond of the Electron Microscopy Unit (Plant Research Centre, Agriculture Canada, Ottawa, ON) and N. Dolson for their technical assistance.

LITERATURE CITED

[Received December 29, 1986. Revision received May 25, 1987. Accepted June 2, 1987]