

Effects on Polysaccharides During Baking and Storage of Bread—In Vitro and In Vivo Studies

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ABSTRACT

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The extent of staling of sour dough bread and conventional yeast bread was evaluated by differential scanning calorimetry during five days of storage. The bread products were made of whole grain wheat flours, one unmalted and one malted. The susceptibility of starch to α -amylase was determined by measuring the rate of an in vitro amylolysis at time intervals during storage. The contents of resistant starch and soluble and insoluble dietary fiber were analyzed. The bulking capacity of dietary fiber and the protein nutritional value were evaluated through balance experiments in rats. The in vitro susceptibility of starch to α -amylase was lower in sour dough bread than in conventional bread. Despite considerable staling,

storage did not alter the amylolysis. The starch availability in sour dough bread was improved after predigestion with pepsin. The use of malted flour resulted in a lower retrogradation, in particular in conventionally fermented bread. The sour dough fermentation did not affect the dietary fiber content or the distribution of insoluble and soluble components. Furthermore, the bulking capacity and protein utilization of sour dough bread were similar to those of conventional yeast bread. It is evidently possible to reduce staling in bread by the addition of malted flour and still maintain important nutritional and quality characteristics.

In many western countries a great part of the bread consumed is industrially baked, and as a consequence the deterioration in bread quality due to staling has become an economic problem. Although staling involves changes in both crumb and crust, leading to a hard crumb and a soft leathery crust, the term staling today usually refers only to changes in the crumb (Lin 1982).

The primary cause of staling is considered to be retrogradation of starch (Knightly 1977, Kulp and Ponte 1981, D'Appolonia and Morad 1981). The different roles of the two starch components, amylose and amylopectin, have been discussed intensively over the years. Amylose has a greater tendency to retrograde and was therefore considered to be the main cause of staling until Schoch and French (1947) suggested that aggregation of amylopectin was the factor responsible for crumb staling. The observation of Kim and D'Appolonia (1977), that most of the amylose was retrograded already during the first hours after baking, supported this theory. Lin (1982) concluded that amylopectin plays a more important role in bread staling than amylose. The role of amylopectin in retrogradation of starch was further confirmed in other studies by the use of differential scanning calorimetry (Russel 1983, Eliasson 1985).

Many attempts have been made to retard staling, for instance by adding α -amylase from different sources (Schultz et al 1952, Miller et al 1953, Beck et al 1957, Zobel and Senti 1959, Silverstein 1964, Dragsdorf and Varriano-Marston 1980). They all show that bread with enzyme supplementation stays soft longer than unsupplemented controls. An increased amount of extractable dextrins was noted in bread after enzyme supplementation. It was suggested that these dextrins interfere with the crystallization of starch and thus prevent staling (Schultz et al 1952, Miller et al 1953, Beck et al 1957, Zobel and Senti 1959, Silverstein 1964). However, the dosage of α -amylase imposes a significant practical problem, as a too high level will result in an unsatisfactory product. Another method of adding α -amylase is by using malted flour, which has a high intrinsic α -amylase activity.

It has often been claimed that sour dough bread stays soft longer than a conventionally fermented product. The preparation of sour dough bread includes a process where the cereal is exposed to

microbial attack and low pH for several hours. It is possible that the microbial amylases are capable of producing low molecular weight dextrins. However, the low pH values of the sourdough repress the activity of intrinsic amylases (Spicher and Stephan 1982). This instead contributes to a decrease in the formation of dextrins, which according to the suggestion above (Schultz et al 1952, Miller et al 1953, Beck et al 1957, Silverstein 1964) could result in increased staling.

In addition to deterioration of eating quality, the phenomenon of staling may have nutritional implications. The rate of digestion and absorption of starch, and hence its effect on blood glucose response after a meal, is of considerable interest both in diabetic and nondiabetic subjects. It has been suggested that retrogradation of starch reduces the susceptibility to enzymic degradation (Schultz and Landis 1932; Jackel et al 1952, 1953; Volz and Ramstad 1951). However, data in the literature on the effect of staling during storage of bread are contradictory. In some reports, storage reduced enzyme susceptibility (Schultz and Landis 1932; Jackel et al 1952, 1953; Volz and Ramstad 1951), whereas in others the opposite was found (Abelin and Biderbost 1932, Habs 1943, Habs and Plagemann 1943).

Furthermore, during baking of bread, a small fraction of the starch is rendered totally resistant to amylolytic enzymes unless solubilized in KOH or dimethyl sulfoxide (Englyst et al 1982, Johansson et al 1984, Siljeström and Asp 1985). The resistant starch is believed to consist of retrograded amylose (Siljeström and Asp 1985, Berry 1986), although no direct evidence is available. This enzyme-resistant starch fraction adds to the dietary fiber value, because most methods for dietary fiber analysis used today are dependent on an efficient, enzymic removal of starch. In some methods (Englyst and Cummings 1984) a dimethyl sulfoxide solubilization step is included to remove resistant starch. In vivo experiments with rats, however, indicate that the modified starch fraction formed during baking acts as an easily fermentable fiber component in the large intestine (Björck et al 1986) and should thus be included in the dietary fiber concept.

Bran and whole grain cereals with a large amount of insoluble dietary fiber are known to reduce the transit time in the bowel and to increase the fecal bulk. The increment in fecal dry weight is mainly due to dietary fiber, which is resistant to bacterial degradation in the large intestine (Cummings 1981). The breakdown is dependent on several factors, in particular the solubility and the chemical structure of the fiber (Cummings 1982). The sour dough process may affect the chemical or physical structure of the dietary fiber and thus be of importance for the susceptibility of the fiber to bacterial enzymes in the large intestine. Very few studies are available on the effects of food processing in

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this respect. The bacteria of a sourdough are also capable of a proteolytic activity (Spicher and Nierle 1984), which might affect the dietary utilization of the proteins.

The purpose of the present investigation was to study in vitro and in vivo effects on polysaccharides during baking of sour dough bread and conventional yeast bread. The extent of staling during storage of the bread products was evaluated as well as the in vitro susceptibility of starch to α -amylase during storage. Resistant starch and the contents of soluble and insoluble dietary fiber were analyzed in the different bread products as well as in the corresponding raw materials. In addition, the bulking capacity of the dietary fiber in raw material and in the corresponding baked products was evaluated through balance experiments in rats. Two different whole grain wheat flours were used, one unmalted and one malted.

MATERIALS AND METHODS

Bread Products

The raw materials used were unmalted whole grain wheat flour (water content 12.2%, protein 9.6%, falling number 256 sec) or malted whole grain wheat flour (water content 9.9%, protein 10.8%, falling number 62 sec), both obtained from a commercial source (Nordmills AB, Malmö, Sweden). The bread was produced by a sour dough process or by conventional yeast fermentation. The formulas used were as described below:

Conventional yeast bread. The formula for conventional yeast bread consisted of 270 g of water, 10 g of NaCl, 10 g of baker's yeast, and whole grain wheat flour. The amount of flour in the dough was adjusted to give a ratio between flour (dwb) and water of 1.29. The ingredients were mixed and the dough was fermented at 32–34°C (60% rh) for 30 min. A loaf (650 g) was formed, proofed at 32–34°C (60% rh) for 40 min, and then baked for 30 min at 230°C with addition of steam during the first 2 min.

Sour dough bread. The sourdough was made in two steps. First, equal amounts of flour and tap water were mixed and a starter culture containing approximately 5×10^7 living cells per gram of dough was added. The starter culture was a homo-fermentative lactic acid bacteria (*Lactobacillus plantarum*) isolated from a Swedish commercial sourdough, made of wheat flour (sourdough A [Spicher and Lönner 1985]). The mixture was put at rest for 24 hr at 30°C. In the second step, 22 g of the sourdough from the first step was mixed with 500 g of tap water and 300 g of flour, and the mixture was left to rest for another 20 hr at 30°C.

The bread dough was made of 350 g of the fully developed sourdough, 50 g of water, 10 g of NaCl, 10 g of baker's yeast, and whole grain wheat flour. The ratio of flour to water was adjusted as described above. Fermentation and baking were carried out in the same way as for the conventional yeast bread.

Four types of bread were evaluated in this study: sour dough bread made of unmalted whole grain wheat flour (SB); sour dough bread made of malted whole grain wheat flour (SMB); bread made of unmalted whole grain wheat flour by conventional yeast fermentation (RB); and bread made of malted whole grain wheat flour by conventional yeast fermentation (RMB).

Characterization of Sourdoughs and Bread

Determination of pH. During the sour dough fermentation process, the pH was continuously determined by a combined glass-calomel electrode and a pH-meter PHM 62 (Radiometer, Copenhagen, Denmark), and the values were recorded. The pH in the final bread was also measured.

Determination of the acid equivalent. Ten grams of the dough or the bread was mixed with 90 ml of distilled water and homogenized. The mixture was titrated with 0.1N NaOH to pH 8.5. The acid equivalent, S° , is expressed as the amount of NaOH consumed in milliliters (Spicher and Stephan 1982).

Lactic acid and acetic acid. The amounts of lactic acid (measured as both L[+] and D[+] lactate) and acetic acid were determined enzymically in the sourdoughs and in the bread (Boehringer Mannheim, GmbH-Biochemica, Mannheim, FRG). The samples were prepared as follows: a 10-g sample was mixed with 100 ml of

distilled water and homogenized; the mixture was heated to 60°C, held for 5 min, and was then cooled to approximately 20°C in an ice bath. After adjustment to pH 7, the mixture was stirred at ambient temperature for 30 min. The suspension was centrifuged and then filtered through a 0.45- μ m membrane filter (Millipore) and stored at -18°C until analysis.

Viable counts. The colony-forming units (cfu) were determined in the sourdough after the first and the second step of the preparation using the poured plate method. The counts of lactic acid bacteria were made on MRS medium (Oxoid Limited, Basingstoke, UK). The incubations were conducted for 2 days at 30°C.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to measure the retrogradation of starch in the different bread products. After cooling the bread for 2 hr at room temperature, each loaf was packed in a polyethylene bag and stored at $22 \pm 0.5^\circ\text{C}$ for 1, 2, or 5 days. The loaves were weighed before and after storage. To obtain samples for DSC measurements, each loaf was cut in halves and samples were cut from the center of each half. Pieces of bread (typically 4–7 mg) were put in a preweighed sample pan (coated DuPont pan), which was sealed and reweighed. The sample pan was put in the calorimeter (Perkin-Elmer DSC-2) at 22°C, allowed to equilibrate for a couple of minutes, and then heated to 92°C at a rate of 10°C/min. An empty pan was used as a reference. The evaluation of transition temperatures and enthalpies was made with a computerized system developed for DSC measurements (Eliasson 1986). The transition enthalpy (ΔH_c) was expressed as joules per gram of starch in the bread. The dry matter content of each individual sample was determined after the DSC scan by puncturing and drying the pan at 105°C for 16 hr. Each result reported is the mean value of three scans.

Chemical Analyses

After cooling the bread at room temperature, two slices were cut from the middle section. One slice was analyzed for dietary fiber content and the other for starch content and starch susceptibility in fresh bread. The rest of each loaf was put in a polyethylene bag and stored at ambient temperature up to 6 days.

Dietary fiber. An enzymic gravimetric method was used as described by Asp et al (1983). The bread slices were dried at 40°C in a vacuum oven and milled to pass a 0.5-mm screen. The enzymic hydrolysis of protein and starch was carried out in three steps with Termamyl (Novo A/S, Copenhagen, Denmark), pepsin, and pancreatin. The fiber was divided into insoluble and soluble components and filtered off, using Celite as filter aid. All dietary fiber values were corrected for indigestible protein (Kjeldahl N \times 6.25) and ash (ignition at 550°C for at least 5 hr) associated with the fiber. All values are mean values of triplicate analyses. Dietary fiber was analyzed in fresh bread and in bread stored for 6 days.

Resistant starch. The resistant starch determination was performed on the residue from the dietary fiber analysis as described by Siljeström and Asp (1985). The residue was solubilized in 2M KOH, and after neutralization incubated with amyloglucosidase (crystal suspension, 10 mg/ml, Boehringer Mannheim, GmbH-Biochemica, Mannheim, FRG), and analyzed for glucose with glucose oxidase peroxidase reagent (Glox-Novum, Kabi Diagnostica, Sweden). Starch content was expressed as polymer weight (0.9 \times monomer weight). Resistant starch was calculated as the amount of starch available after solubilization in KOH minus the amount of starch directly available to amyloglucosidase, i.e., without prior KOH treatment. The amount of directly available starch (residual starch) in the fiber preparation was small, less than 0.2% (dwb) in all samples.

Starch. Starch content was analyzed according to Holm et al (1986) in fresh bread. The sample was incubated first with Termamyl, a thermostable α -amylase, and then with amyloglucosidase. The amount of liberated glucose was analyzed with glucose oxidase peroxidase reagent. Starch content was expressed as polymer weight (0.9 \times monomer weight) and calculated on a dry weight basis.

Susceptibility of starch to α -amylase. The rate of starch hydrolysis was measured by incubating the bread, suspended in buffer, with an α -amylase solution (porcine pancreatic α -amylase, Sigma Chemical, St. Louis, MO). Samples were withdrawn at intervals for up to 1 hr and mixed with dinitrosalicylic acid reagent for determination of reducing sugars (Holm et al 1985). Maltose was used as standard, and the degree of hydrolysis was expressed in maltose equivalents.

In another experiment, a preincubation with pepsin was performed prior to the incubation with α -amylase as described by Holm et al (1985). The subsequent incubation with α -amylase was performed as described above. Corrections were made for reducing substances present in the enzyme preparation and for the small amounts of reducing sugars formed during the pepsin incubation due to the lowering of the pH.

Animal Experiments

Material. Unmalted whole grain wheat flour, conventional yeast bread, and sour dough bread made of unmalted whole grain wheat flour were used. The bread was sliced, frozen at -20°C , freeze-dried, milled to a particle size of less than 0.5 mm, and stored frozen until incorporation into the test diets. The flour was milled

to the same particle size.

Animals. Male Sprague-Dawley rats weighing approximately 80 g were divided into groups of five and kept individually in metabolic cages in a room maintained at 23°C (50–60% rh). Feed intake was restricted to 10 g of dry matter per day, whereas water was provided ad libitum. After a 4-day adaptation period to the diet, feed residues, urine, and feces were collected during 5 days. The feces were collected dry, removed every day, and frozen at -20°C . Feces were then freeze-dried, weighed, milled to a particle size less than 0.5 mm, and stored at -20°C until analysis of nitrogen. The urine was collected in H_2SO_4 (20 ml/L) and then analyzed for nitrogen.

Diets. The diets contained 5% corn oil, 10% sucrose, 4.8% mineral mixture, and 1% vitamin mixture, including choline chloride (Table I). The bread was used as the only protein source at a concentration of 1.5% N (dwb). However, to get the same fiber and nitrogen content in all diets, casein had to be added to the diet containing the flour. Corn starch was used to adjust the dry matter content. The dietary fiber content was 7.9% (dwb) in all diets.

Nitrogen balance. Feed, urine, and feces were analyzed for total N by using the Kjeldahl method with concentrated H_2SO_4 at 400°C with selenium as catalyst. True digestibility, biological value, and net protein utilization were calculated using the Thomas-Mitchell

TABLE I
Composition of the Diets in Rat Experiments
(% of dry matter)

Component	Reference Bread	Sour Dough Bread	Raw Material ^a
Wheat product	77.3	77.5	69.1
Sucrose	10.0	10.0	10.0
Corn oil	5.0	5.0	5.0
Minerals ^b	4.8	4.8	4.8
Vitamins ^c	0.8	0.8	0.8
Choline chloride	0.2	0.2	0.2
Corn starch	1.9	1.7	9.0
Casein	1.1

^aUnmalted whole grain wheat flour.

^bContained (g): $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 8.6; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 32.0; KH_2PO_4 , 7,780; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 4,024; CaCO_3 , 7,600; KI, 1.6; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2,000; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 180; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 80; CoCl_2 , 0.46; NaCl, 2,382.

^cContained (g): Menadione, 2.5; thiamin hydrochloride, 10.0; riboflavin, 10.0; pyridoxine hydrochloride, 5.0; calcium pantothenate, 25.0; nicotinic acid, 25.0; folic acid, 1.0; inositol, 50.0; *p*-aminobenzoic acid, 5.0; biotin, 0.2; vitamin B₁₂ (cyanocobalamin), 0.015; vitamin A, 0.86; vitamin D, 0.025; vitamin E, 100; wheat starch, 3,765.

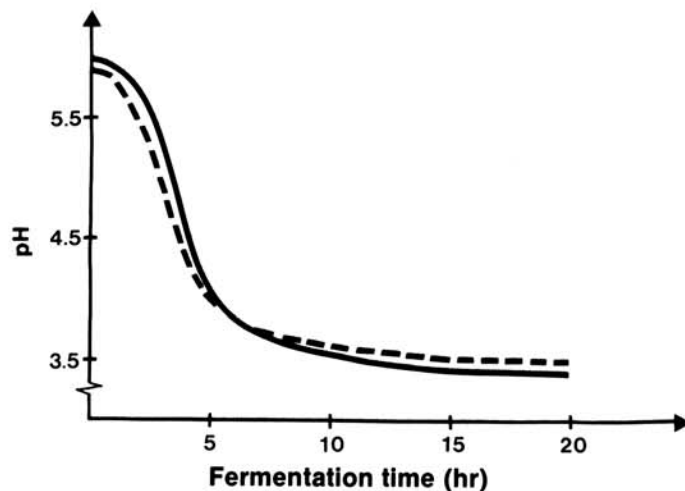


Fig. 1. Changes in pH in sourdoughs (step 2) made of unmalted whole grain wheat flour (—) and malted whole grain wheat flour (---).

TABLE II
Characteristics of Sourdoughs and Bread Products

Sourdough/ Bread Product ^a	pH	S ^o ^b	Viable Counts (cfu/g)	L-Lactic Acid (g/100 g)	D-Lactic Acid (g/100 g)	Acetic Acid (g/100 g)
SD						
Step 1, 24 hr	3.6	17.5	1×10^9
Step 2, 0 hr	6.0	2.2	5×10^7
20 hr	3.4	14.0	1×10^9	0.44	0.70	0.02
SB						
Stored 1 day	4.5	9.6	...	0.24	0.33	0.02
Stored 2 days	4.5	9.8	...	0.23	0.35	0.02
Stored 5 days	4.5	9.8	...	0.25	0.36	0.04
SMD						
Step 1, 24 hr	3.6	22.3	2×10^9
Step 2, 0 hr	5.9	3.4	7×10^7
20 hr	3.5	17.8	2×10^9	0.46	0.93	0.03
SMB						
Stored 1 day	4.5	13.6	...	0.22	0.43	0.04
Stored 2 days	4.5	12.9	...	0.22	0.44	0.01
Stored 5 days	4.4	13.3	...	0.24	0.47	0.04
RB	6.2	3.6	...	<0.01	0.01	0.01
RMB	6.2	6.4	...	<0.01	0.01	0.02

^aSD = Sourdough of unmalted flour, SB = sour dough bread of unmalted flour, SMD = sourdough of malted flour, SMB = sour dough bread of malted flour, RB = conventional yeast bread of unmalted flour, RMB = conventional yeast bread of malted flour.

^bAcid equivalent.

equations (Eggum 1973). Correction factors for basal excretion of urinary N and metabolic fecal N were determined by using 4% (dwb) egg protein and 5% (dwb) cellulose in the diet.

Statistical evaluation. All statistical evaluations of data from the animal experiment were made with the two-tailed Student's *t* test.

RESULTS AND DISCUSSION

Characteristics of Sourdoughs and Bread

The pH changes in the two sourdoughs made of unmalted (SD) or malted flour (SMD) were similar (Fig. 1). In the final sourdoughs, the pH was 3.5 in SMD and 3.4 in SD (Table II). The acid equivalent and the viable counts were a little higher in SMD than in SD, 17.8 and 2×10^9 versus 14.0 and 1×10^9 , respectively. Obviously the malted flour had a better buffering capacity, probably partly due to a higher proteolytic activity, which resulted in a higher level of free amino acids and a greater amount of short peptides.

The pH values in both types of sour dough bread were 4.5 (Table II). The acid equivalent was a little higher in SMB than in SB, and consequently the amount of lactic acid was higher in SMB. The amounts of acetic acid were very low in all products. The starter culture used was a homofermentative lactic acid bacteria, producing mostly D-lactic acid. The amounts of D-lactic acid produced were about twice the amounts of L-lactic acid. Storage did not affect the amounts of acid in the sour dough bread. The pH values in both RB and RMB were 6.2, and the acid equivalent 3.6 and 6.4, respectively. Lactic acid and acetic acid were almost not detectable after conventional fermentation.

The height of the sour dough bread, SB and SMB, was clearly above that of RB and RMB (Fig. 2). The former types also had cracks in the surface to a certain extent. The porosity of RMB was not satisfactory because the crumb was too dense. The tastes of the four types of bread were very different. The malted whole wheat flour gave RMB and SMB a bitterness and a mawkish taste. In contrast, the bread baked of unmalted flour, RB and SB, tasted much better and in particular the sour taste in SB contributed to an extra intensity in aroma.

Staling as Measured by DSC

The retrogradation in bread is easily followed by DSC. When

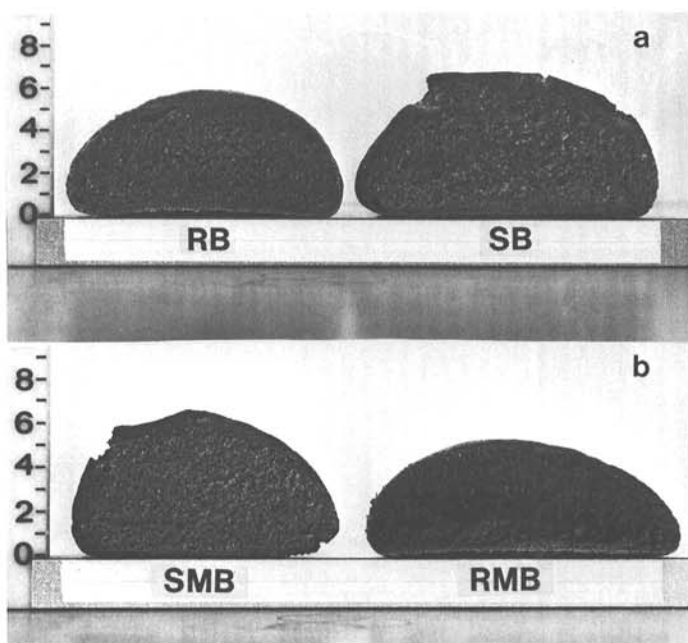


Fig. 2. Cross sections of the different bread types. **a**, Breads of unmalted flours; **RB** = yeast leavened bread, **SB** = sour dough bread. **b**, Breads of malted flours; **SMB** = sour dough malted bread, **RMB** = yeast leavened malted bread.

stale bread is heated in the DSC, a "staling endotherm" (Russel 1983) appears on the DSC thermogram, a result of the melting of crystallized amylopectin (Eliasson 1985). The enthalpy calculated from this endotherm is thus proportional to the amount of retrograded amylopectin.

When the fresh bread samples were heated in the DSC, only a baseline could be observed in the DSC thermograms, indicating the absence of retrograded amylopectin. However, for bread that had been stored prior to analysis, an endotherm was observed in the temperature range 55–75°C (Fig. 3). The yeast-leavened bread baked of malted flour (RMB) had to be stored for two days before

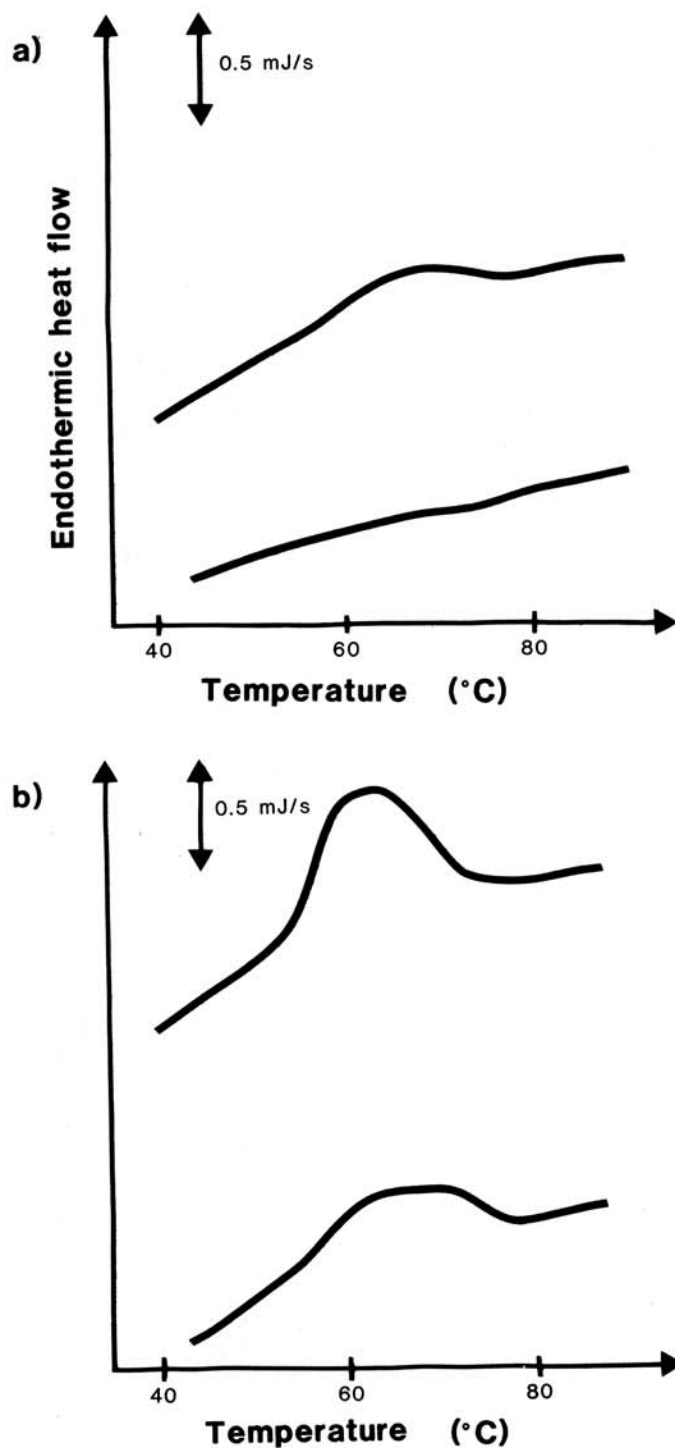


Fig. 3. Differential scanning calorimetry thermogram showing the retrogradation of two of the bread products: **a**, malted yeast leavened bread and **b**, unmalted yeast leavened bread. The lower curves show the staling endotherm after one day of storage, and the upper curves after five days at +22°C.

this staling endotherm could be observed, in contrast to the three other products where one day of storage was enough.

The rate and extent of staling in the four bread types as measured by DSC are illustrated in Figure 4. The moisture contents of the baked breads were similar. The weight loss during storage was measured, and the maximum weight loss was 0.5% (dwb), which was found in the two types of sour dough bread stored for 5 days. As the starch content differed somewhat between the bread types (Table III), the enthalpy (ΔH_c) was calculated and expressed as joules per gram of starch. The ΔH_c values of the staling endotherm during storage show that the retrogradation was greater in bread baked of unmalted flour than in bread baked of malted flour. With the unmalted whole grain wheat flour, the method of baking did not significantly affect the retrogradation during storage, i.e., RB and SB retrograded at approximately the same rate and to the same extent. It is possible that the differences in staling between

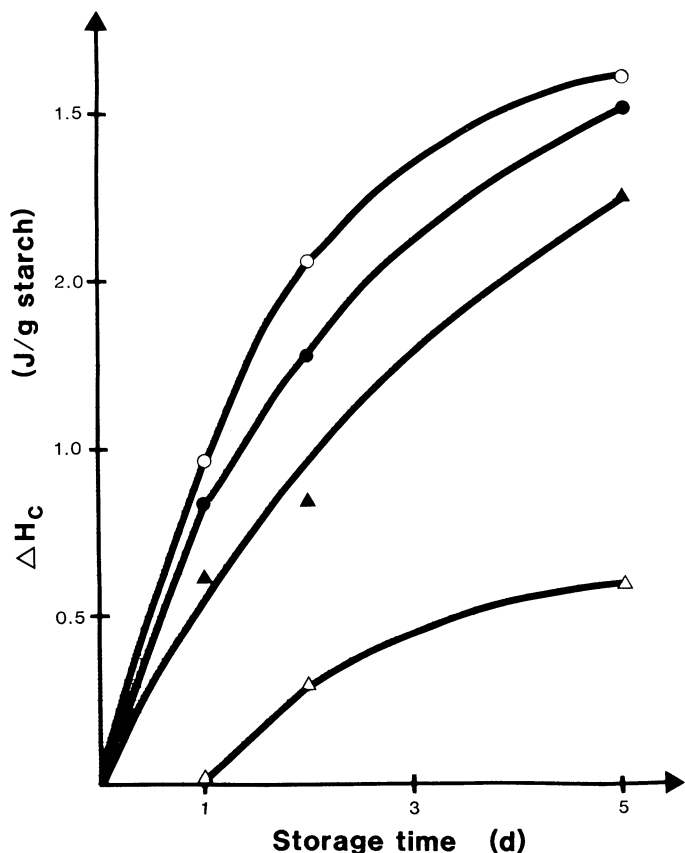


Fig. 4. Changes in transition enthalpy (ΔH_c) over time for bread stored at +22°C. ○—○ = Yeast leavened bread; ●—● = sour dough bread; △—△ = yeast leavened bread of malted flour; ▲—▲ = sour dough bread of malted flour.

TABLE III
Total Starch Content, Dietary Fiber and Resistant Starch in Bread Products, and the Corresponding Raw Materials (% of dry matter)

Bread ^a or Raw Material	Total Starch Content	Dietary Fiber		Resistant Starch in Fiber Fraction	
		Insoluble	Soluble	Insoluble	Soluble
RB	69.9	8.1	2.2	0.3	<0.1
SB	68.3	8.2	2.0	0.3	<0.1
RMB	62.4	10.3	2.3	<0.1	<0.1
SMB	61.6	10.5	2.1	<0.1	<0.1
Unmalted flour	72.7	9.1	2.4
Malted flour	66.6	11.1	2.1

^aRB = Conventional yeast bread of unmalted flour, SB = sour dough bread of unmalted flour, RMB = conventional yeast bread of malted flour, SMB = sour dough bread of malted flour.

sour dough bread and conventional bread would have been more explicit if changes in other properties, such as taste and texture, had been investigated. The sour dough bread made of malted flour seemed to retrograde somewhat more slowly than the products made of unmalted flour, but the extent of retrogradation, ΔH_c , at infinite time would probably be the same. The retrogradation of RMB, however, was very slow indeed (Fig. 4), and the ΔH_c value seemed to level off at a much lower level than for the three other types of bread. Thus, the products from malted flour were very much affected by the method of baking.

The obvious difference in retrogradation between the two malted bread products seems to confirm the suggestion that dextrinization of starch prevents staling (Schultz et al 1952, Miller et al 1953, Beck et al 1957, Silverstein 1964). It appears as if the low pH in the sourdough will repress the intrinsic amylase activity in SMB, thus diminishing the formation of dextrans and enhancing the staling compared to RMB. A lower level of reducing substances in SMB than in RMB is also reported below.

Susceptibility of Starch to α -Amylase During Storage

The availability of starch for amylolysis after storage at room temperature for 1 day is shown in Figure 5. Bread made of malted flour showed a higher initial level of hydrolysis. The reducing activity was most prominent in RMB, corresponding to an initial degree of starch hydrolysis of about 30%, versus about 17% in the sourdough variety, SMB. Thus, the amylases present in the malted flour were somewhat less active during fermentation and the initial stages of baking in the case of sour dough bread. The high level of intrinsic amylases present in malted flour caused a high initial degree of hydrolysis in conventionally fermented bread (RMB), but did not significantly affect the rate of digestion compared to bread baked of unmalted flour (RB). The extent of starch digestion

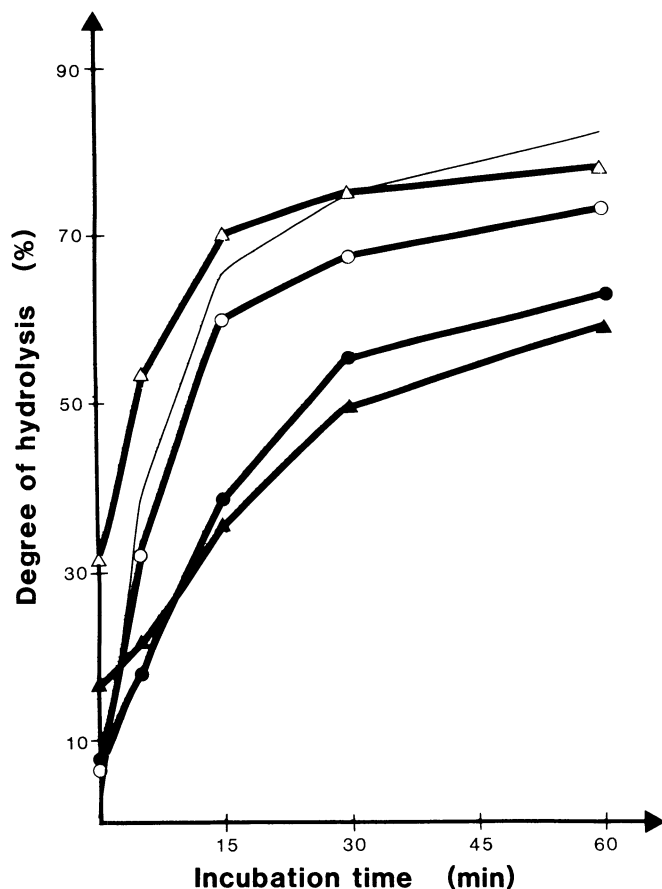


Fig. 5. Susceptibility of starch in bread products to α -amylase after one day of storage at room temperature. Similar results were obtained after two, three, and six days of storage. ○—○ = Yeast leavened bread; ●—● = sour dough bread; △—△ = yeast leavened bread of malted flour; ▲—▲ = sour dough bread of malted flour; — = control (Zulkowsky soluble starch).

by pancreatic α -amylase decreased in the following order: RMB > RB > SB \approx SMB. Thus, both sour dough bread products were less susceptible to amylolysis than conventionally fermented bread.

The bread products were analyzed in the same way after two, three, and six days of storage at room temperature. The behavior after the sixth day of storage was the same as after the first day. Thus, storage did not affect susceptibility to α -amylase even though the bread products studied covered a wide range regarding the extent of staling, as judged from the DSC thermograms (Fig. 4). In contrast, earlier studies suggested that staling of white wheat bread is accompanied by a decrease in the susceptibility of starch to amylases (Schultz and Landis 1932; Jackel et al 1952, 1953). However, in these reports, starch availability was determined indirectly by measuring the ability of the amylolytic products formed to maintain yeast fermentation (Schultz and Landis 1932). Our results imply that staling, which predominantly involves amylopectin, does not affect the availability of starch to pancreatic α -amylases.

In a second series of experiments, a preincubation with pepsin was performed prior to α -amylase digestion. The starch in RMB still showed the most rapid rate of amylolysis (Fig. 6). However, predigestion of the protein matrix significantly improved the susceptibility of starch in the sour dough bread products, to the same level as seen in RB. No effect of pepsin was noticed in the other products. This can be interpreted as if the protein matrix in the sour dough bread formed a barrier towards attack by α -amylase. A possible mechanism could be that the protein, due to denaturation, is rendered more rigid during processing at low pH. Further, enclosure of pepsin in the *in vitro* system did not reveal any effect of staling on enzyme susceptibility of starch. The finding that starch in sour dough bread is enclosed in a matrix, thus

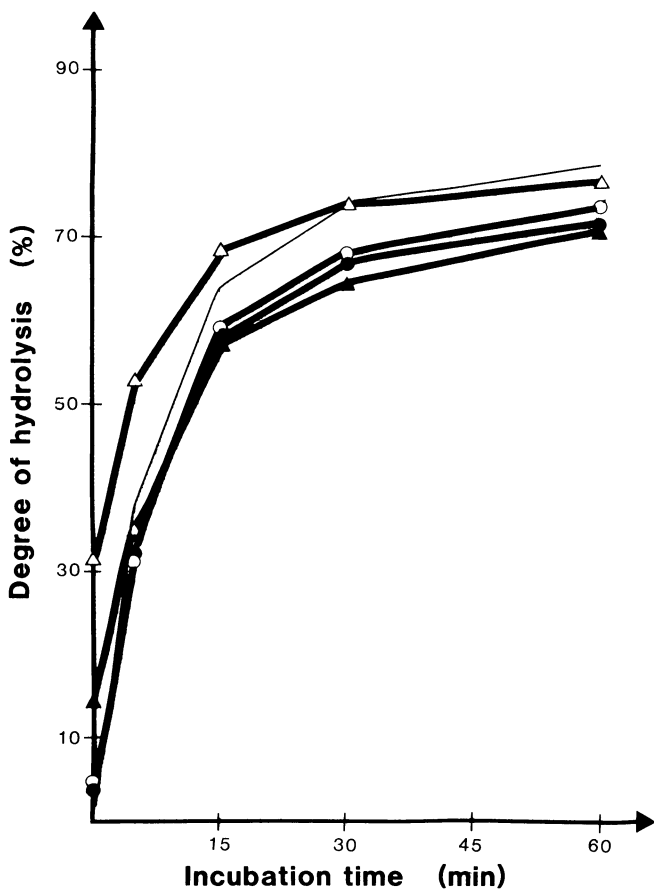


Fig. 6. Susceptibility of starch in bread products to α -amylase after one day of storage at room temperature and after preincubation of the sample with pepsin. Similar results were obtained after two, three, and six days of storage. ○—○ = Yeast leavened bread; ●—● = sour dough bread; △—△ = yeast leavened bread of malted flour; ▲—▲ = sour dough bread of malted flour; — = control (Zulkowsky soluble starch).

protected from enzymic attack (Holm et al 1985), is probably of no consequence *in vivo*. However, the use of *in vitro* amylolysis to predict the rate of digestion *in vivo* may lead to false conclusions. Furthermore, in the oral cavity, salivary amylases hydrolyze ingested starch to some extent. The products formed are then metabolized by the microorganisms present in the dental plaque, resulting in a pH drop on the tooth surface (Björck et al 1984a,b). It is possible that starch in sour dough bread is a less available substrate, as proteolytic enzymes are not present in human saliva.

Resistant Starch

Starch that had become resistant to amylolytic enzymes was detected in small amounts in unmalted whole grain wheat breads (RB and SB), but not at all in bread of malted flour (RMB and SMB), or in the raw materials (Table III). The method of baking, sour dough or conventional yeast fermentation, had no influence on resistant starch formation. The formation of resistant starch in bread of unmalted whole grain wheat flour (0.3% dwb) was considerably lower than in previous studies (Englyst et al 1983, Johansson et al 1984, Siljeström and Asp 1985), where amounts of 1–3% (dwb) were found in baked products. One possible explanation could be the high dry matter content of the dough in the present investigation. A decreased flour-water ratio has been suggested to increase the formation of resistant starch (Siljeström and Asp 1985, Berry 1986). In an earlier study Johansson et al (1984) reported a tendency to lower amounts of resistant starch in bread baked of whole grain flour than in white flour breads. Hence it must be noted that the recipe and baking conditions used are obviously of importance to the amount of resistant starch formed during baking.

In the malted bread products in the present investigation, no resistant starch was formed. It has been suggested that resistant starch consists of retrograded amylose (Siljeström and Asp 1985). Berry (1986) reported a close relationship between amylose content and yields of resistant starch in autoclaved starch suspensions. In materials with a high amylose content, for instance amylo maize and purified potato amylose, a high amount of resistant starch was found, versus only small amounts in waxy maize. He concluded that formation of resistant starch, in bread and other baked products, probably is a process of amylose retrogradation occurring rapidly, due to favorable conditions of temperature and starch-water ratio. In one experiment (Berry 1986), debranching of the amylopectin fraction with pullulanase and subsequent autoclaving of the debranched mixture produced a considerably higher yield of resistant starch than did autoclaving of the corresponding undigested material. The longer fragments (degree of polymerization 45–60) produced by the enzymic degradation were proposed to be active in the formation of the resistant starch. It is probable that the intrinsic amylases in the malted flour, in the present investigation, produced dextrans with other degrees of polymerization and thus prevented the formation of resistant starch in the malted bread products (SMB and RMB).

In agreement with previous studies (Johansson et al 1984, Siljeström and Asp 1985) storage of the bread did not affect the amount of resistant starch, despite the obvious staling observed, as measured by DSC, in the products that were stored the longest time. Berry (1986) also reported that resistant starch in wheat bread was present immediately after baking and that no increase was noticed during storage.

Dietary Fiber

The content of dietary fiber in the bread products is shown in Table III. The sour dough fermentation had apparently no solubilizing effect on the fiber polysaccharides, as no differences in total fiber content or in the distribution of soluble and insoluble components were detected between SB and RB or SMB and RMB, respectively. However, the distribution of insoluble and soluble fiber in the malted flour was different from that in the unmalted flour. The malted flour contained a higher amount of insoluble dietary fiber, which is reflected in the bread products. A storage period of up to six days did not affect the fiber content of the bread,

suggesting that the retrograded amylopectin is not recovered as dietary fiber.

Animal Experiments

Fecal dry weights. The fecal dry weights obtained with the two bread products (RB and SB) in the diet were similar, 3.5 g and 3.6 g for reference bread and sour dough bread, respectively (Table IV). However, relative to the raw material there was a small but significant decrease ($P < 0.05$) in apparent fecal dry weight after baking. This was caused by a higher intake of the raw material, and no significant differences could be detected in dry matter digestibility (Table IV). The similar dry matter digestibilities strongly suggest that the amount of fiber excreted also is similar, because fecal dry weight increment of resistant types of fiber, such as whole grain cereal fibers, mainly are due to unfermented fiber (Nyman et al 1985).

Previous investigations show that some processes, such as extrusion cooking and popping, increase the amount of soluble fiber and as a consequence the fermentability of the fiber (Björck et al 1984d, Nyman et al 1987). This redistribution of insoluble fiber to soluble after extrusion cooking and popping could be due to the cleavage of glycosidic bonds (Assarsson et al 1959). Sour-dough preparation involves a process where the fiber polysaccharides are exposed to microbial attack for several hours, and hence the fiber could be expected to be partly degraded, thus affecting the bulking capacity. However, in this study no redistribution of dietary fiber due to baking was observed, nor did the process of baking have any effect on the bulking capacity. Thus, the two baking processes seemed to be similar regarding the fecal dry weights of rats given bread products or the corresponding raw whole grain wheat flour.

In previous studies of fiber from whole grain wheat using the same rat model (Nyman et al 1985, 1987), considerably higher (approximately 30%) fecal dry weights were found. The dietary fiber intake, the fiber content in the cereal, and the distribution between soluble and insoluble fiber were similar to that in the present investigation. Thus, it seems that other factors may also affect the fermentability of the fiber and consequently the bulking capacity.

Protein nutritional value. Results from the nitrogen balance experiments with the two bread products made of unmalted whole grain flour are shown in Table V. No significant differences were detected either in true digestibilities, biological values, or in the net protein utilization. The results are similar to those reported earlier for whole grain wheat flour (Björck et al 1984c, Pedersen and Eggum 1983). The utilization of the proteins was obviously not

affected by the proteolytic activity exercised by the sour dough bacteria. The protein nutritional value was not determined in the raw material, because casein was added to the diet.

CONCLUSIONS

The method of baking (sour dough or conventional yeast fermentation) was only of importance to staling, as measured by DSC, in the bread products made of malted flour. The low pH during sour dough fermentation seemed to repress the intrinsic amylase activity somewhat. Consequently there was a decrease in the amount of dextrins formed, as judged from a lower level of reducing carbohydrates in the sour dough bread. This might explain the higher rate of retrogradation in SMB than in RMB.

The starch in sour dough bread was less susceptible to amylolysis by α -amylase, *in vitro*, than the starch in conventionally fermented bread, probably because of a more rigid protein matrix enclosing starch in the sour dough bread. This barrier was overcome by introducing a predigestion of the sample with pepsin. Storage of the bread products did not alter either the rate or the extent of amylolysis. Our results imply that neither staling, *i.e.*, retrogradation of amylopectin, nor the method of baking would be expected to affect the susceptibility of starch to pancreatic α -amylases *in vivo*.

In contrast to our previous studies, the amount of resistant starch formed during baking was negligible, suggesting that the baking conditions or recipe affect the yield of retrograded amylose.

Sour dough fermentation apparently had no significant effect on the nonstarch polysaccharides, as judged from the similar dietary fiber values and the similar bulking capacities of the fibers in rat experiments. Nor did sour dough fermentation affect the protein nutritional value of the sour dough bread compared to conventionally yeast leavened bread baked under similar conditions.

In conclusion, by using sour dough fermentation, it is evidently possible to use flour with a higher α -amylase activity and reduce the rate of staling in bread, as measured by DSC, without affecting the starch availability *in vitro* or deteriorate the quality characteristics of importance for the consumer.

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TABLE IV
Fecal Dry Weight and Dry Matter Digestibilities in Rats
Fed Diets Containing Conventionally Yeast Leavened
or Sour Dough Bread Made of Unmalted Flour
(mean values and standard deviations)

Diet	Fecal Dry Weight ^a (g/5d)	Dry Matter Digestibility ^a (%)
Yeast bread	3.5 ± 0.5 a	92.3 ± 0.6 a
Sour dough bread	3.6 ± 0.8 a	92.4 ± 1.2 a
Raw material	4.2 ± 0.4 b	90.8 ± 1.4 a

^a Values in the same column followed by different letters are significantly different ($P < 0.05$) from each other.

TABLE V
Protein Utilization by Rats in Bread Products
(mean values and standard deviation)

Bread	True Digestibility (%)	Biological Value (%)	Net Protein Utilization (%)
Yeast bread	90.7 ± 2.0	51.8 ± 1.6	46.6 ± 1.9
Sour dough bread	90.0 ± 3.6	48.7 ± 3.6	43.9 ± 4.8

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