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Effect of added pentosans on some properties of wheat bread*

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The effects of water-soluble (WS-W) and water-insoluble (WI-W) wheat pentosans and water-soluble (WS-R) rye pentosans on some baking characteristics of wheat flour and staling of bread were examined. All three pentosan preparations markedly increased the farinograph water absorption. While addition of WS-W and WS-R (at 2%, w/w) pentosans increased the specific loaf volume, WI-W did not significantly affect this parameter. At a constant dough consistency, pentosan-supplemented breads had higher moisture contents and water activity values. Higher retrogradation rates of the amylopectin, as measured by differential scanning calorimetry, were shown for breads supplemented with pentosans, presumably due to their higher moisture content. Water-soluble pentosans retarded the aggregation process between amylose molecules, as evidenced by the amount and type of water-extractable carbohydrates from bread crumb. Susceptibility of bread crumb to pancreatic α-amylase slightly decreased with the addition of pentosans, particularly upon storage; WI-W was the most effective in this respect.

INTRODUCTION

The nonstarch polysaccharides (pentosans) of wheat and rye, because of their ability to bind large quantities of water, are important functional ingredients in dough and bread systems (Kulp & Bechtel 1963; Jelaca & Hlynka, 1972; Casier *et al.*, 1973). However, there are some controversial reports as to the exact role of pentosans, both in breadmaking and during postbaking storage of baked goods. Casier *et al.* (1973) have shown beneficial effects of water-insoluble pentosans on bread volume and textural properties of the crumb. These observations, however, were not confirmed when US wheat varieties were studied (Kulp & Bechtel, 1963; Hoseney *et al.*, 1971). Jelaca & Hlynka (1972) also reported an increase in the loaf volume when water-soluble pentosans were added. While the starch fraction is generally considered to be responsible for staling of baked products (Kulp & Ponte, 1981), pentosans have been shown to retard the retrogradation of starch gels (Kim & D'Appolonia, 1977) and the staling rate of bread (Kim & D'Appolonia, 1977; Jankiewicz & Michniewicz, 1987), as assessed by firmness measurements. In contrast, the addition of pentosans to starch gels did not change the kinetics of starch crystallization during aging, as measured by differential scanning calorimetry (DSC) (Longdon & LeGrys, 1981).

The main objective of this study was to examine the effect of the addition of three different pentosan preparations, isolated from wheat and rye, on some baking characteristics of wheat flour and the bread staling process.

MATERIALS AND METHODS

Flour

Flour was prepared from the grain of a pure cultivar of Canada Western Red Spring wheat (Katepwa) on a Buhler pneumatic laboratory mill after tempering to 16·5% moisture content. The yield of straight grade flour was 72% (protein content 12·1%, ash 0·42%, total pentosans 0·42%). More detailed chemical, milling and breadmaking characteristics of this flour and dough were reported elsewhere (Michniewicz *et al.*, 1990).

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Preparation of pentosans

Water-soluble (WS-W) and water-insoluble (WI-W) wheat pentosans were obtained from the Katepwa flour according to Izydorczyk *et al.* (1990) and Michniewicz *et al.* (1990), respectively. Water-soluble rye (WS-R) pentosans were isolated from rye, using the procedure of Jankiewicz & Michniewicz (1987). Following enzymatic digestion of starch, the pentosan preparations were heated in a boiling water bath for 30 min to inactivate the enzymes and were then freeze-dried; no residual amylolytic activity was detected in these preparations. The protein content and monosaccharide composition of pentosans were determined according to Lowry *et al.* (1951) and Izydorczyk *et al.* (1990), respectively.

Breadmaking

All microbaking trials were performed according to the AACC straight dough procedure (AACC, 1983; method 10-09), with slight modifications. A 2-h bulk fermentation, a 20-min intermediate proofing, a 40-min proof period at 30°C and a 13-min bake at 220°C were used. After bulk fermentation, the dough was divided, followed by intermediate proof, sheeting moulding, and deposition of dough loaves into pans. The baking formula, based on flour weight, was as follows: 3% bakers' compressed yeast, 2·5% sugar, 1% salt, 0·3% malt, 0·1% phosphate and 15 ppm potassium bromate. All doughs contained 35 g flour and were mixed in a mixograph bowl at an absorption equivalent to a consistency of 500 B.U., previously determined by microfarinograph. WS-W, WI-W and WS-R pentosan preparations, in amounts equal to 2% of flour, were blended in the dry state with the flour before making the dough.

Following baking, the breads were allowed to cool for 30 min and were stored in double plastic bags at 3°C. The moisture content of the bread crumb was determined, using the AACC method (AACC, 1983; approved method 44-15A). The crust was removed from bread at different storage-time intervals. The crust-free bread was frozen immediately in liquid nitrogen and freeze-dried. Preliminary experiments indicated that rapid freezing of crumb in liquid nitrogen did not affect the amount of retrograded starch determined calorimetrically; i.e. the magnitude of the staling endotherm was dependent only on storage time at 3°C. Crumb samples were collected from loaves that were stored for 1 h and 1, 3, 5 and 8 days after baking. The freeze-dried crumb was ground on a Wiley mill to pass through a 0·5 mm sieve and stored at –20°C prior to analysis.

Determination of water activity

Water activity (a_w) was determined for the bread crumb stored for 1, 3, 5, 8 and 10 days by a CX-1 Water Activity System (Decagon Devices, Pullman, WA), as described by Czuchajowska & Pomeranz (1989).

DSC

DSC was performed on freeze-dried crumb samples, using a DuPont 9900 Thermal Analyzer, equipped with a DuPont 910 cell base and a low pressure calorimeter cell, according to the method of Biliaderis *et al.* (1985). Analyses were performed in triplicate using sample weights of 8 to 12 mg (30% aqueous suspensions of freeze-dried crumb). The samples were heated in the calorimeter at a rate of 10°C/min. Apparent enthalpy (ΔH; J/g) and onset and peak temperatures of the staling endotherm were determined using the DuPont software.

Other analytical procedures

Water soluble extracts of freeze-dried bread crumb (0·5 g in 20 ml H_2O) were prepared by mechanical shaking (30 min, 22°C); and centrifugation (5000 × *g*) for 15 min. Total carbohydrates in the extracts were determined by the phenol–sulfuric acid method (Dubois *et al.*, 1956), while pentose-containing carbohydrates were estimated by the Bial's method (Dische, 1962). Absorbances of soluble starch–iodine complexes were measured at 525 and 640 nm, as described by Ebermann & Schwarz (1975). Freeze-dried water-soluble extracts were solubilized in water (20 mg/5 ml) and subjected to gel filtration on a Sepharose CL 4B column (2·6 × 80 cm), eluted with 0·3% NaCl at 20°C. Total carbohydrates (phenol–sulfuric acid), pentoses (Bial's reaction) and absorbance of the iodine–starch complexes were determined on the eluates.

Digestion of starch *in vitro* with α-amylase

The resistance of starch to α-amylolysis was determined, using a porcine pancreatic α-amylase preparation (Type I-A, Sigma Chemical Co., St. Louis, MO). To 500 mg of freeze-dried and ground bread crumb, 0·05M Na phosphate buffer (50 ml), pH 6·9, containing 0·04% (w/v) NaCl and diluted α-amylase preparation (containing 32 or 320 nKat of activity) were added; 1 nKat of enzyme liberates (from soluble starch) reducing power equivalent to 1 nmol/s maltose (pH 6·9; 20°C). Enzyme digestion was carried out at 37°C. Aliquots (0·1 ml) were taken at different time intervals and analyzed for reducing sugars, using 3,5-dinitrosalicylic acid reagent as described by Hostettler *et al.* (1951); maltose was used as standard. The degree of starch hydrolysis was expressed as the percentage of starch hydrolyzed to maltose (Bjorck *et al.*, 1986).

Statistical analysis

Analysis of variance was carried out to determine differences among samples in conjunction with the Duncan's multiple range test.

RESULTS AND DISCUSSION

Baking experiments

Table 1 summarizes the composition of the three pentosan preparations used in this study. Despite the multiple treatments with α-amylase and amyloglucosidase employed during isolation of these materials, appreciable amounts of glucose were detected. Glucose may originate from β-glucans and/or residual α-D-glucans that are not accessible to amylolysis.

The effects of pentosans on the baking performance of Katepwa flour are shown in Table 2. The specific volume of bread was altered by the addition of pentosans, especially for breads supplemented with the two water-soluble pentosan preparations.

The results of the present study confirmed earlier observations (D'Appolonia *et al.*, 1970; Jelaca & Hlynka, 1972) that water-soluble pentosans have a significant positive effect on loaf volume. The addition of WI-W pentosans, on the other hand, did not seem to affect significantly the loaf volume and the structure of bread crumb (Fig. 1). These findings are in contrast to those of Casier *et al.* (1973) and support the findings of Kulp & Bechtel (1963) and Hoseney *et al.* (1971). The reasons for such contradictory results published by various workers may lie in differences in baking characteristics of the base flours of different wheat cultivars, differences in chemical composition of pentosans and the way pentosans were incorporated into the dough.

Table 1. Chemical composition of pentosans

Type	Protein (%)	Molar ratio			
		Ara	Xyl	Gal	Glu
WS-W	6·0 ± 0·1[a]	1·00	0·95	0·55	0·22
WI-W	10·1 ± 0·5	0·67	1·00	trace	0·33
WS-R	4·3 ± 0·3	0·72	1·00	trace	0·49

[a] $n = 3 \pm$ S.D.

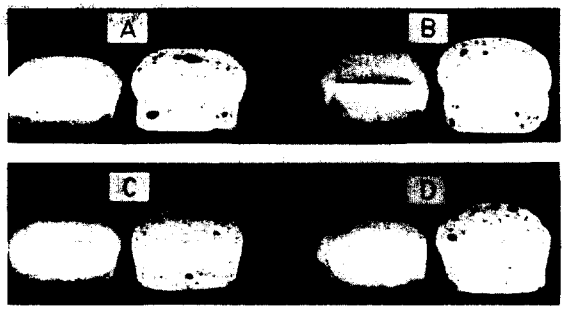

Fig. 1. Minibreads baked from wheat flour (A), and wheat flour supplemented with: 2% WS-W (B), 2% WI-W (C) and 2% WS-W (D) pentosans.

Water activity and moisture content of bread crumb

The relatively low level of moisture in all experimental breads (Table 2) was most likely due to the minibaking procedure used in this work. Addition of pentosans caused an increase in both the water absorption of dough and the moisture content of bread crumbs in comparison with the control samples. The loss of moisture from the breads was faster during the first 3 days than for the remainder of the storage period. It is well known that rapid redistribution of moisture between crumb and crust occurs early during storage (Kulp & Ponte 1981; Czuchajowska & Pomeranz, 1989).

In the context of shelf life stability of control and pentosan-supplemented breads, water activity (a_w) measurements were conducted over an extended period of storage (Table 3); according to Czuchajowska & Pomeranz (1989), substantial changes in this parameter do not occur during the first 24 h of storage. Significant differences in a_w between control and breads supplemented with pentosan preparations were observed. There was also a decrease in a_w for all crumb samples during storage. Despite similar losses in moisture content among the samples, the highest rate in a_w decrease after 8 or 10 days of storage was seen for the breads supplemented with WS-W and WS-R pentosans. It is of interest also to note that the highest water content of dough supplemented with WS-R pentosans (mixed

Table 2. Water absorption of dough, weight, specific volume and moisture content of bread loaves baked from wheat doughs supplemented with several pentosan preparations

Sample	Water absorption[a] (%)	Weight[b] (g)	Specific volume[c] (cm³/10 g flour)	Moisture content in crumb (%)		
				0·5 h	75 h	192 h
Control	62·3	14·3[d1]	63·7[d1]	31·3	28·7	28·1
+2% WS-W	71·3	14·3[1]	74·5[2]	35·2	32·7	31·9
+2% WI-W	71·9	14·5[1]	61·3[1]	34·2	31·9	31·2
+2% WS-R	76·8	14·3[1]	84·8[3]	34·0	31·6	30·7

[a] Measured at 500 B.U. by microfarinograph.
[b] Weighed 30 min after baking.
[c] Measured 30 min after baking.
[d] Means of quadruplicate measurements; values in each group followed by the same numeral (column) are not significantly different ($\alpha = 0{\cdot}05$).

Table 3. Water Activity (a_w) of bread crumb during storage[a]

Sample	Time of storage (days)				
	1	3	5	8	10
Control	0·942^{a1}	0·938^{ab1}	0·937^{ab1}	0·934^{b1}	0·931^{b1}
2% WS-W	0·972^{a2}	0·963^{b2}	0·948^{c3}	0·942^{c4}	0·941^{c2}
2% WI-W	0·969^{a2}	0·966^{a2}	0·963^{a2}	0·957^{b3}	0·954^{b3}
2% WS-R	0·968^{a2}	0·963^{ab2}	0·960^{b2}	0·949^{c2}	0·939^{d2}

[a] Means of quadruplicate measurements; values in each groups followed by the same letter (row) or numeral (column) are not significantly different (α = 0·05).

Table 4. Enthalpy values (J/g) of staling endotherm of bread crumb stored at 3°C[a]

Days	*Control*	*WS-W*	*WI-W*	*WS-R*
0	—	—	—	—
1	0·7^{a1}	1·4^{b1}	1·2^{b1}	1·5^{b1}
3	1·9^{a2}	1·9^{a1,2}	1·9^{a2}	2·5^{b2}
5	2·2^{a2,3}	2·8^{b3}	2·7^{b3}	2·6^{b2}
8	2·6^{a3}	2·7^{a3}	3·6^{c4}	3·2^{b3}

[a] Means of quadruplicate measurements; values in each group followed by the same letter (row) or numeral (column) are not significantly different (α = 0·05).

to optimum) was not reflected in the a_w value of the respective bread crumb. Much smaller changes in a_w upon storage were exhibited by breads supplemented with WI-W. The reasons for the difference in the hydration properties of WS (wheat and rye) and WI-W pentosans may lie in the ability of the former to participate effectively in composite hydrated film networks, together with gluten proteins. On the other hand, hydrated WI-W pentosans are expected to exist as discrete particles in the dough. The exact chemical and structural bases for the hydrating behavior of pentosans are unknown and require further investigation.

Since the water-soluble pentosans markedly increased the specific loaf volume of bread (Table 2), part of their improving effect on firming of bread, as previously reported by Jankiewicz & Michniewicz (1987), may be due to the finer grain of the bread crumb and their contribution to elasticity of the gluten network. Moreover, the higher water absorption of the water-soluble pentosan-supplemented doughs and breads may contribute to a softer crumb texture due to the plasticizing effect of water on the gluten/starch network (Levine & Slade, 1990).

DSC

Because of earlier reports on the retarding effects of pentosans on the firming process of starch gels (Kim & D'Appolonia, 1977), it was of interest to examine starch retrogradation in bread by calorimetry (Table 4). Ordering of the outer short degree of polymerization (DP) chains (*c.* 15) of the amylopectin molecule has been implicated in the development of the staling endotherm at 45° to 60°C (Russell, 1983). The enthalpy of this transition increased most rapidly during the first 3 days of storage and was somewhat slower thereafter. The enthalpy values for the bread crumb supplemented with pentosans were higher than those for control bread. Taking into account the higher moisture content of pentosan-supplemented breads, these data are in accord with the calorimetric findings of Zeleznak & Hoseney (1986) on the moisture content dependence of starch retrogradation. Using wheat starch gels and bread, these authors have shown that between 20 and 40% water there is an acceleration in the retrogradation rate with increasing moisture; within this range, even small changes in water content greatly affected the kinetics of the process.

In the moisture range of 0 to 40%, water acts as a plasticizer of the amorphous starch matrix (as in freshly baked products), enhancing molecular mobility and thereby kinetics of state transformations of starch (Levine & Slade, 1990). It is apparent from those studies as well as from our data that, in assessing the functional role of pentosans in starch recrystallization, it is necessary to take into account variations in moisture content among samples and their influence on the crystallization kinetics. Moreover, the reported reduction in firmness (upon storage) of breads supplemented with pentosans (Kim & D'Appolonia, 1977; Jankiewicz & Michniewicz, 1987) may be a direct consequence of a higher moisture content of these systems (for water-plasticized materials the rigidity modulus is inversely related to the moisture content) rather than reflecting changes in the rate of amylopectin recrystallization.

Means of onset and melting peak temperatures of the transition endotherm of bread crumb samples are shown in Fig. 2. In general, the transition temperatures obtained in the present study were slightly lower than those typically observed for the staling endo-

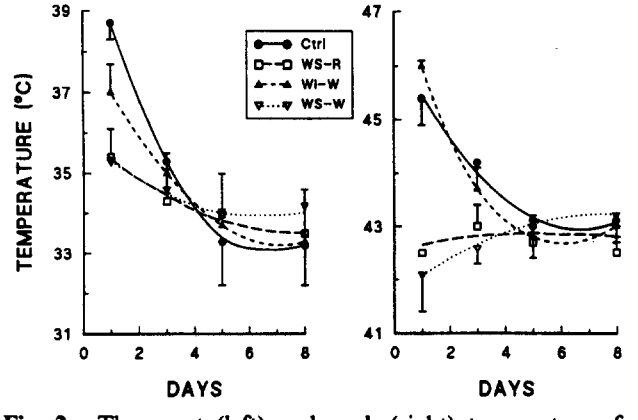

Fig. 2. The onset (left) and peak (right) temperatures for melting of recrystallized amylopectin in bread crumb supplemented with different pentosan preparations and stored for up to 8 days (3°C).

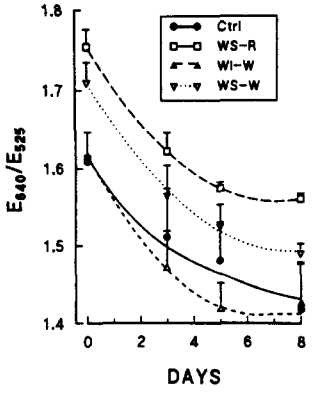

Fig. 3. The values of E_{640}/E_{525} factor for water extracts of bread crumbs prepared from control and pentosan-supplemented (2%, w/w) wheat flours.

therm (Russell, 1983; Zeleznak & Hoseney, 1986; Czuchajowska & Pomeranz, 1989), presumably because of the relatively low (3°C) storage temperature (Soulaka & Morrison, 1985). Although low temperature of storage accelerates the retrogradation rate (Longdon & LeGrys, 1981), the crystallites of retrograded amylopectin are less organized. It was interesting also to note that, in the presence of WS pentosans (both wheat and rye), the onset and peak temperatures of the transition were slightly lower during the first 3 or 4 days of storage in comparison with the control and samples containing WI-W pentosans; a lower melting peak temperature of crystallized amylopectin is indicative of less organized (less perfected) crystallites. After this time, differences among samples were minimized. There were no changes in the region of melting (*c.* 100–110°C) of amylose–lipid complexes on addition of pentosans (data not shown).

In order to examine the changes in the physical state of starch due to aggregation processes involving amylose and amylopectin molecules during storage of bread, the E_{640}/E_{525} ratio was determined for the iodine–starch complex in water-soluble fractions extracted from bread crumb (Fig. 3). The values of this factor are indicative of the relative proportion of amylose to amylopectin in the extracts (Ebermann & Schwarz, 1975). The E_{640}/E_{525} ratios decreased during storage for all bread crumb samples, indicating a progressive insolubilization of the amylose component. The rate of decrease was approximately the same for all samples. However, crumbs of breads supplemented with water-soluble pentosans (rye and wheat) exhibited the highest E_{640}/E_{525} values. Our results are in agreement with earlier observations of Morad & D'Appolonia (1980), which showed that the amount of soluble amylose decreases during storage of bread. They also indicate that water-soluble pentosans, in contrast to insoluble pentosans, inhibit (during storage) interassociations between amylose molecules in the composite

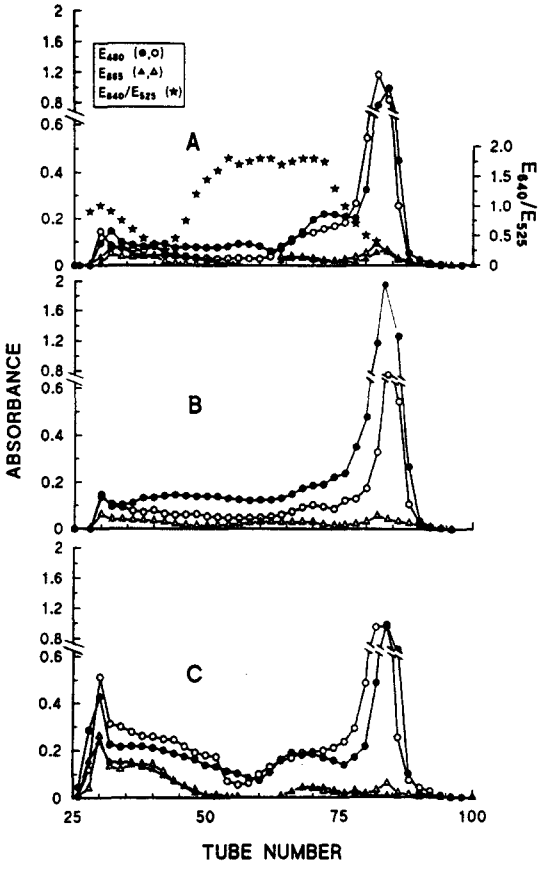

Fig. 4. Carbohydrate elution profiles of water extracts of bread crumb supplemented with pentosan preparations: control (A); added 2% of WI-W (B); added 2% of WS-R (C). Absorbances at 480 and 665 nm correspond to eluting total carbohydrates and pentose-containing fractions, respectively; solid symbols refer to fresh bread extracts, while open symbols correspond to stored bread (8 days, 3°C).

starch/gluten matrix and thereby yield greater amounts of water-extractable amylose.

The gel filtration profiles of water-soluble extracts of bread crumb (Fig. 4) shows several carbohydrate regions; amylopectin elutes around V_0, small molecular weight dextrins and glucose at V_t, while amylose and pentosans have intermediate elution volumes. There were some minor differences between the elution profiles of extracts of different crumb samples, especially in the range of fractions 40–75. The larger proportion of eluting carbohydrates in fractions 25–50 for the WS-R bread (Fig. 4(c)), compared with other samples, is due to the incorporated water-soluble pentosans. Differences in the amount of solubilized carbohydrates between fresh and stored (8 days) bread crumbs were less pronounced in the case of bread containing water-soluble pentosans than for control and WI-W supplemented breads. These findings concur with the data of Fig. 3 and further support the notion that water-soluble pentosans retard, to some extent, the time-dependent aggregation processes of starch (presumably amylose) in bread crumb.

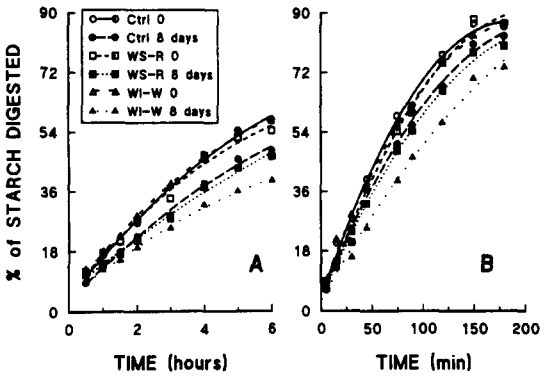

Fig. 5. Susceptibility of bread crumb starch to digestion by pancreatic α-amylase. A, 64 nKat/g of freeze-dried crumb; B, 640 nKat/g of freeze-dried crumb. Solid and open symbols as in Fig. 4.

α-Amylolysis of starch from bread crumb

The starch in aqueous bread crumb suspensions is highly susceptible to hydrolysis by amylolytic enzymes immediately after baking. The effect of WS-W, WS-R and WI-W pentosans on the susceptibility of bread crumb starch hydrolysis by pancreatic α-amylase (at 64 nKat/g freeze-dried crumb) is shown in Fig. 5(a). The degree and rate of hydrolysis of starch in fresh crumb was about the same for the control and pentosan-supplemented samples. Storage of bread for 8 days caused a decrease in susceptibility of starch to α-amylolysis for all samples; the WI-W supplemented bread showed the greatest resistance. Higher concentrations of enzyme (640 nKat/g freeze-dried crumb) caused increases in both the initial rate and extent of starch hydrolysis (Fig. 5(b)); differences in susceptibility between the control and pentosan supplemented samples were less pronounced in comparison with those at the lower enzyme concentration. It would appear from these data that addition of water-soluble pentosans to dough (at 2%, w/w) reduces, only slightly, the degree of α-amylolysis of the crumb.

Finally, the results of this study are in general agreement with the findings of earlier reports (Volz & Ramstad, 1951; Jackel *et al.*, 1953; Jankiewicz & Michniewicz, 1987), which indicated that staling of bread reduces the susceptibility of starch to α-amylase.

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