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Changes in Wheat Starch Crystallinity During Staling of Flat Breads: Effects of Protein on Retrogradation

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ABSTRACT: The crystallinity of wheat starch in two types of flat breads was studied using Differential Scanning Calorimetery. Three types of flours with different proteins of 9.4, 11.6 and 13.5% were prepared and two different types of flat breads with thicknesses of 2 and 3 millimeters were baked from each flour. The crystallization enthalpy (ΔH), peak temperature (Tp) and onset point temperatures (To) of the DSC thermograms were analyzed on the first and 3rd days of storage in all types of breads. Comparison of the results showed that the baking process and time influenced on the extent of starch crystallinity. In spite of the breads with 3mm thickness, the loss of crispiness in breads with 2 mm thickness proceeded over shorter times. In very thin flat breads (thickness of 2 mm), a temperature of about 100 C was rapidly riched, but due to the short time of baking process, starch was not fully gelatinized and the latter caused slower retrogradation upon the storage. The results indicated lower starch recrystallization enthalpy. Under the experimental conditions, high protein breads showed lower extent of retrogradation.

Keywords: *Flat Bread, Protein, Retorgradation, Starch Crystallinity.*

Introduction

Starch is a major component of flour and its gelatinization induced major structural changes during bread baking (Keetels, 1996). The characterizations of starch in bread crumb and staling have been extensively studied (Hug Iten, 1999). Starch seems to be a key factor in the staling phenomenon. Native starches have been presented as semi crystalline granules (Biliaderis, 1992).

Gelatinization of starch occurs when foods are heated in water. During the gelatinization, starch granules swelled and lost their molecular order. (Carcea, 1996).

During the baking, the starch was gelatinized, and the semi crystalline structure was changed into an amorphous structure. During cooling and subsequent storage of the bread, the crystalline structure of the starch was recovered at the short range scale, which was commonly known as retrogradation (the recrystallization of gelatinized starch). This process caused significant changes in the mechanical properties of the crumb and affected the sensory appreciation (Keetels, 1996). This is, however, not a case for very thin flat breads. In this case, the presence of intact and non swollen starch granules has been reported (Primo martin, 2007).

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Retrograded starch is the most important and common starch fraction in breads from the nutritional and technological points of view. Processing techniques and storage conditions may affect both gelatinization and retrogradation (Garica Alonso, 1999).

Several works showed that bread staling was closely associated to starch retrogradation (Danonan, 1979; Cauvain, 1998). Some other works suggested that the mechanism of bread staling, deals with the interactions between starch granules and proteins, and it was indicated that the staling rate can be delayed by increasing the amount of protein that will delay the retrogradation of starch components (Martin, 1991).

Prediction of the starch crystallinity level during storage was evaluated by several methods, such as wide-angle X-ray diffraction (Abd Karim, 2000), DSC (Differential Scanning Calorimetery) and HNMR relaxometery. DSC was recently used to investigate the crystallization and retrogradation of starch (Abd karim, 2000).

In this work the crystallinity of starch in flat breads with different thicknesses which were produced by three different flours varied in protein contents, was studied.

The crystallinity of starch was determined using DSC technique. In addition, the state of starch (gelatinization and retrogradation) in very thin flat breads and normal flat breads produced from three different flours was studied.

Materials and Methods

Chemical Analysis of flours

Three types of flours with 9.4. (P_1), 11.6 (P_2) and 13.5% (P_3) proteins were prepared and the protein, ash and moisture content of flours as well as farinograph test were determined and carried out according to AACC procedures; 46-12,08-01, 16-44 A, and 54-21 respectively (AACC, 2000).

Bread baking and formulation

Two flat breads with the thicknesses of 2 mm (B_2) and 3 mm (B_3) were prepared from flours. Flat breads were prepared at the baking lab using wheat flour (l000g), dry yeast (4g) and salt (15g) in the formulation (Primo martin, 2007).

All ingredients except water were blended at low speed (150 rpm) for 1.5 min. Next, water was added and mixed at medium speed (200-220 rpm) for 6 min until final dough was prepared. After mixing, the dough was allowed to rest for 45 min and was then divided and rounded. The dough was allowed to rest for 12 min. Proofing was performed at 30-32°C and 80% RH until a fixed volume of gas was produced. Breads with 2 mm thickness were baked at 330°C for 75 sec and Breads with 3 mm thickness were baked at 315°C for 90 Sec. Baked breads were allowed to cool at ambient temperature (25°C). Breads were packed in sealed polyethylene films and stored for 1 and 3 days in plastic bags (25°C).

Differential Scanning Calorimetery (DSC)

30±5 mg of bread samples were weighed in a hermetically sealed aluminum pan and analyzed. The samples were heated at 25-200 °C at the rate of 5 °C/min. The onset (T_o) and peak (T_p) temperatures and the transition enthalpy (J/g) of recrystallization (retrogradation) were calculated. Rate of enthalpy increase was calculated by:

$$\Delta(H_3 \quad H_1) = \Delta H_3 \quad \Delta H_1$$

Statistical analysis

The results were means of the analyses of 3 replicates. The significance of each treatment in the samples was analyzed using the analysis of variance (ANOVA), and if ANOVA was significant, Tukey test and 2 tailed T-test (significance of differences at P<0.05), were used. SPSS V.13 was performed at the 95% confidence level.

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Results and Discussion

The relative recrystallization of starch (showed by ΔH) in B_2 and B_3 breads is shown in Table 1.

The DSC thermograms acquired in 1 day storage and 3 days storage showed that the wheat starch recrystallization enthalpy was increased during the storage time in both samples which showed retrogradation. However, the amount of retrogradation in staled breads differed considerably for the two kinds of breads. Retrogradation of starch in B_3 was higher as compared with B_2 breads.

This can be explained by the fact that during baking of B_2, starch is not fully gelatinized due to the short time of baking therefore the semi gelatinized starch, regains its crystallinity slower, during the short time of storage, and the ΔH ,which shows the recrystalization enthalpy (or retrogradation), is lower.

In B_3 breads, which were thicker and having crumb, the baking time was longer and the starch had more time to be gelatinized. The gelatinized starch in B_3, regained its crystallinity much faster and during the short time of storage, showed higher recrystallization enthalpy (Table 2) (Primo Martin, 2007).

Table 1. Enthalpy variations during storage

Sample	Storage time	ΔH (J/g)
B_2	1 day storage	193.47^a
	3 days storage	341.34^b
B_3	1 day storage	318.35^a
	3 days storage	481.35^b

ΔH: Recrystallization enthalpy showing retrogradation
**All values are the means of three replicates.*
** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

Table 2. Enthalpy variations in B_2 and B_3 breads

Storage time	Sample	ΔH (J/g)
1 day	B_2	193.47^a
	B_3	318.35^b
3 days	B_2	341.34^a
	B_3	481.35^b

**All values are the means of three replicates.*
** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

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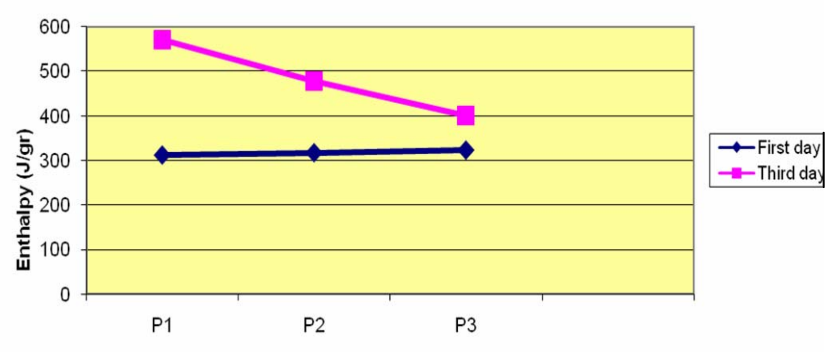

Fig. 1. Effect of bread storage on enthalpy and retrogradation.
P_1: Flour with 9.4% protein
P_2: Flour with 11.6% protein
P_3: Flour with 13% protein

Table 3. Peak and onset point temperatures of breads during the storage time

Sample	1 day storage		3 days storage	
	T_p (°C)	T_o (°C)	T_p (°C)	T_o(°C)
B_2	96.79^a	63.48^a	103.63^a	72.26^a
B_3	102.45^b	96.79^a	112.03^b	85.125^b

Tp: peak temperature, T_o: onset point Temperature
**All values are the means of three replicates.*
** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

Figure 1 Shows the effect of bread staling on enthalpy or retrogradation and also indicates the increase in enthalpy during bread staling (Ribotta, 2007).

During the storage, crystals have time to be recovered and regain their crystallinity. Therefore by increasing the storage time, there could be much more recovered crystals which need high energy for melting (ΔH) (Jocobs, 1998; Keetels, 1996).

Table 3 shows the peak temperature and onset point temperatures in B_2 and B_3 breads.

The typical DSC thermograms of the starch-water mixture showed three endothermic peaks (Fukuoka, 2002). The first peak (55-60°C) corresponded to the gelatinization of starch crystallities. The second peak (about 65-75 °C) was the melting of remaining crystallities and the third peak (about l00°C) had been attributed to dissociation of amylase- lipid complex.

DSC thermograms for bread samples were a little different. B_3 bread thermograms showed a single peak at about l00°C, which was due to dissociation of recovered crystals (recrystals). The absence of the first and second peak, and the presence of the third peak at the temperatures about l00°C indicated that the starch had completely been gelatinized during baking of B_3 breads.

B_2 thermograms showed a peak at lower temperatures than B_3, which corresponded to the starch crystals that had not been gelatinized during the baking (Table 3). DSC thermograms of the flours showed a gelatinization peak at about 65-70°C while DSC thermograms of bread showed the melting of remaining crystals (which had higher melting temperatures and could not

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be gelatinized during the baking) or the melting of recrystals (that needed the highest melting temperatures).

The difference in the melting temperatures in thermograms could be attributed by the occurrence of different crystalline granules that differ in stability.

The extent of starch gelatinization was governed by the moisture content and temperature history (Fukuoka, 2000; Cunin, 1995)

During baking of B2 bread, due to the short time of baking which was not sufficient to gelatinize all starch granules, only the less stable crystals would be gelatinized. The lower peak and onset point temperatures in B_2 bread showed the presence of remaining and ungelatinized starch granules that were melted faster than recrystallized granules.

B_3 Bread had higher peak and onset point temperatures than B_2. This fact showed that due to the longer time of baking, more starch granules were gelatinized, therefore in these breads after the storage time (1 day and 3 days) most crystals were recovered crystals, which were stronger and had higher melting temperatures, reflecting in high peak and onset point temperatures.

The effect of gluten on the extent of retrogradation was studied by DSC (Tables 4 and 5).

It was found that the recrystallization enthalpy of starch as measured by DSC, was decreased with increasing levels of protein (Eliasson, 1995).

This was explained by the distribution of water within the mixed system (Table 6).

Table 4. Enthalpy variations in B_2 bread produced by flours having different protein contents in both days of storage

	Enthalpy (J/g)	
Sample	1 day storage	3 days storage
P_1	220.02 ^{c}	400.52^{b}
P_2	172.25^{a}	316.39^{a}
P_3	196.29^{b}	328.39^{a}

P_1: flour with 9.4% protein, P_2: flour with 11.6% protein, P_3: flour with 13% protein.

**All values are the means of three replicates.*

** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

Table 5. Enthalpy variations in B_3 bread produced by flours having different protein contents in both days of storage

	Enthalpy (J/g)	
Sample	1 day storage	3 days storage
P_1	312.69^{a}	571.42^{c}
P_2	317.68^{a}	478.29^{b}
P_3	323.62^{a}	400.24^{a}

**All values are the means of three replicates.*

** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

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Table 6. Farinograph results

sample	Farinograph parameters			
	Water absorption (%)	Dough development time (min)	Dough resistancy (min)	Dough softening (20 min)
p_1	64.05[a]	1.875[a]	2.25[a]	117[ab]
p_2	66.05[b]	3.625[b]	3.62[b]	117[a]
p_3	74.50[c]	3.625[b]	4.50[c]	110[a]

**All values are the means of three replicates.*
** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

As can be seen, increasing the protein content of flours caused an increase in the water absorption of flour. Therefore, protein could alter the amount of water available to the starch. (Eliasson, 1997; Osella, 2007). It might be suggested that this was due to the dilution of starch by increasing protein, and therefore the retrogradation was decreased (Ottenhof, 2004).

Table 7 showed that the minimum and maximum enthalpy (retrogradation) was observed in breads which were produced by P_3 and P_1 flours respectively.

Table 7. Total enthalpy variation of breads produced by flours having different proteins

Sample	E_T (J/g)	
	B_2	B_3
P_1	310.27[c]	442.05[c]
P_2	244.32[a]	397.99[b]
P_3	262.24[b]	361.93[a]

E_T: Total enthalpy
**All values are the means of three replicates.*
** In each column averages with the same characters (a,b,..) have no significant differences at 5%level according to Tukey test.*

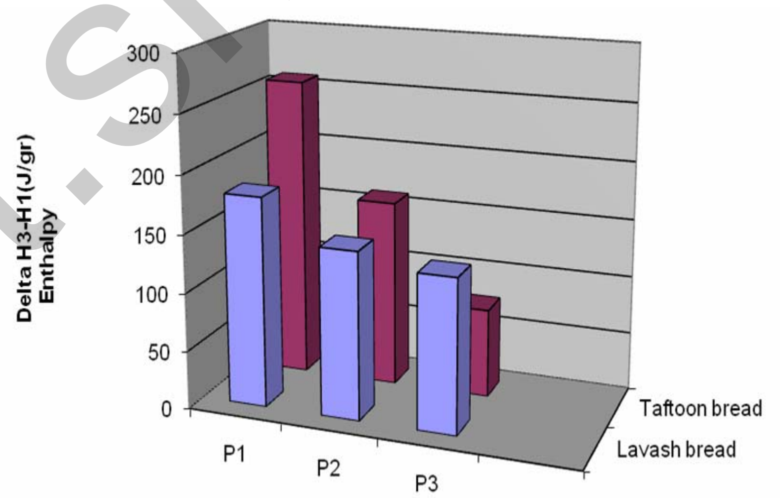

Fig. 2. Rate of enthalpy changes in B_2 and B_3 breads having different proteins

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Figure 2 Shows the rate of enthalpy increase $\Delta(H_3-H_1)$ in two kinds of breads produced by different protein contents. It was recognized that the recrystallization enthalpy was lower in B_2 bread as compared to B_3, and also this figure shows that high protein flours produce breads with lower ΔH and retrogradation.

Conclusion

The results showed that the recrystalliztion enthalpy, the peak temperature (T_p) and the onset point temperature (T_o) were lower in the thin flat breads (B_2) as compared to the thicker one (B_3). It was indicated that due to the shorter baking time for thin flat bread (B_2), starch granules could not be fully gelatinized and consequently ΔH, T_p and T_o were lower. DSC results indicated the lower recrystallization enthalpy (ΔH) for breads with higher protein contents.

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