On the Large-Scale Isolation of Water-Insoluble Cell Wall Material from Wheat Flour

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Nonstarch polysaccharides (NSPs) are believed to have an influence on the baking performance of wheat flours due to their high water-binding capacity. Much attention has been given to the isolation and characterization of water-soluble NSPs. The chemical structure as well as the physicochemical and baking properties of the water-insoluble NSPs have been less well characterized. In order to study the relation between chemical structure and physicochemical and baking properties, large amounts of water-insoluble cell wall material (WIS) are necessary.

Various procedures have been reported for the isolation of WIS. Most isolations involve centrifugation of flour-and-water suspensions (Yamazaki 1955, Markwalder 1975, Kim and D'Appolonia 1976, Abdel-Gawad 1982) or dough washings (D'Appolonia and MacArthur 1975), resulting in a sludge layer that is further purified. In order to get a distinct sludge layer, high centrifugation speeds must be applied, which means that only small volumes can be centrifuged at a time. Alternative isolation methods are based on wet-sieving and ultrasonication in aqueous ethanol (Mares and Stone 1973) or removal of starch and intracellular protein by organic solvents (Selvendran and Du Font 1980). Most of the isolation methods yield cell wall material that contains large amounts of intracellular protein and/or starch. Usually quantities up to 5 g of cell wall material are obtained. The centrifugation of large amounts of material as a first step of the isolation procedure hinders rapid upscaling of the procedure. Therefore, when larger quantities of WIS are required these methods become very labor-intensive.

Recently we reported the isolation of highly purified WIS based on dough washing followed by wet sieving (Gruppen et al 1989). This method circumvents the labor-intensive centrifugation as a first step of the isolation procedure. In this study we describe the upscaling of our method to yield 100-g quantities of WIS.

MATERIALS AND METHODS

Flour

Wheat flour was prepared from grain of *Triticum aestivum* cv. Arminda (1986 harvest), a soft milling wheat variety, using a Bühler MLU 202 laboratory mill. Six flour fractions were combined to obtain a 71% net extraction rate of straight-run flour. On a dry weight basis the flour contained 10.8% protein (N × 5.7).

Isolation of WIS

Figure 1 shows a schematic diagram of the WIS isolation procedure. A dough was prepared by mixing 5 kg of flour and 1.5 L of distilled water in a Hobart type D 300 mixer. After 30 and 50 sec, respectively, 0.76 and 0.38 L of distilled water were added. The dough was kneaded for 7 min. Next, distilled water was added to the dough at a flow rate of 100 ml/min for 1.5 min. After this addtion, the dough was kneaded for 1 min to allow the water to be absorbed. This addition and absorption process was repeated three times. Then the addition and absorption times were changed to 1 and 0.75 min, respectively. After 50 min this procedure was stopped. Next the slurry of gluten, starch, and WIS was diluted with 10 L of distilled water and further separated as described by Weegels et al (1988). Following this procedure, the slurry was pumped onto a Vortair vibrating sieve (HEUB 90-S4, BBC KEG;4) onto which a stack of five sieves of 250, 125, 90, 50, and 32 μm was mounted. Fresh tap water was sprinkled over the sieves for washing.

Five runs of 5 kg flour were processed sequentially and the fractions on the 32- and 50-μm sieves were combined (crude WIS slurry). Residual starch in this fraction was removed as described previously (Gruppen et al 1989). For this purpose, the crude WIS slurry was diluted to 10 L, buffered to pH 6.5, and heated to

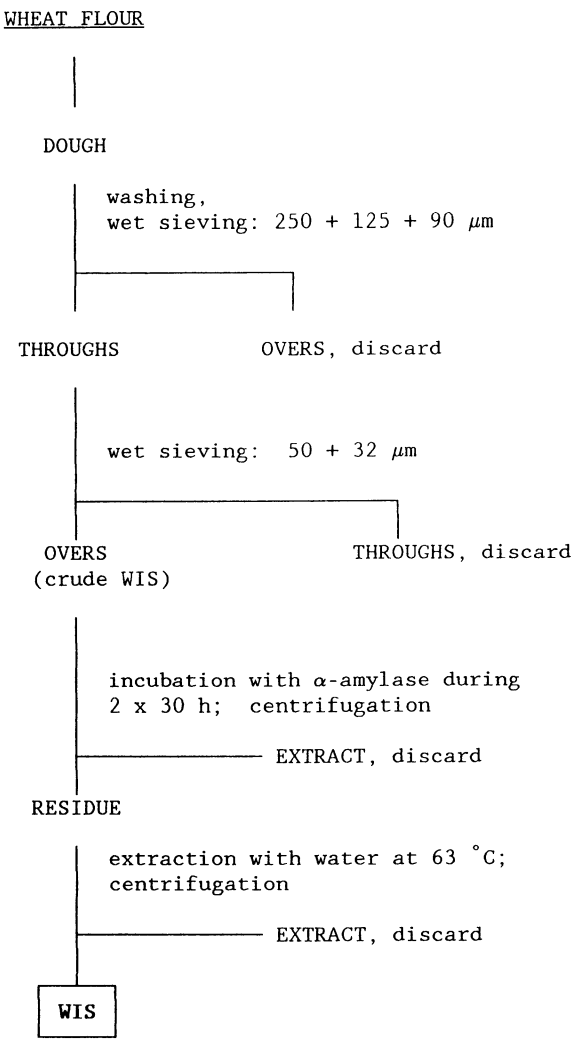

Fig. 1. Schematic diagram for the large-scale isolation of water-insoluble cell wall material (WIS) from wheat flour.

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63° C for 1 hr. After cooling to 30°C, the mixture was incubated for 30 hr with 33 mg of α-amylase (Merck no. 16312) and centrifuged. The α-amylase activity amounted to 303 U/mg. One unit was expressed as the amount of enzyme liberating 1 μmol of reducing endgroups per minute from soluble potato starch (Merck no. 1252) at pH 6.5 (sodium maleate buffer) and 30°C under saturated substrate conditions. Maltose (Merck no. 5912) was used as a reference. The resulting residue was resuspended in buffer, incubated with α-amylase, and centrifuged. The residue was four times extracted with water (2 L, 63°C) and freeze-dried (WIS). The α-amylase showed no activity towards arabinoxylan and (1→3),(1→4)-β-glucan.

Analysis

The WIS fraction was analyzed for starch content, protein content (N × 5.7), lipid content, ferulic acid content, neutral sugar composition and amino acid composition as described previously (Gruppen et al 1989). (1→3), (1→4)-β-Glucan content was determined enzymatically using the test kit of Biocon Ltd.

RESULTS AND DISCUSSION

Table I shows the yield and composition of the WIS isolated on a large scale together with the previously published data on WIS from small-scale isolation. The two fractions differed mainly in residual starch content; the large-scale isolation resulted in a lower starch content of 0.2%. This was attributed to the extensive washing that could be performed using the pilot plant sieving set. Previous studies (Gruppen et al 1989) revealed that extensive washing results in a lower residual starch content.

The NSP composition of both fractions was very similar. When isolated on a large scale, the WIS contained about 95% NSP. This NSP consists mainly of xylose, arabinose, and glucose, which are present as arabinoxylans and β-glucans (Mares and Stone 1973). The figures in parentheses represent the amount of glucose found on 1*M* H_2SO_4 hydrolysis after correction for the residual starch content, indicating it to be of noncellulosic origin. This is in correspondence with the (1→3),(1→4)-β-glucan content (7.7%, data not shown) we found for WIS isolated on a large scale with the Biocon test kit. Therefore, approximately half of the glucose is present as (1→3),(1→4)-β-glucans, the other half probably being a mixture of glucomannans and cellulose (Mares and Stone 1973).

Fractions isolated on a large and small scale had similar protein contents of 2.4 and 2.5%, respectively (Table I). The amino acid data for both fractions (Table II) likewise showed similar compositions except for somewhat lower glycine and glutamic acid contents of WIS isolated on a large scale. The porcine α-amylase was tested for proteolytic activities under the conditions used to remove starch. Although it showed some breakdown of casein, no solubilization of wheat gluten could be measured with E280. Analysis of protein according to Sedmak and Grossberg (1977), determined that less than 0.02% of wheat gluten was solubilized. Therefore, the relatively high glycine content is likely to be typical for wheat cell wall material (Gruppen et al 1989).

The large-scale yield of WIS is approximately 0.42% of the flour. With small-scale isolation using only a 32-μm sieve, we found a yield of 0.76% (Gruppen et al 1989). The reason for this difference is that a substantial part of the cell wall material was retained on the larger sieves (250, 125, and 90 μm) used in the present study. These sieves not only retain gluten particles that are loosened during the dough washing but also retain larger WIS particles. We only used material retained on the 32 and 50-μm sieves (these were visually free of gluten particles) and thereby introduced a lower yield of WIS. Highly purified WIS has been previously isolated by Kim and D'Appolonia (1976) by making use of centrifugation and alkali treatment. They obtained a WIS yield of 0.2%, starting with 200 g of flour. The NSP composition of their WIS resembled ours. However, a consequence of alkali treatment is that ester bonds within the WIS are broken during the isolation. Therefore substantial information about alkali-sensitive linkages (e.g. feruoyl and acetyl groups) in the WIS is lost. In the present study all alkali-sensitive linkages are still present in the WIS.

These results show that for Arminda flour we can isolate highly purified WIS on a large scale. With five runs of 5 kg of flour we prepared 2–3 L of crude WIS suspension within one day, yielding 100 g of purified WIS after α-amylase treatment. The main advantage of this method is that by making use of the pilot plant sieving set, the labor-intensive centrifugation of large amounts of flour-water slurry can be avoided. In addition, the extensive washing necessary for obtaining highly purified WIS can be done. The resulting crude WIS suspension has a relatively small volume and can further be purified. This purification includes prolonged α-amylase treatment followed by removal of the starch digest by washing with water using centrifugation. Although time-consuming, the α-amylase incubation requires minimal manual input. The subsequent removal of the starch digest is more labor-intensive but can, due to the relatively small volume of crude WIS suspension, easily be carried out on laboratory scale.

We also prepared WIS from a commercial soft wheat flour blend (Zeeuwse bloem) and analyzed starch and protein content. With this flour we found similar low amounts of residual starch and protein, 0.15 and 2.3%, respectively. In order to see whether WIS could be isolated from hard wheat varieties we also used Katepwa flour (Canadian Red Spring) and a Northern Spring

TABLE I
Composition of Isolated Water-Insoluble Cell Wall Material (% Weight)

Material	Large-Scale Isolation	Small-Scale Isolation[a]
Nonstarch polysaccharides[b]	94.7	93.1
rhamnose	0.2	0.1
arabinose	28.2	27.5
xylose	49.6	47.3
mannose	2.3	2.0
galactose	0.7	0.7
glucose	13.7 (8.3)	15.5 (8.6)
Starch	0.2	2.5
Protein	2.4	2.5
Lipids	0.6	0.7
Ferulic acid	0.3	0.3
Total	98.1	99.0
Yield[c]	0.42	0.76

[a] Data from Gruppen et al (1989).
[b] Sugars released on 72% 1*M* H_2SO_4 hydrolysis; the figures in parentheses represent the glucose content determined with 1*M* H_2SO_4 hydrolysis.
[c] Percentage of water-insoluble cell wall material recovered from flour.

TABLE II
Amino Acid Composition (mol %) of Water-Insoluble Cell Wall Material

Amino Acid	Large-Scale Isolation	Small-Scale Isolation[a]
Alanine	9.0	8.4
Arginine	5.5	5.6
Aspartic acid	7.9	7.7
Glutamic acid	9.2	10.7
Glycine	14.1	17.1
Histidine	2.6	3.1
Hydroxyproline	1.5	1.2
Isoleucine	3.2	2.9
Leucine	8.7	8.1
Lysine	4.8	4.7
Methionine	1.7	1.1
Phenylalanine	3.3	3.3
Proline	8.1	6.7
Serine	6.7	5.4
Threonine	5.3	5.5
Tyrosine	1.9	2.5
Valine	6.3	5.9

[a] Data from Gruppen et al (1989).

wheat flour blend. In both cases we isolated large amounts of crude WIS (before α-amylase digestion) indicating a good separation of gluten and nonstarch polysaccharides. This study indicated that highly purified water-insoluble cell wall material can be obtained in large quantities by making use of dough washing followed by wet sieving and α-amylase treatment.

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