ELSEVIER

PII: S0308-8146(96)00216-6

Food Chemistry, Vol. 58, No. 1-2, pp. 75-80, 1997

0308-8146/97 $17.00+0.00

Effects of enzyme preparations for baking, mixing time and resting time on bread quality and bread staling

S. Sahlström* & E. Bråthen

MATFORSK, Norwegian Food Research Institute, Osloveien 1, N-1430, Ås, Norway

(Received 26 January 1996; revised version received 2 May 1996; accepted 2 May 1996)

Breads were made using four fungal α-amylase preparations, three different mixing times and three different resting times to study their effect on bread quality and staling. All the four enzyme preparations caused significantly darker crusts. A significant effect of resting time on crust darkness was also obtained. A tendency towards increased bread volume by enzyme addition was observed and the presence of added α-amylases seemed to decrease the effect of resting and mixing time on bread volume.

Bread supplemented with enzyme preparations firmed at a lower rate, while only small effects of enzyme addition on retrogradation of starch, as expressed by enthalpy measured by differential scanning calorimetry (DSC), were observed. After 7 days, enthalpy values were significantly lower for the two shorter resting times.

No significant correlation between crumb firmness and enthalpy per gram starch was found. Storage of bread increased the amount of non-susceptible starch. The lowest increase was observed for breads baked without added enzymes. The effects of mixing time and resting time on non-susceptible starch were slight. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Wheat flour contains various enzymes, such as α- and β-amylases, proteases, lipases, phosphatases and oxidases (Reed & Thorn, 1971). The enzymes are either indigenous (constituents of the wheat), endogenous (produced by micro-organisms naturally present in the flour or as added cultures) or exogenous (added) (Fox & Mulvihill, 1982). The original purpose for supplementing flour with α-amylases was to generate fermentable compounds, mainly maltose (Kulp, 1986), in the dough. However, other changes, such as increased bread volume (Rubenthaler *et al.*, 1965; Mainder & Joergensen, 1983; Kuracina *et al.*, 1987), improved crumb grain (Mainder & Joergensen, 1983; Cauvain & Chamberlain, 1988), crust colour and flavour (Kulp, 1993) are also obtained.

Commercial α-amylases aimed at the baking industry are obtained from cereals, fungi and bacteria. The main difference between these enzymes lie in their temperature optima, the fungal enzymes having the lowest (50–60°C) and bacterial the highest (70–80°C) (Hamer, 1992). In addition to α-amylase most commercial preparations contain various amounts of protease, pentosanase, cellulase and/or oxidase activity. The processing of bread can be divided into three basic operations mixing, fermentation (resting and proofing) and baking. The simplest bread-making procedure is a straight-dough system where all bread formula ingredients are mixed into a developed dough. After resting, the dough is divided into loaf-sized pieces, rounded, moulded, placed on a baking tray, proofed and baked.

When a loaf of bread is removed from the oven after baking, a series of changes starts that eventually leads to deterioration of quality. Those undesirable changes that occur with time are collectively called staling. Very little attention has been paid to the effects of bread process variables on staling. Starch is considered to play a major role in bread staling (Kulp & Ponte, 1981). Schoch & French (1947) suggested that bread staling was caused by heat-reversible aggregation of amylopectin and not by retrogradation of amylose which occurs during the initial cooling period. In accordance with the supposed importance of starch in bread staling, the addition of α-amylases retards staling (Herz, 1965; Kulp & Ponte, 1981). The results of Martin & Hoseney (1991) and Akers & Hoseney (1994) suggest that short chain dextrins, DP 4–9, retard bread staling. Breads supplemented with bacterial and fungal α-amylases firmed at a slower rate than when no enzymes were added. Cereal α-amylases had no antifirming effect and

*To whom correspondence should be addressed.

the extracts from breads treated with these enzymes did not contain the short-chained dextrins. However, the presence of cereal α-amylase activity has been observed to induce a decrease in amylopectin recrystallisation in bread crumb as measured by differential scanning calorimetry (DSC) (Siljeström *et al.*, 1988).

When studying the effect of reheating on the firmness of bread crumb, Ghiasi *et al.* (1984) found that changes in starch crystallinity, as measured by DSC, did not correlate well with changes in staling, measured as crumb firmness. This indicates that firming is not solely a result of amylopectin retrogradation. Factors other than amylopectin retrogradation have been considered as possible contributors to bread staling. Martin *et al.* (1991), suggested that bread staling could be caused by progressive cross-binding between protein and swollen residues of starch granules, mediated by amylose molecules leached out during baking. However, Inagaki and Seib (1992) found that firming occurred even when the starch contained no amylose and they instead proposed that interaction between swollen starch granules and the gluten matrix might occur during ageing of the bread. Siljeström *et al.* (1988), implied that staling, does not affect the availability of starch to pancreatic α-amylases.

The aim of the present investigation was to study the effect of different mixing times, resting times and enzyme preparations on bread quality and to investigate their effect on bread storage, as revealed by bread firmness, DSC and susceptibility of starch to fungal α-amylase. The aim was also to investigate the relationship between bread firming, starch recrystallisation and susceptibility of starch to fungal α-amylase.

MATERIALS AND METHODS

Flour

The flour used was a commercial standard white wheat flour (78% extraction rate), containing 30 ppm ascorbic acid, obtained from Møllesentralen i/s, Oslo, Norway. Water absorption at 500 BU was 56.7%, with dough development time 6 min and dough stability 18 min, as measured by Farinograph (model no 8 101, Brabender OHG, Duisburg, Germany) according to ICC Standard no 115 (1972). Protein and moisture contents were 14.9 and 14.7%, respectively.

Enzyme preparations

The α-amylase preparations used (Table 1) were of commercial quality. The enzymes were added to the dough along with the water. Fungal α-amylases (Grindamyl) were supplied by Grinsted Products A/S (Braband, Denmark). The Panlyve series were obtained from Lyven (Cagny, France). All enzyme products were described by the producers as α-amylases however some of the products were said to contain side activities. The specific α-amylase activity measured with p-nitrophenol maltoheptaoside as substrate (Ceralpha: α-amylase assay kit, Megazyme, North Rocks, Sydney, Australia) (Table 1), showed the same relationship between the products as that given by the producers. In addition, both the flour and the enzyme preparations had β-amylase activity (Betamyl: β-amylase assay kit, Megazyme, Australia) (Table 1).

Test baking

The individual baking experiments were randomised, with one repeat. The recipe is given in Table 2. The water temperature was adjusted to give a dough temperature of 28°C after mixing. The doughs were mixed in a Diosna SP 40 F (Dierks & Söhne, Osnabrück, Germany), for 2 min at low speed followed by either 6, 8 or 10 min mixing at high speed.

The doughs were divided into three and the pieces rested at 25°C for 10, 30 and 50 min, respectively. At the end of the resting period three 300 g and six 600 g pieces were moulded by hand and

Table 1. Commercial enzyme products used in the experiments

Trade name (abbreviation)	Type of enzyme(s)	Specific activity (SKB[a]/g)	Recommended dosage (g/100 kg flour)	Used dosage (g/100 kg flour)	Specific α-amylase activity (units[b]/g)	Specific β-amylase activity (units[c]/g)
Grindamyl A-1000, (GA)	α-amylase	10 000	2–5	5	2 521	5 959
Grindamyl S-100, (GS)	α-amylase, hemicellulase	2 000	10–20	20	500	1 063
Panlyve AMY 10, (PA)	α-amylase	45 000	0.2–1	1	20 413	38 774
Panlyve NP2 (PN)	α-amylase, protease	4 500	1–5	5	1 688	6 027
Standard wheat flour (ST)					0.1	666

[a]Sandstedt-Keen-Blish, SKB unit. Values given by the producers.
[b]One unit of activity is defined as the amount of enzyme, in the presence of excess α-glucosidase and glucoamylase required to release one micromole of p-nitrophenol from p-nitrophenol maltoheptaoside in one minute.
[c]One unit of activity is defined as the amount of enzyme required, in the presence of excess α-glucosidase, to release one micromole of p-nitrophenol from p-nitrophenol maltopentaoside in one minute.

Table 2. Baking recipe

Ingredients	Gram
Flour	10 500
Water	6 000[a]
Yeast	330
Salt	123
Fat, marine oil	330
Enzyme product	as given in Table 1

[a]Optimum as determined by Farinograph.

placed in iron pans (bottom: 80×80 mm, top: 132×132 mm, height: 100 mm) or on a baking-tray, respectively. The loaves were proved for 50 min at 35°C and 85% relative humidity (RH) and baked at 220°C for 25 min in a rotating fan oven (type BEX 1.0 Bago line, Faaborg, Denmark), with steam added during the first 30 s. Before measurements the breads were cooled for 3 h at room temperature. Breads were stored for 1 or 7 days in polyethylene bags at room temperature.

Analysis of bread

Loaf volume was measured on the 300 g loaves using a rape seed displacement method (AACC, 1983, Method 72–10). Crust darkness was determined on the same loaves as 100-L* (100-L* = 0, white and 100-L* = 100, black) from the CIE 1976, L*a*b* system (Francis & Clydesdale, 1977). Crumb firmness was measured on the larger loaves according to AACC (1983), procedure 74–09, using a Texture analyser (Stable Microsystems, Haslemere, England).

Thermal properties of bread crumb were measured by differential scanning calorimetry (DSC) using a Mettler DSC 30S (Mettler Toledo AG, Schwerzenbach, Switzerland) at a scanning rate of 5°C/min. About 30 mg bread crumb was heated from 20–90°C in a steel cell, with an empty cell as the reference. The first enthalpy peak between 50–80°C was used to calculate the recrystallization of starch (Russel, 1983; Eliasson, 1985). Total starch (TS) and starch susceptible to fungal α-amylase at 40°C (SS) in the bread crumb were determined with a Total Starch Assay Kit and Starch Damage Assay Kit, respectively, (Megazyme, North Rocks, Sydney, Australia). Percent non-enzyme susceptible starch (NSS) was calculated as [(TS-SS)/TS]×100.

Statistical analysis

All measurements were performed twice. The data were analysed by ANOVA using the GLM procedure of SAS (SAS, Propietary Software release, 6.08. Copyright 1989 SAS Institute Inc, Cary, NC.). Tukey's test ($p = 0.05$) was performed to determine minimum significant differences (MSD).

RESULTS AND DISCUSSION

Crust darkness

Addition of enzyme preparations caused significantly darker crusts (46.4–46.8) compared to breads without enzymes (39.9). This can be explained by increased formation of reducing sugars when α-amylases are added. No differences were found between the various enzyme products. Thus the proteolytic activity reported present in enzyme preparation PN (Table 1) did not produce enough free amino groups to influence the crust darkness.

Average crust darkness values for resting times, 10, 30 and 50 min were significantly different, 45.2, 47.4 and 43.1, respectively. The higher values for the intermediate resting time may be explained by assuming that, during the initial fermentation, the production of low-molecular weight sugars exceeded that metabolised by the yeast. As fermentation proceeds the amount of yeast increases. Finally, sufficient yeast is present to ferment all the available sugar.

Without added enzyme, no significant effect of mixing time on crust darkness was observed. With added enzyme, there was a slight tendency towards darker crust with decreasing mixing time (PA, mixing time 6 min. crust darkness 49.7, mixing time 10 min. crust darkness, 45.6; GS mixing time 6 min. crust darkness 47.8, mixing time 10 min. crust darkness 42.6). This is contrary to what could be expected from the study of Stear (1990), who found that prolonged mixing results in unwinding of the proteins, making more NH_2-groups available for Maillard reactions. However, since increased mixing time probably results in larger carbohydrate fragments (Hamer, 1992), the changes could be explained if these longer fragments either react more slowly in the Maillard reaction or are less prone to caramelization than low-molecular weight carbohydrates.

Bread volume

Addition of fungal enzymes gave no significant increase in bread volume. However, there was a slight tendency towards increased loaf volume (without enzyme addition 921 ml, with enzyme addition 941–1014 ml). This is in agreement with results reported from other laboratories (Mainder & Joergensen, 1983; Kuracina *et al.*, 1987; Cauvain & Chamberlain, 1988; Valjakka *et al.*, 1993). Cauvain & Chamberlain (1988) attributed this to longer duration of owen rise.

Mixing time significantly affected bread volume only for bread baked without enzymes (Table 3). This is in accordance with claims (Rugbjerg, 1987), that addition of fungal enzyme makes the loaf volume less sensitive to variations in mixing time. No effect of resting time was found.

Table 3. Effect of varying mixing and resting time on bread volume (ml) for the different enzyme products tested

Mixing time	Enzyme preparation[1,2]				
	GA	GS	PA	PN	ST
Resting time 10 min					
6	912	943 a	950 a	930 a	921 ab
8	950 a	1001 a	980 a	968 a	923 ab
10	889 a	1033 a	1011 a	987 a	915 ab
Resting time 30 min					
6	930 a	965 a	969 a	964 a	905 b
8	986 a	998 a	943 a	1000 a	949 ab
10	968 a	999 a	976 a	1144 a	979 a
Resting time 50 min					
6	928 a	1138 a	951 a	908 a	882 b
8	938 a	1023 a	991 a	962 a	896 b
10	967 a	1022 a	973 a	1091 a	919 ab
MSD[3]					72

[1]See Table 1.
[2]Values in the same column followed by different letters are significantly different ($p \leq 0.05$).
[3]Minimum significant difference.

Crumb firmness

A significant difference in crumb firmness of freshly baked breads, was found between enzyme preparations GA and PN (Table 4). Breads supplemented with PN were less firm than breads supplemented by GA. Previously, proteases added to the dough have been shown to give less firm crumb (Stauffer, 1987). This may therefore provide an explanation since PN, but not GA, contains proteolytic activity.

During storage, crumb firmness increased (Table 4). After one day breads containing GS, PA and PN had significantly softer crumbs than bread baked without added enzyme product. This was also the case after 7 days and in addition breads containing GA had become significantly softer than breads without added enzymes. However, GA gave a firmer crumb than GS. Enzyme product GS is reported to contain hemicellulase as side activity (Table 1). Rugbjerg (1987) reported that modification of pentosans can retard staling. Also addition of pentosans has been seen to decrease the rate of staling (Kim & D'Appolonia, 1977). Valjakka *et al.* (1993), observed that 0.015% (based on flour weight) of fungal α-amylase gave a lower crumb firmness after 7 days of storage than 0.02%. In the present study, no effect of enzyme activity level on crumb firmness was observed. No significant effects of mixing or resting time on crumb firmness were found.

Staling as measured by DSC

The retrogradation of starch in bread can easily be followed by DSC-analysis. When stale bread is heated a 'staling endotherm' appears on the DSC thermogram (Russel, 1983) as a result of melting of the crystallised amylopectin (Eliasson, 1985). The enthalpy calculated from this endotherm is proportional to the amount of retrograded amylopectin. The moisture content in bread crumb has been shown to decrease during storage (Herz, 1965) and, therefore, the enthalpy was calculated and expressed as joules per gram starch. For breads with enzymes added, there were tendencies towards lower enthalpy values and thereby less retrogradation, after one day of storage compared with breads without enzymes added (Table 5). However, only PA gave a significantly lower enthalpy value than ST. The effect of the added enzymes disappeared after 7 days of storage. This indicates that the α-amylases do not produce sufficient low-molecular weight dextrins to significantly alter the staling rate.

The major effect on enthalpy values in the present study was due to variations in resting time. Average enthalpy values for all enzyme preparations after 7 days of storage, were 0.598 J/g starch and 0.606 J/g starch for resting times 10 and 30 min, respectively. These values were significantly lower than the enthalpy of 0.620 J/g starch obtained after a resting time of 50 min. Thus, less retrogradation had occurred during storage in breads made with the two shorter resting times. Increased resting time can be expected to reduce the amount of low-molecular weight dextrins (Hamer, 1992), substances which have been shown to retard the firming rate (Martin & Hoseney, 1991; Akers & Hoseney, 1994).

No significant difference in enthalpy values between different mixing times was observed. It thus seems that changes in mixing time are of little importance considering starch retrogradation during storage.

Table 4. Effect of enzyme supplementation on crumb firmness after 3 h, 1 and 7 days

Enzyme preparation[1]	Crumb firmness (N) after 3 h	Crumb firmness (N) after 1 d	Crumb firmness (N) after 7 d
GA	2.1 a[2]	4.8 ab	10.8 b
GS	2.0 ab	4.4 b	9.9 c
PA	2.0 ab	4.3 b	10.5 bc
PN	1.8 b	4.3 b	10.4 bc
ST	2.0 ab	5.5 a	11.5 a
MSD[3]	0.20	0.91	0.66

[1]See Table 1.
[2]Values in the same column followed by different letters are significantly different ($p \leq 0.05$).
[3]Minimum significant difference.

Table 5. Effect of enzyme supplementation on enthalpy, ΔH and non-susceptible starch (NSS) in bread crumb stored for 1 and 7 days

Enzyme preparation[1]	ΔH, day 1 (J/g starch)	ΔH, day 7 (J/g starch)	NSS, day 1 (%)	NSS, day 7 (%)
GA	0.25 ab[2]	0.62 a	47.9 a	72.3 a
GS	0.24 ab	0.60 a	48.2 a	72.2 a
PA	0.22 b	0.62 a	50.3 a	72.9 a
PN	0.24 ab	0.64 a	50.1 a	73.1 a
ST	0.28 a	0.63 a	52.2 a	75.1 a
MSD[3]	0.06			

[1]See Table 1.
[2]Values in the same column followed by different letters are significantly different ($p \leq 0.05$).
[3]Minimum significant difference.

Susceptibility of starch to α-amylase during storage

Storage of bread, from 1 to 7 days at room temperature increased the amount of non-susceptible starch (NSS) in the bread crumb by approximately 45–50% (Table 5). Less availability of starch after bread storage is in agreement with earlier studies (Schultz & Landis, 1932; Jackel *et al.*, 1952, 1953), but contradictory to Siljeström *et al.* (1988) who reported no effect of storage of bread on the availability of starch to pancreatic α-amylases. These differences may be a result of different methods used for measuring starch susceptibility. In the present study storage of bread thus caused increases in both NSS and enthalpy i.e. retrogradation of starch. Except for enzyme preparation GA, no significant effects of mixing and resting time on NSS were detected after one day of storage. For GA, the shortest mixing time (6 min) caused higher NSS (53–55%) than the longer mixing times (8 min, 42.7–43.7% and 10 min, 42.1–48%). After 7 days of storage, no significant effects of mixing and resting time were found, except for PA where 6 min mixing time and 10 (75.4% NSS) or 50 min (74.4% NSS) resting time produced bread crumbs with higher amounts of NSS than when using 10 min mixing time and 50 min resting time (70.4% NSS). This is in general in agreement with the results for GA after one day, and may indicate that enzyme-susceptibility of starch in enzyme supplemented breads are smallest when short mixing times are applied.

CONCLUSION

Crust darkness was significantly affected by addition of commercial α-amylases. Independent of added α-amylase, resting time influences crust darkness, with maximum crust darkness obtained after 30 min. Addition of α-amylase reduced the dependence of loaf volume on mixing time, but gave no significant increase in bread volume. All four enzyme preparations reduced the firming rate, but to a different degree. The α-amylases had only small effects on recrystallization of amylopectin as measured by DSC. Also as stated by Ghiasi *et al.* (1984), the mechanisms governing crumb firmness and the retrogradation of amylopectin seemed to be different. After 7 days of storage, enthalpy for resting times 10 and 30 min. respectively, were significantly lower than for resting time 50 min. Thus, less recrystallization occurred during storage in breads made with the two shorter resting times. The amount of non-enzyme-susceptible starch increased during storage, but was unaffected by addition of α-amylase. Although both firmness, retrogradation and non-susceptible starch increased with time of storage, there is not necessarily any causal relationship. The lack of a causal relationship is supported by the lack of correlations when the different storage periods are kept apart.

ACKNOWLEDGEMENTS

The authors wish to thank Anne Birgit Bævre, Leif Arne Fardal, Åshild Longva and Alf Nielsen for technical assistance.

REFERENCES

Akers, A. A. & Hoseney, R. C. (1994). Water-soluble dextrins from α-amylase-treated bread and their relationship to bread firming. *Cereal Chem.*, **71**, 223–226.

American Association of Cereal Chemist. (1983). Approved Methods of the AACC. Method 72-10, approved April 1961, revised October 1982; Method 74-09, approved October 1986, revised November 1987 and October 1988. The Association: St. Paul, MN.

Cauvain, S. P. & Chamberlain, N. (1988). The bread improving effect of fungal amylase. *J. Cereal Sci.*, **8**, 239.

Eliasson, A.-C. (1985). Retrogradation of starch as measured by differential scanning calorimetry. In *New Approaches to research on Cereal Carbohydrates*. eds R. D. Hill & L. Munck. Elsevier Science, Amsterdam, p. 93.

Fox, P. F. & Mulvihill, D. M. (1982). Enzymes in wheat, flour and bread. In *Advances in Cereal Sciences and Technology*, Vol. 5, ed. Y. Pomeranz. American Association of Cereal Chemistry, St. Paul, MN, pp. 107–156.

Francis, F. J. & Clydesdale, F. M. (1975). *Food Colorimetry: Theory and Applications*. The Avi Publishing Company, Westport, CO.

Ghiasi, K., Hoseney, R. C., Zeleznak, K. J. & Rogers, D. E. (1984). Effect of waxy barley starch and reheating on firmness of bread crumb. *Cereal Chem.*, **61**, 281–285.

Hamer, R. J. (1992). Enzymes in the baking industy. Friends or foes? In: *Cereal Chemistry and Technology: A Long Past*

and Bright Future, ed. P. Felliet. Institute National de la Recherche Agronomique, 9th International Cereal and Bread Congress, Paris, 1992.

Herz, K. O. (1965). Staling of bread — a review. *Food Technol.*, **19**, 90.

Inagaki, T. & Seib, P. A. (1992). Firming of bread crumb with cross-linked waxy barley starch substituted for wheat starch. *Cereal Chem.*, **45**, 339.

Jackel, S. S., Schultz, A. S., Schoonover, F. D. & Schaeder, W. E. (1952). The decrease in susceptibility of bread crumb starch to beta-amylase during staling. *Cereal Chem.*, **29**, 190.

Jackel, S. S., Schultz, A. S. & Schaeder, W. E. (1953). The *in vitro* indigestibility of the starch in fresh and stale bread. *Cereal Chem.*, **30**, 326.

Kim, S. K. & D'Appolonia, B. L. (1977). Bread staling studies. III. Effect of pentosans on dough, bread, and bread staling rate. *Cereal Chem.*, **54**, 225.

Kulp, K. (1986). Influence of liquid ferments on quality characteristics of white pan bread. *Research Department Technical Bulletin* **7**(9), American Institute of Baking, Manhattan, KS.

Kulp, K. (1993). Enzymes as dough improvers. In: *Advances in Baking Technology*, eds. B. S. Kamel. & C. E. Stauffer. Blackie Academic and Professional, London, pp. 152–178.

Kulp, K. & Ponte, J. G. Jr. (1981). Staling of white pan bread. Fundamental causes. *CRC Crit. Rev. Fd Sci. Nutr.*, **15**, 1.

Kuracina, K. A., Lorenz, K. & Kulp, K. (1987). Starch functionality as affected by amylases from different sources. *Cereal Chem.*, **64**, 182.

Mainder, M. & Joergensen, O. B. (1983). Interactions of starch and fungal alpha-amylase in bread making. *Starch*, **35**, 419.

Martin, M. L. & Hoseney, R. C. (1991). A mechanism of bread firming. II. Role of starch-hydrolyzing enzymes. *Cereal Chem.*, **68**(5), 503–507.

Martin, M. L., Zeleznak, K. J. & Hoseney, R. C. (1991). A mechanism of bread firming. I. Role of starch swelling. *Cereal Chem.*, **68**(5), 498–503.

Reed, G. & Thorn, J. A. (1971). Enzymes. In: *Wheat chemistry and technology*, 2nd edn, Vol. 3, ed. Y. Pomeranz. American Association of Cereal Chem, St. Paul, MN, pp. 453–491.

Rubenthaler, G., Finney, K. F. & Pomeranz, Y. (1965). Effects on loaf volume and bread characteristics of alpha-amylases from cereal, fungi and bacterial sources. *Food Technol.*, **19**, 239.

Rugbjerg, U. (1987). Applications of enzymes in baking. II *Meeting on Industrial Applications of Enzymes*, Barcelona, Spain.

Russel, P. L. (1983). A kinetic study of bread staling by differential scanning calorimetry and crompressibility measurements. The effect of added monoglyceride. *J. Cereal Sci.*, **1**, 297.

Schoch, T. J. & French, D. (1947). Studies on bread staling I. The role of starch. *Cereal Chem.*, **24**, 231.

Schultz, A. S. & Landis, Q. (1932). The hydrolysis of starch in bread by flour and malt amylase. *Cereal Chem.*, **9**, 305.

Siljeström, M., Björck, I., Eliasson, A.-C., Lönner, C., Nyman, M. & Asp, N. G. (1988). Effects on polysaccharides during baking and storage of bread — *in vitro* and *in vivo* studies. *Cereal Chem.*, **65**, 1–8.

Standard methods of the international association for cereal chemistry (ICC). (1972). Wienna, ICC Standard no. 115.

Stauffer, C. E. (1987). Proteases, peptidases and inhibitors. In: *Enzymes and Their Role in Cereal Technology*, eds J. E. Krueger, D. Lineback & C. E. Stauffer. American Association of Cereal Chemist, St. Paul, MN, pp. 201–238.

Stear, C. A. (1990). Elements of the baking process and their control. In: *Handbook of Breadmaking Technology*, Elsevier Applied Science, London, pp. 553–595

Valjakka, T.-T., Ponte, J. G.Jr. & Kulp, K. (1993). Studies of a raw digesting enzyme. I. Comparison to fungal and bacterial enzymes and an emulsifier in white pan bread. *Cereal Chem.*, **71**(2), 139–144.