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A DSC study of the effect of ascorbic acid on bound water content and distribution in chitosan-enriched bread rolls during storage

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Abstract Changes of tightly and loosely bound water relative content in bread were studied using differential scanning calorimetry method. Combination of chitosan with ascorbic acid changes water redistribution between starch and gluten and in such a way can be related to bread quality and sensory properties. The decrease of the water vaporization temperatures, melting temperatures and enthalpies in fresh bread containing chitosan were detected when ascorbic acid was added in combination with chitosan. The decrease of melting peak temperature has been attributed to the increase of interaction of loosely bound water and the decrease of vaporization peak temperature has been attributed to the decrease of interaction of tightly bound water with bread components as a result of ascorbic acid addition. Freezable water amount and total water amount in crumb decrease more rapidly during first stage of staling and more slowly at the second stage of staling in the bread nutritionally fortified with chitosan.

Keywords Bread · Chitosan · Ascorbic acid · Bound water · Staling

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Introduction

Differential scanning calorimetry (DSC) is an appropriate method to determine freezable and nonfreezable water contents in food products from water melting and evaporation endothermic peak areas and temperatures [1–4].

Staling of bread is a highly complex chemical and physical process in a highly complex heterogeneous unstable dynamic system. The precise mechanism of staling is not yet fully understood. Various mechanisms of bread staling have been proposed [5] and conflicting results are reported. Evidently during bread staling, multiple mechanisms operate at different sites and at different times. Water plays a critical role in bread staling [1, 3, 6–9]. One important mechanism appears to be migration of moisture. Softening of the crust and hardening of the crumb are related to moisture redistribution (crumb-to-crust migration) during storage [3, 8, 9].

The role of antistaling additives may be to function as plasticizers, and/or to retard the redistribution of water between components [5].

Chitosan was considered as an additive in a number of food products [10, 11]. Chitosan, similarly to other dietary fibers, significantly reduces the risk for many human diseases. Chitosan was reported to exhibit antibacterial, antioxidative, and health beneficial effects [10, 11]. Chitosan significantly decreases low-density lipoprotein cholesterol while increasing high-density lipoprotein cholesterol [12–14]. It can reduce blood serum cholesterol levels with no apparent side effects and can be used as a nutritional supplement to lower plasma lipid level. Cholesterol-lowering therapy reduces the risk of coronary heart disease. The combination of chitosan with ascorbic acid enhances chitosan's fat binding, as reflected by increased fecal fat excretion [15]. Ascorbic acid promotes the dissolution of

chitosan in the stomach and intestine and its transformation into a fat absorbing gel. The presence of ascorbic acid in the lower digestive tract also lowers its pH and results in reducing the risk of large bowel cancer development.

However, flour replacement by dietary fibers to promote beneficial physiological effect is often accompanied by adverse technological effects [16] including reduction in starch availability for gelatinization and decreased loaf volume. In food technology in dough systems, addition of ascorbic acid to the flour increases bread loaf volume [17]. The sulfhydryl-disulfide interchange reaction is used to explain the action of ascorbic acid. The network of disulfide bonds formed in the gluten structure plays a significant role in retaining the carbon dioxide produced during fermentation and results in higher volume and improved texture [18]. So the use of ascorbic acid in combination with chitosan can synergistically improve both technological properties of bread and health beneficial properties.

Chitosan changes water migration and redistribution between starch and gluten and in such a way increases the rate of bread staling [19]. Water properties also change over bread storage time and can be related to food quality and sensory properties [20]. The interactions between water and macromolecules in food products affect food quality. Water is bound to macromolecules with differing strengths and in different amounts. Information on water binding and distribution within the food is, therefore, important while studying quality changes during processing and storage. During bread storage, moisture migrated from the crumb to the crust, which was associated with the firming of the crumb. It was found that freezable water content and total water content in bread crumb decrease during staling more rapidly in the presence of chitosan. Two stages of bread crumb staling were indicated. Chitosan increases the rate of bread staling during both stages. It was suggested that during bread staling chitosan accelerates water migration from crumb to crust, prevents amylose–lipid complexation, and increases dehydration rate both for starch and gluten [19].

The objective of this study was to study the effect of the synergistic combination of chitosan with ascorbic acid on water properties in nutritionally enriched fresh bread and the changes of the water properties and tightly and loosely bound water relative content in the bread over storage time.

Materials and methods

Sample preparation

Dough was prepared based on the following formulation: 500 g wheat flour, 35 g sugar, 17 g dry yeast, 8 g salt, 10 g butter, 10 g chitosan, 2 g ascorbic acid, and 260 g water.

Chitosan with viscosity 29 cP and degree of deacetylation 97% was kindly supplied by Primex (Siglufjordur, Iceland). Dough mixer "MONO" (Swansea, UK) was used to make dough at two stirring rates during 10 min. The dough was allowed to proof for 25–30 min at 38 °C and relative humidity 72% and cut to pieces with weight 65 g. The rolls were baked in confectionery oven "MONO" (Swansea, UK) at 200 °C for 12 min. The rolls were then cooled at room temperature for 30 min and packed in polypropylene bags.

Thermal analysis

Differential scanning calorimetry thermograms were obtained using a differential scanning calorimeter DSC "Mettler 300" (Mettler-Toledo AG, Schwerzenbach, Switzerland). Approximately 10 mg bread samples were placed into aluminum pans, and then a lid was secured by crimping. The pans were not hermetically sealed but rather crimped in such a way as to prevent pressure buildup and to allow vaporized moisture to escape easily from the pan during the heating cycle. The samples were placed in the sample compartment at $T = 25$ °C and cooled to $T = -50$ °C at 10 °C/min and held at $T = -50$ °C for 5 min. Samples were then scanned from $T = -50$ °C to $T = 25$ °C with a heating rate of 5 °C/min to determine the melting endotherm of water and then scanned from $T = 25$ °C to $T = 150$ °C with a heating rate of 10 °C/min to volatilize the water. Each measurement was performed in triplicate.

Results and discussion

Bread contains water under confined conditions both in discontinuous starch and continuous gluten phases. The behavior of such a system will depend on characteristics of the dispersed and the continuous phases, and on the interactions between them as well as on the migration and redistribution of water. The experimental results show that, to prevent staling, it is more important to slow down the dehydration phenomena rather than to increase the initial moisture content in the bread and a correlation between bread staling kinetics and water migration was proposed [9]. The water at interphases in the bread crumb is more mobile and accelerated migration of moisture from crumb to crust contributes to the bread staling by tightening crumb structure and increasing formation of hydrogen bonds between protein and starch [5, 21].

Water in various polymer and biopolymer systems, including food products, can be subdivided into free water (bulk water), which freezes at the usual freezing point and is not influenced by the biopolymer surfaces, the unfrozen bound water (tightly bound water), which does not freeze

and intermediate water (loosely bound water), which freezes below the usual freezing point. In the vicinity of a polar or dipolar region of a macromolecule, the dipolar water molecule has a non-random average orientation and thus a lower potential energy than it does in bulk. The surface of polymer molecules affects the energy and orientation of nearby water molecules. This disturbance of the attracted water extends several molecular diameters from a hydrophilic surface [22]. The loosely bound water is connected to the hydrophilic polymer groups and melts at a lower temperature due to its interaction with the polymer [23]. It was shown that in low moisture cereals and cookies water mobility (and not the amount of water) in the bread crumb can change due to various chemical interactions in the system (hydrogen bonding, starch retrogradation, and glassy/rubbery equilibrium) [24]. The temperatures of melting can determine the type of water—bulk water or loosely bound water. The water with melting temperature 0 °C was considered as free bulk water. Bread has no free water-melting temperatures are well below 0 °C. If melting temperature was shifted to lower temperatures the water was considered as loosely bound water, and if water do not freeze, but can be detected due its vaporization, such water was considered as tightly bound water.

Figure 1 shows typical water vaporization endotherms in fresh bread enriched with chitosan in combination with ascorbic acid and in bread enriched with chitosan in combination with ascorbic acid after 7 days storage at room temperature. Bread can be considered as interpenetrating network of biopolymer macromolecules—starch and gluten. It was shown that enthalpy of water is proportional to equilibrium water content for polymer networks swollen by water [25]. The same relationship was also observed for water in cheese spreads [26].

Temperatures of endothermic vaporization peaks can be related to the mobility of non-freezable tightly bound water and temperatures of endothermic melting peaks can be related to the mobility of freezable loosely bound water. The mobility of bound water characterized by the decrease of the temperature of vaporization peak increases over storage time for control bread, as shown in Fig. 2. Decrease of the temperature of vaporization peak in the fresh bread containing ascorbic acid can be due to the formation of disulfide bonds in the gluten in dough system. The network of disulfide bonds formed in the gluten structure enables the dough to retain carbon dioxide produced by the yeast and improves loaf volume and bread texture [27]. Decrease of the temperature of vaporization peak over storage time, Fig. 2, can be associated with the decrease in the interaction of tightly bound water with crumb components. Decrease of enthalpy over storage time can be associated with the decrease of the amount of tightly bound water in crumb over storage time, Fig. 3. So the content of tightly bound water and its interaction with gluten and starch molecules decreases in the presence of ascorbic acid.

Decrease of interaction of tightly bound water with crumb components as well as decrease of amount of tightly bound water is more intensive during first 2 days of storage in the presence of chitosan. In control bread, the processes of dehydration did not finish during first 2 days.

Prevention of water sorption by starch due to preferable interaction of chitosan with starch granules during first 2 days of staling can be proposed to explain these experimental data. Similarly to the effect of surfactants on moisture migration from the crumb to the crust [28], in the experiments, the adsorption of polycationic chitosan onto the starch granule surface and the complex formation between starch and chitosan prevented starch from taking

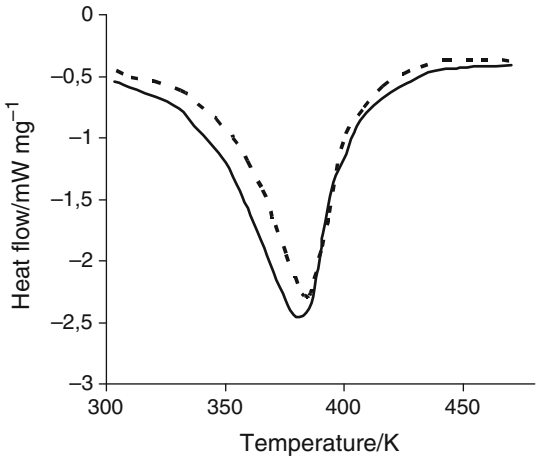

Fig. 1 Typical water vaporization endotherms in fresh bread enriched with chitosan in combination with ascorbic acid (*solid lines*) and in bread enriched with chitosan in combination with ascorbic acid after 7 days storage at room temperature (*dotted lines*)

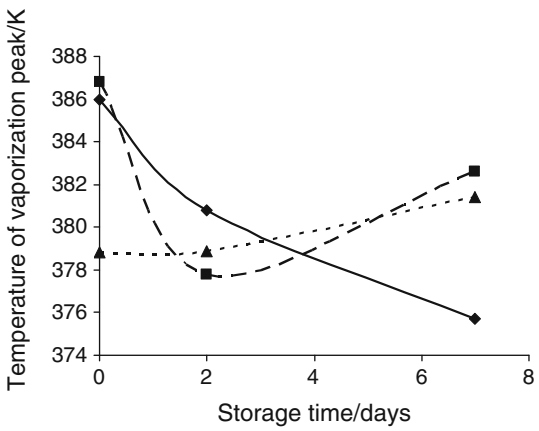

Fig. 2 Changes of the temperature of the endothermic vaporization peak for the water in bread crumb over the storage time at room temperature: control bread (*filled diamond*); bread containing chitosan (*filled square*); bread containing chitosan in combination with ascorbic acid (*filled triangle*)

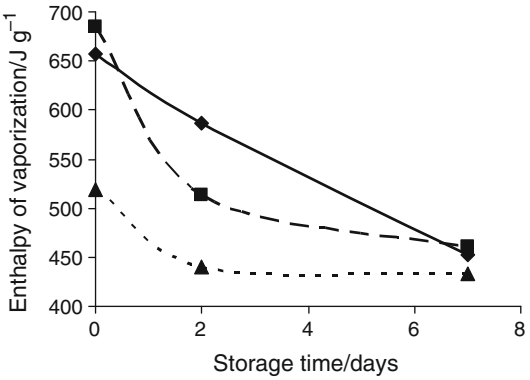

Fig. 3 Changes of the enthalpy of the endothermic vaporization peak for the water in bread crumb over the storage time at room temperature: control bread (*filled diamond*); bread containing chitosan (*filled square*); bread containing chitosan in combination with ascorbic acid (*filled triangle*)

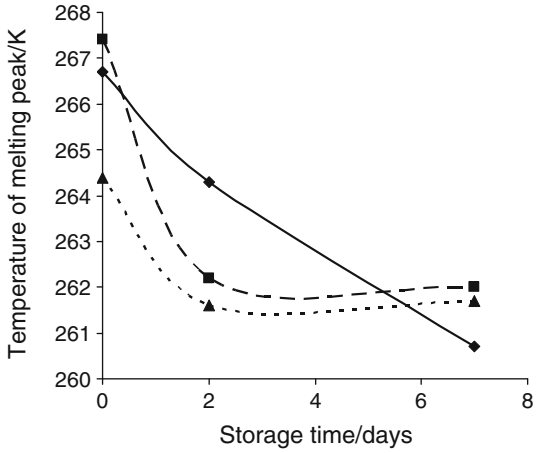

Fig. 4 Changes of the temperature of the endothermic melting peak for the water in bread crumb over the storage time at room temperature: control bread (*filled diamond*); bread containing chitosan (*filled square*); bread containing chitosan in combination with ascorbic acid (*filled triangle*)

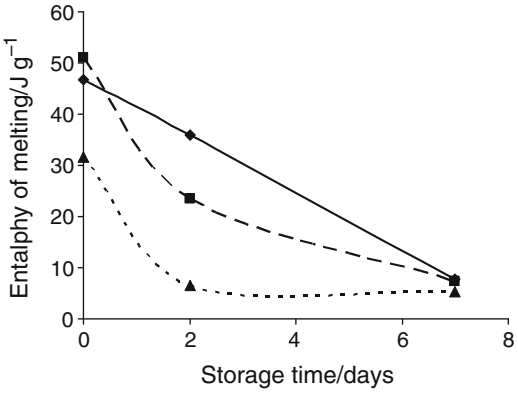

Fig. 5 Changes of the enthalpy of the endothermic melting peak for the water in bread crumb over the storage time at room temperature: control bread (*filled diamond*); bread containing chitosan (*filled square*); bread containing chitosan in combination with ascorbic acid (*filled triangle*)

up water released from gluten during bread aging. This water migrates to the crust of the bread. At the same time, chitosan prevents release of water from starch granules. This water affects the rate and extent of starch retrogradation process [29].

During the second stage of staling, changes of vaporization enthalpy are less intensive in the presence of chitosan compared to the changes of vaporization enthalpy for control bread. The temperature of vaporization peak increases in the presence of chitosan and decreases for control bread during second stage of staling. So it can be supposed that in the presence of chitosan the total amount of water decreases, but its interaction with gluten increases, because only tightly bound and less mobile water molecules remain in gluten in the second stage of staling.

The decrease of the temperature of melting peak in fresh bread containing ascorbic acid, Fig. 4, can be due to a stronger interaction of loosely bound water with fresh bread components and the decrease of melting enthalpy, Fig. 5, can be due to a decreased amount of the water loosely bound to fresh bread components.

So the DSC data show a weaker interaction of tightly bound water and a stronger interaction of loosely bound water with fresh bread components in the presence of ascorbic acid.

Decrease of the temperature of melting peak over storage time, Fig. 4, can be associated with the increase in the interaction of loosely bound water with crumb components. During storage of bread, some water migrates from amorphous to crystalline starch, where it is more tightly bound [21]. Decrease of enthalpy over storage time can be associated with the decrease of the amount of loosely bound water in crumb over storage time, Fig. 5.

Increase in the interaction of loosely bound water with crumb components as well as decrease of the amount of loosely bound water is more intensive during first 2 days of storage in the presence of chitosan.

Temperature of melting peak and enthalpy of melting decrease during the second stage of staling of control bread, Figs. 4 and 5, indicating increase of interaction of loosely bound water with crumb components and decrease of loosely bound water content. But temperatures of melting peak do not change during second stage of staling for breads containing chitosan and chitosan in combination with ascorbic acid, indicating no changes in the interaction strength of loosely bound water with gluten. Enthalpy of melting decreases slower for bread containing chitosan if compared with control bread and enthalpy of melting for bread containing chitosan in combination with ascorbic acid do not change.

Conclusions

Two stages of bread crumb staling have been observed. It was found that in the presence of chitosan freezable water content and total water content in bread crumb decrease more rapidly during first stage of staling and more slowly at the second stage of staling.

The use of the combination of chitosan with ascorbic acid as synergistic natural health beneficial additive for fresh bread results in the decrease of the water vaporization and melting temperatures peaks and the enthalpies of water vaporization and melting in fresh bread. So it is possible to conclude about the more pronounced decrease of water content during baking if ascorbic acid is used as health beneficial additive in fresh bread compared to control bread.

The decrease of melting temperature is due to the increase in the interaction of loosely bound freezable water with bread components and decrease of vaporization temperature is due to the decrease of interaction of tightly bound nonfreezable water with bread components as a result of ascorbic acid addition.

During second stage of staling changes of vaporization enthalpy are less intensive in the presence of chitosan compared to the changes of vaporization enthalpy for control bread. Temperature of vaporization peak increases in the presence of chitosan and temperature of vaporization peak decreases for control bread during second stage of staling. So it can be supposed that in the presence of chitosan the amount of water decreases but its interaction with gluten increases during second stage of staling because only tightly bound and less mobile water molecules remain in gluten at the second stage of staling.

Water redistribution is an important characteristic for the staling of bread and therefore knowledge of the water redistribution obtained from experimental DSC data could lead to a better understanding of the impact of ascorbic acid addition (in combination with chitosan) on the staling rate and related mechanisms.

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