Resistant Starch Formation During Baking – Effect of Baking Time and Temperature and Variations in the Recipe

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Bildung von resistenter Stärke während des Backens in Abhängigkeit von Backzeit, Backtemperatur und Rezeptvariationen

Zusammenfassung. Die Menge an Ballaststoffen, die nach einer enzymatisch-gravimetrischen Methode bestimmt wurde, war in weißem Weizenbrot fast 20% höher als in dem entsprechendem Mehl. Die Erhöhung konnte zum großen Teil auf die Bildung von „resistenter Stärke" zurückgeführt werden, d.h. auf eine Stärkefraktion, die nur nach Auflösung mit 2 *m*-KOH für Amyloglucosidase zugänglich ist. Die resistente Stärke wird während des Backens oder während der Abkühlung des Brotes gebildet, und die Menge scheint vom Wassergehalt des Teiges, aber nicht vom Fettgehalt abhängig zu sein. Die Resultate dieser Untersuchung weisen darauf hin, daß die resistente Stärke retrogradierte Stärke, möglicherweise Amylose, ist.

Summary. The dietary fibre content in white wheat bread, measured with an enzymatic, gravimetric method, was almost 20% higher than in the corresponding flour. The increment was largely explained by the formation of resistant starch, i.e. starch available to amyloglucosidase only after solubilization with 2 *m*-KOH. The resistant starch was formed in the oven or upon cooling of the finished bread. The water content in the dough seemed to influence the extent of the resistant starch formation, whereas changes in the fat content had no effect. The results indicate that the resistant starch might be hard retrograded starch, possibly amylose.

Introduction

Processing of food products, such as cereals and potatoes has been reported to alter the characteristics of the carbohydrates. The changes occurring are quite varied and consequently their significance, nutritionally and for the taste and texture of the product, vary [1–5]. One of these changes is the formation of starch resistant to hydrolysis by amylolytic enzymes [6–9]. Although a minor component, it plays an important role in both enzymatic starch analysis and dietary fibre analysis.

The removal of starch is a critical step in all methods of dietary fibre analysis, and is usually performed by enzymatic hydrolysis. Any portion of starch made resistant to enzyme attack will thus add to the dietary fibre value. This contribution will become especially important when analysing products from starch-rich materials with low or medium dietary fibre content, such as white wheat flour or potatoes.

The physiological role of the resistant starch is not yet clear. However, some reports indicate an incomplete absorption of starch from processed starchy foods, such as mashed potatoes [10] and bread [11]. Although the resistant starch is a minor constituent of the food product, one might assume that man consumes quite appreciable amounts, since starchy foods are our major carbohydrate source.

In a previous study [9] the extent of resistant starch formation during baking was investigated. We found that the time of storage (up to 4 weeks) did not alter the amount of resistant starch in frozen or dried bread compared with fresh bread. The purpose of the present study was to investigate some of the factors that might influence resistant starch formation in bread.

Materials und Methods

Bread samples

The bread was baked from wheat flour with an extraction rate of approximately 80%. Two slightly different recipes were used.

Recipe 1 (Laboratory scale)

230 g flour, 125 g water, 8 g yeast, 1.9 g salt, 5 g sugar, and 10 mg ascorbic acid. – The ingredients were mixed and the dough was fermented for 40 min, divided into pieces of 100 g each, transferred to pans and proofed for another 40 min. The bread was then baked at 230 °C for 20 min in a laboratory oven.

Recipe 2 (Large scale)

1800 g flour, 1000 g water, 85 g yeast, 30 g salt, and 30 g sugar. – The ingredients were mixed and the dough was fermented for 25 min, divided into 100 g pieces, transferred to baking sheets and proofed for

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40 min. The bread was then baked in a convection oven at 200 °C or 230 °C at an air velocity of 1 m/s. The temperature in the centre of the bread was measured with thermocouples in two of the rolls.

After cooling, the crumb and crust were separated, and the crumb was dried, ground and analysed. In the storage study, the bread was left to cool and then kept in sealed plastic bags for 1 to 7 days.

Dietary Fibre Analysis

An enzymatic, gravimetric method was used, as described by Asp et al. [12]. The samples were milled in a Cyclotec mill (Tecator AB, Höganäs, Sweden) to pass a 0.5 mm sieve. The enzymatic hydrolysis of protein and starch was then carried out in three steps: (1) partial starch degradation, with a heat-resistant amylase (Termamyl) included in the gelatinization step, at 100 °C for 15 min, (2) hydrolysis of protein with pepsin at pH 1.5 for 1 hour, and (3) final starch and protein hydrolysis with pancreatin at pH 6.8 for 1 hour. Soluble dietary fibre components were precipitated with 4 volumes of 95% ethanol, and the total dietary fibre was separated by filtration using Celite as filter aid. The filtration was carried out in a modified Fibertec apparatus (Tecator AB, Höganäs, Sweden). All dietary-fibre values are corrected for indigestible protein (Kjeldahl Nx6.25) and ash (ignition at 550 C for at least 5 hours) associated with the fibre.

Starch Analysis

Residual starch. Approximately one quarter of the fibre plus Celite residue was suspended in 1 ml of water and boiled for 15 min. After cooling, 0,5 ml of 0.2 *M*-sodium acetate buffer, pH 4.75, and 10 µl of amyloglucosidase (crystal suspension, 10 mg/ml, Boehringer, Mannheim, FRG) were added and the tubes were incubated at 60 °C for 30 min and then centrifuged at 4000 rpm for 10 min. The supernatant liquids were transferred to 5-ml volumetric flasks. The sediment was washed with 1.5 ml of water and recentrifuged. The washing water was combined with the original supernatant liquid and the glucose oxidase-peroxidase reagent diluted to 5 ml with water. One ml of this was mixed with 2 ml of glucose oxidase-peroxidase reagent [5.6 g GLOX-Novum (Kabi-Diagnostica, Stockholm, Sweden) in 100 ml of 0.5 *M*-Tris-buffer, pH 7.0] and incubated at 37 °C for 1 h. Absorbance was measured at 450 nm. Glucose, 25 µl/ml, was used as the standard and water as the blank.

Resistant starch. Approximately one quarter of the fibre plus Celite residue was suspended in 1 ml of water and boiled for 15 min. After cooling, 1 ml of 4 *M*-KOH was added, to give a 2 *M*-KOH solution. The suspension was left at ambient temperature for 30 min (mixed every 5 min), and neutralized with 2 ml of 2 *M*-HCL (6,7). One ml of 0.4 *M*-sodium acetate buffer, pH 4.75, and 20 µl of amyloglucosidase were added. The tubes were incubated at 60 °C for 30 min and then centrifuged. The supernatant liquids were transferred to 10 ml volumetric flasks, the sediment was washed and the combined supernatant liquids diluted to 10 ml with water. Glucose analysis was performed as for residual starch. The amount of resistant starch was then the value obtained with KOH-solubilization less the value for residual starch.

The amount pf resistant starch obtained with 2 *M*-KOH solution for 30 min was not increased by extending the treatment to 60 min nor by increasing the KOH solution concentration to 4 *M*. Solubilization with 1 *M*-KOH for 30 min, however, gave a significantly lower value.

Statistical Evaluation

The statistical evaluations were made with Student's-test. The values in the tables are means of triplicate analyses (dietary fibre) or duplicate analyses (residual and resistant starch). The absolute standard deviation is 0.4 for the dietary fibre analysis and 0.1 for the residual starch analysis and resistant starch analysis.

Results and Discussion

Influence of Storage Time and Temperature

As shown in Table 1, the dietary fibre, residual starch and resistant starch contents were the same in fresh bread as in samples stored at ambient temperature or in a refrigerator. The resistant starch constituted approximately 15% of the dietary fibre in the bread. This is in agreement with our previous results [9] where no differences were found between fresh, frozen or dried bread samples.

The dietary fibre residue from unprocessed wheat flour contained only traces of residual starch and resistant starch. This indicates that the resistant starch is formed during dough-making, in the oven or upon cooling of the finished bread as suggested by Englyst et al. [8].

The resistant starch does not completely account for the increase in dietary fibre. The remainder may be very hard retrograded starch that is not solubilized by the KOH or polysaccharides from the yeast, or due to fermentation, which causes a loss of non dietary fibre constituents of the dough.

Baking time

The temperature profile for the centre of a small round bread roll is shown in Fig. 1. During a few minutes the temperature rose to 98 °C and then remained the same throughout the baking procedure. Table 2 shows the combined data from two experiments where the rolls were baked to different centre temperatures. The resistant starch increased with increasing temperature, but did not increase further after about 10 minutes, when the temperature had reached its plateau (Fig. 2).

It is also evident from Table 2 that the dough-making process is not responsible for the formation of resistant starch. The two dough samples (freshly mixed and fermented) contained only traces of resistant starch.

Table 1. Effect of storage time and temperature on the amount of resistant starch in bread crumb

Sample	Dietary fibre	Residual starch	Resistant starch
	mg/100 mg dry matter		
Wheat flour	3.6	0.2	<0.1
Fresh bread	4.9	0.3	0.8
Stored at ambient temperature			
1 day	4.9	0.3	0.7
3 days	4.6	0.3	0.8
7 days	4.6	0.3	0.7
Stored in refrigerator			
1 day	4.8	0.3	0.7
7 days	5.2	0.3	0.8

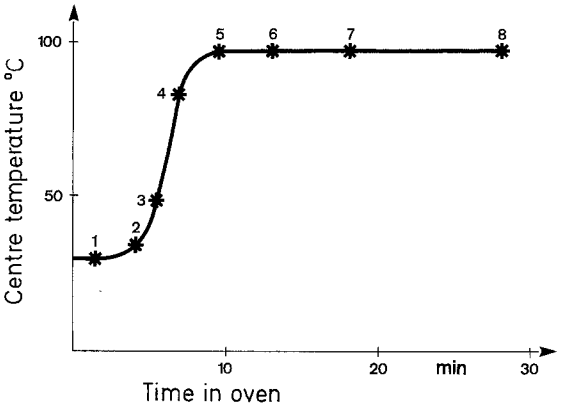

Fig. 1. Time-temperature relationship for the centre of a round wheat bread roll

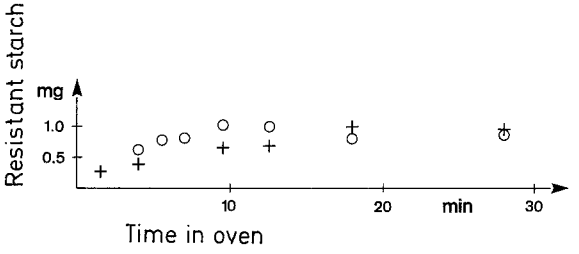

Fig. 2. Resistant starch in bread crumb in mg/100 mg dry matter

Table 2. Resistant starch in the crumb of bread baked to various centre temperatures

Sample	Centre temperature °C	Dietary fibre	Residual starch	Resistant starch
		mg/100 mg dry matter		
Wheat flour		3.3	0.1	<0.1
Dough (freshly mixed)		3.5	0.2	<0.1
(fermented)		3.2	0.1	<0.1
Bread *1*	29	3.9	0.2	0.3
2	35	4.2	0.3	0.6
		4.8	0.2	0.4
3	48	4.6	0.3	0.8
4	83	4.4	0.3	0.8
5	98	4.9	0.4	1.0
		5.0	0.3	0.7
6	98	4.4	0.3	1.0
		5.4	0.3	0.7
7	98	4.8	0.3	0.8
		4.6	0.2	0.9
8	98	4.6	0.3	0.9
		5.1	0.2	0.9

Influence of the Recipe

To investigate the effect of some ingredients in the recipe on resistant starch formation, the following experiments were carried out.

In the first experiment margarine was added (1.3 – 3.9 g/100 g flour) to recipe 1. Table 3 shows that this addition had no systematic effect on either residual or resistant starch.

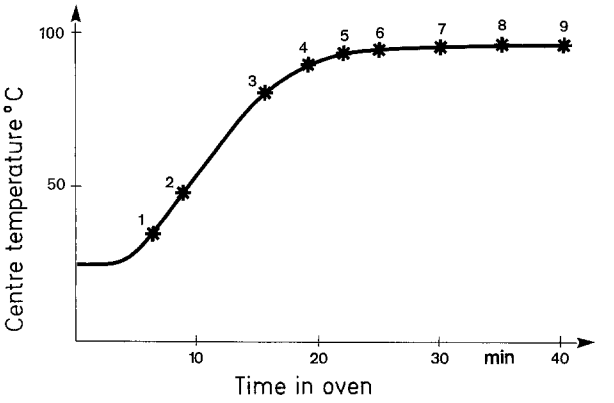

Fig. 3. Time-temperature relationship for the centre of a round wheat bread roll (baking-powder)

Table 3. Effect of margarine added to recipe *1* on the amount of resistant starch in bread

Margarine g/100 g flour	Dietary fibre	Residual starch	Resistant starch
	mg/100 mg dry matter		
–	4.9	0.3	0.7
1.3	4.6	0.3	0.8
2.2	4.5	0.4	0.5
3.0	4.8	0.3	0.8
3.9	4.4	0.2	0.9

Table 4. Flour to water ratio

g flour/ 100 g water	Dietary fibre	Residual starch	Resistant starch
	mg/100 mg dry matter		
180	5.2	0.4	0.9
160	5.4	0.4	0.9
140	5.6	0.4	1.1

The second experiment was an investigation of the influence of an increased amount of water in the dough. A change in the flour to water ratio from 1.8:1 to 1.4:1 tended to increase the dietary fibre, from 5.2% to 5.6% of the dry matter (Table 4). This increase was partly explained by resistant starch formation, an increase from 0,9% to 1.1%.

Baking-Powder Bread

One experiment was made with baking-powder as a leavening agent. The intention was to see if the yeast could be responsible for the formation of resistant starch in yeast leavened bread.

The centre temperature of the bread was measured and plotted against baking time (Fig. 3). The increase

Table 5. Dietary fibre and resistant starch in the crumb of bread made with baking-powder

Sample	Centre temperature °C	Dietary fibre	Residual starch	Resistant starch
		mg/100 mg dry matter		
Flour		3.3	0.1	<0.1
Bread *1*	35	3.2	0.2	0.3
2	48	4.0	0.2	0.4
3	81	4.0	0.2	0.6
4	90	4.2	0.3	0.5
5	94	3.4	0.3	0.6
6	95	3.7	0.3	0.7
7	96	3.5	0.3	0.6
8	97	3.8	0.3	0.7
9	97	3.7	0.2	0.7

in temperature was much slower than in the bread with yeast. Maximum temperature was reached after 20 min, compared with 10 min for the yeast bread. The first bread (baked 5 min) was still doughy in the middle, whilst the yeast bread had a spongy bread-like texture after only a few minutes in the oven. Formation of resistant starch was also seen in the baking-powder bread. However, despite the longer baking time, the resistant starch tended to be lower in the baking-powder bread than in the corresponding yeast bread (Table 5).

General Discussion

Retrogradation of the starch in bread crumb is today considered to be the major factor involved in bread staling [13–15]. According to Schoch and French [16] and Kim and D'Appolonia [17] most of the amylose retrogradation takes place during baking and subsequent cooling of the loaf. The amylopectin retrogrades more slowly and is the main factor involved in crumb firming. The resistant starch found in this investigation could be firmly retrograded amylose, but probably not retrograded amylopectin, since no increase in resistant starch was found during storage of the bread.

In a fresh white pan bread the moisture content of the crumb is about 40–45% [15, 18]. In the close vincinity of the crust the moisture content is even lower. Only part of the starch in such bread is gelatinized during baking, and the rest occurs as partially swollen granules. A higher water content in the dough would allow more of the starch to become gelatinized [19]. A white pan bread with a flour to water ratio of 10:7 had 70% gelatinized starch in the centre of the crumb [18], whilst an Egyptain balady bread (flour to water ratio of 10:7.6) had abount 90% gelatinized starch in the middle crumb [19]. They were both analysed by the method of Varriano-Marston et al. [18]. The increase in resistant starch found in the present investigation when increasing the water content supports the hypothesis that cooling of gelatinized starch produces resistant starch [13].

Lipids are known to form stable complexes with amylose [20]. As shown by Holm et al. [20], the complexes were almost completely hydrolyzed by the thermostable enzyme Termamyl, included in the dietary fibre analysis. Hence it follows that the resistant starch cannot be due to amylose-lipid complexes.

Theander [2, 21] found an increase in glucose-based fibre in wheat flour heat-treated at 180 °C. The increase was more pronounced as the time of treatment increased. Four hours processing yielded twice as much glucose in the dietary fibre as did 0.5 h. He also reported a decreasing starch content in over-processed materials. He attributed the increase in dietary fibre glucans to modified and degraded starch. The main part, he says, is probably degraded and chemically modified oligosaccharides containing 1,6-anhydroglucose end-units. These saccharides are short-lived and can probably react with starch or dietary fibre to form new branched structures. It is unlikely, however, that these new structures, are present in bread in any appreciable amounts, since the 1,6-anhydroglucose is formed mainly at low water concentrations. The finding that the dietary fibre increase is lower in the crust than in the crumb [9], and the fact that the temperature is much higher ans water concentration lower in the crust, do not point to anhydroglucosan formation.

In conclusion our results indicate that the resistant starch might be retrograded amylose. This is supported by the time at which it is formed and the fact that no changes occur during storage. The higher amount in bread with more water also supports this hypothesis. The relationship between flour to water ratio and resistant starch needs to be investigated further, however.

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