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A study of changes in wheat protein during bread baking using SE-HPLC

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Abstract

Samples of bread at different stages of baking were divided into three sections viz. crust, center and base. The sections were freeze-dried, ground and sieved, and changes in protein were followed using SE-HPLC. Sonication was used to solubilize proteins in SDS buffer pH 6.9. A decrease in extractable protein with time of baking was shown by the decrease in total area of the SE-HPLC profile. The center section of bread showed the maximum decrease in extractable protein as compared to the crust and base, which could be due to the differences in rate of temperature change as well as moisture content in the different sections of bread. To check the nature of cross-links formed during baking causing insolubility of proteins, a freeze dried sample of crust (20 min baking) was reduced with β-mercaptoethanol (ME) at room temperature. Almost complete reduction of protein into subunits suggested that disulfide bonds are the major cross-links formed during baking.
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Keywords: Bread; Gluten; Changes during baking; SE-HPLC; Reduction

1. Introduction

During baking, different ingredients such as oxidizing agents, salts, etc. are used to improve bread quality. The action of ingredients during baking can be controlled if the chemical and physical changes during the baking process are well understood. It is expected that increased temperature would promote the formation of protein cross-links, causing setting of the loaf during baking. A number of studies have been conducted to study these changes. Slumier (1986, Chap. 7.5) studied the heat and moisture transfer in dough during bread baking. It was established that during baking temperature inside the bread reaches 100 °C in 30 min whereas the crumb starts to form at 70 °C. The effects of heating on gluten at different moisture contents have been studied in detail by Weegels, Verhoek, Groot, and Hamer (1994a, 1994b) who showed that glutenin aggregation affects the extractability of gluten. Danno and Hoseney (1982) showed that the extractability of protein increases during mixing. SE-HPLC examination of SDS extractable protein by Borneo and Khan (1999) has revealed that polymeric proteins (peak 1) tend to decrease, while low molecular weight proteins (peak 3) tend to increase during bread making (up to proofing stage). However the behavior of gluten has been reported to depend on subunit composition (Lefebvre, Popineau, Deshayes, & Lavenant, 2000). Lavelli, Guerrieri, and Cerletti (1996), Ewart (1988) and Kohler, Belitz, and Weiser (1993) have studied the structural aspects of gluten showing that intra- and inter-molecular disulfide bonds of these gluten proteins are important in forming the gluten matrix in dough. Xu et al. (1999) has reported a study on controlled stepwise reduction of disulfide bonds in bread baking, showing at least two types of disulfide bonds. No significant changes were detected during dough preparation; in contrast, baking caused significant modification of flour protein. Menkovska, Pomeranz, Lookhart, and Shogren (1987) showed using HPLC and SDS–PAGE, that the hydrophobic gliadins are more affected during baking.

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Extractability of protein has limited the study of changes in protein during baking. However, with the advent of sonication which can help solubilize protein (Singh, Donovan, Batey, & MacRitchie, 1990) and size exclusion HPLC (Bietz, 1985) it has been possible to extract and study structural changes in protein in a different way (Singh & MacRitchie, 2001). In the present study the molecular changes in protein during baking has been investigated process using SE-HPLC, sonication and reduction.

2. Materials and methods

2.1. General

All chemicals and buffers were commercial products of highest grade. Untreated flour from ADM Milling Company (12.8% protein and 14% moisture) was used for all the studies.

2.2. Bread protein sample preparation

The baking procedure of MacRitchie and Gras (1973) using 35 g of flour were followed with flour (100%) water (60%) yeast (2%) and salt (2.2%). Loaves were baked in duplicate. Breads were taken out of the oven at 4, 8, 12, 16 and 20 min, cooled at room temperature and divided into three sections, crust (2 mm), center (7, 6 mm away from top and bottom) and base (3 mm). The control was the sample after proofing without baking (referred to as 0 min). All samples were freeze-dried, ground in a coffee grinder and sieved (132 μm) to obtain a uniform particle size.

2.3. Size exclusion-HPLC

SE-HPLC was conducted using a HP 1100 system (Hewlett Packard) with automatic injection. Protein was extracted from baked samples (as prepared above, 10 mg/ml) using SDS buffer pH 6.9 (0.5% SDS, 0.05 M Na_2HPO_4). The sample was vortexed and sonicated at 15 W for 45 s using a sonic dismembrator (Fisher Scientific, Pittsburgh, PA). All samples were centrifuged at 3000*g* for 15 min and filtered through a 45 μm syringe filter. The proteins were fractionated on a Biosep-SEC-S4000 column with column volume of 103.5 ml (Phenomenex, Torrance, CA). The eluting solvent was acetonitrile and water (1:1) containing 0.05% TFA at a flow rate of 0.5 ml/min.

2.4. Reduction studies

β-Mercaptoethanol (ME, 5%) was used to reduce the samples at 80 °C for 30 min in a water bath. One sample of fully baked crust was reduced at room temperature by stirring 100 mg/10 ml protein with 50 μl of ME in a small conical flask (25 ml). Samples were taken out at regular time intervals, centrifuged at 3000*g* for 15 min, syringe filtered using 45 μm filters and injected on the HPLC column (20 μl).

3. Results and discussion

3.1. Protein extraction and HPLC study

The SE-HPLC areas of different protein extracts shows a regular decrease in total extractable protein with an increase in the time of baking (Table 1, Fig. 1), indicating formation of polymers during the baking process. Not only were the fractions of protein represented by the peak area at elution times ranging 13–17 min (corresponding to 200–70 k) decreased in the HPLC chromatogram, but the gliadin was also affected as seen

Table 1
The percentage loss in extractability of protein (sonicated 15 W/45 s) as compared to 0 min baking control from different sections of bread at different times of baking

Percentage protein lost in different sections of bread after baking			
Time of baking (min)	Crust	Center	Base
4	10.8	13.8	6.96
8	18.2	38.7	38.4
12	34.1	70.7	49.4
16	50.5	76.2	57.2
20	68.3	80.1	67.1

*Calculated as: lost peak area during baking = area of control − area of baked samples.

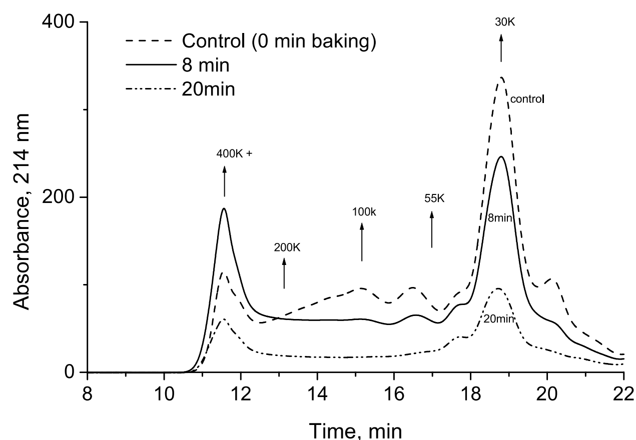

Fig. 1. SE-HPLC chromatograms of the protein extracted after sonication of the crust protein at 6 W/30 s, at different stages of baking control at 8 and 20 min, suspended again in SDS buffer, pH 6.9 and sonicated at 15 W for 45 s. Arrows show the molecular weight range at different retention times. Arrows indicate the molecular weight (e.g. 30 K = 30,000 D) at those retention times.

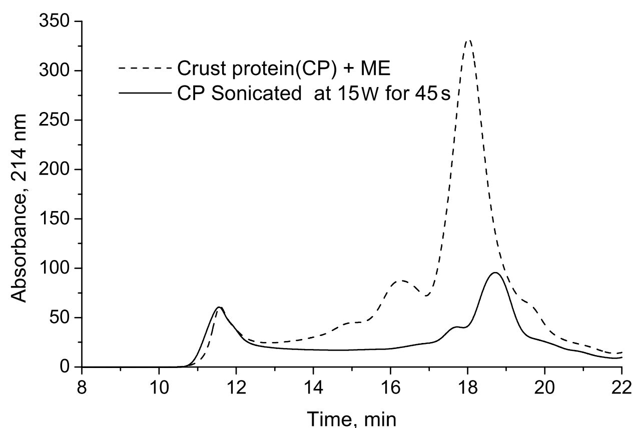

Fig. 2. A comparison of the SE-HPLC profile of crust protein from a fully baked sample sonicated 15 W for 45 s and under reducing condition using β-mecaptoethanol (ME).

by a decrease in area of the peak at 19 min elution time (corresponding to 40 k). A study conducted by Schofield, Bottomley, Timms, and Booth (1983) showed that ω-gliadins, which are known to lack cysteine residues, were unaffected by heat treatment. This suggested that disulfide bond formation is responsible for the polymerization or aggregation reaction during heating. Further, in our experiments, sonication, which may disaggregate polymeric proteins, did not release gliadin (comparing gliadin peak in Fig. 2 (sonicated) and Fig. 1 (0 min)).

To further understand the process, protein in different sections of bread was compared by HPLC using higher power of sonication (15 W for 45 s). For the control (without baking), the total area under the HPLC profile was considered to be the area for comparison after extracting the protein from baked samples. An interesting result was that the soft central part of bread showed a maximum decrease in extractable protein as compared to the crust and base. The temperature-moisture profile may be the determining factor for cross-linking of proteins during baking. The temperature in the center of the bread was 60 °C (5 min baking) and reached 90 °C (20 min of baking).

The unextractable protein (i.e. the protein left after sonication at 15 W for 45 s) was calculated by subtracting the total peak area at a given time of baking from the total peak area of the unbaked control. The unextractable protein increased with time of baking (Table 1).

3.2. Bread protein reduction study

An aim of this study was to investigate the changes of protein cross-linked with disulfide bonds or non-disulfide bonds. The increase in unextractable protein during baking can be ascribed to the formation aggregates or protein cross-linking. Normally sonication can break some disulfide bonds (Khan, Tamminga, & Lukow, 1989; Singh & MacRitchie, 2001). If some other cross-links are present they should be unreducible with ME. The total protein from the fully baked sample (crust) was suspended in SDS buffer and reduced by heating with ME at 80 °C for 30 min and after centrifuging injecting the aliquot on the HPLC column. Comparison of HPLC profiles for sonicated and reduced protein from the crust of fully baked samples is shown in Fig. 2. The total peak area after reduction was almost 2.5 times that obtained with sonication, indicating that not all of the disulfide bonds are broken by sonication.

Reduction is also a chemical reaction to which all the disulfide bonds may not respond in the same way. As shown in a study by Xu et al. (1999), disulfide bonds have different reduction potentials, indicating that there may be a threshold for different kinds of disulfide bonds requiring different amounts of energy to break. Therefore, further investigation of the reduction of crust protein was conducted. The crust protein from the fully baked sample was suspended in SDS buffer containing ME and stirred at room temperature to check the increase in protein solubilization with time. The samples showed very little solubilization of protein immediately after addition of ME. Molecules in the range of dimers and intermediate polymers were least soluble, which suggests that most of these molecules are aggregated or cross-linked to form large polymers (Fig. 3). In 1 h the total peak area increased rapidly in between 17 and 19 min retention time, where LMW subunits and gliadins elute. After 24 and 48 h the area continued to increase. The total peak area of the chromatogram after reduction was equal to that of the control.

The results indicate that disulfide bonds are the major cross-links to form during baking of bread that cause aggregation and polymerization of protein, as shown by

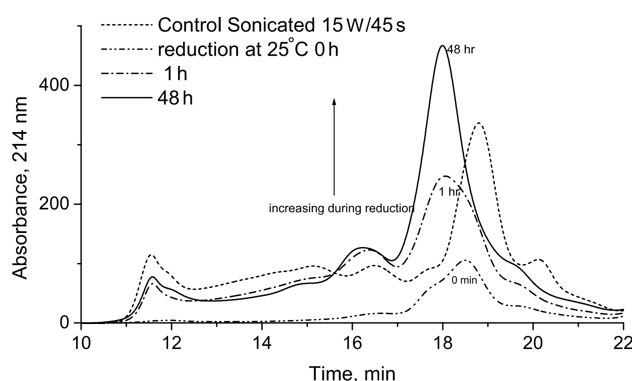

Fig. 3. SE-HPLC chromatograms at different times for study of room temperature reduction of crust protein (100 mg/10 ml in SDS buffer pH 6.9) using β-mercaptoethanol (ME, 50 μl) at different times and its comparison with sample sonicated at 15 W for 45 s.

near complete recovery of solubility following reduction with ME.

4. Conclusion

Decrease in protein solubility due to aggregation and/or cross-linking was observed with time of baking. The effect was greater in the central section than in the crust or base of the bread, indicating that the temperature-moisture profile governs cross-linking during baking. Sonication was not able to break all the disulfide bonds in the protein from the baked samples, indicating very high molecular weights in the insoluble fraction. SE-HPLC showed that ME was able to reduce most of the proteins which indicated that disulfide bonds are mainly responsible for protein insolubility.

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