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**RESEARCH REVIEW**

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# Role of Water in Bread Staling: A Review

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**Abstract** Bread is an essential food consumed worldwide. Bread rapidly loses its desirable texture and flavor qualities associated with freshness through a process known as staling. The shelf life of bread is limited by this staling leading to economical losses in the range of one billion dollars per year. There are a number of mechanisms thought to be related to the staling process, such as water migration and redistribution, starch retrogradation, and gluten transformation. In this review, roles of water and water migration on bread staling are summarized and discussed.

**Key words:** bread, staling, water, water dynamics, starch retrogradation

## Introduction

Bread is a composite material with heterogeneous continuous and discontinuous phases. Understanding molecular-structure functions of the components involved in the staling process is needed. In the previous article, textural analysis for bread staling has been summarized and discussed (1). This review focuses on the role of water on bread staling and analytical methodology for water dynamics in bread staling.

Distribution of water among various regions maybe one of the key factors and the direction of water migration in molecular level, for example, from starch to gluten or *vice versa*, could be another important factor influencing physical properties during bread staling. Microscopic pictures reveal that starch is embedded on a continuous gluten matrix in bread (2-4). Starch crystals in staled bread are in fact a discontinuous phase, which embedded within a continuous and amorphous matrix of starch and gluten. From the material research stand point, bread is a composite foam material with both crystalline and amorphous immiscible components. Characterization of bread and describing it by a single glassy-rubbery transition is therefore hardly adequate. There is a clear need to further characterize the composite and phase separated material.

Dehydration is one of the key problems in staling (5). The firming rate was found to slow down at higher moisture content. Separation of crumb from crust to minimize moisture redistribution maintained the crumb moisture during storage but it did not prevent firming of crumb (6). Vodovotz *et al.* (7) reported the difference of thermomechanical properties between dried and aged breads in which the aged breads show a broader overlapping tan δ transition.

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Schoch and French (8) reported that bread staling was mainly related to the retrogradation of starch. However, bread staling is not synonymous with starch retrogradation (9-14). Bread firming might be caused mainly by the formation of cross-links between partially solubilized starch and gluten proteins due to moisture loss (6,13).

It is generally known that the bread crust becomes softer and more leathery during staling, probably due to moisture migration from crumb. In addition to crumb-to-crust migration, water can be transferred to and from each component. Willhoft (15) and Breaden and Willhoft (16) reported that gluten underwent a transformation releasing water from gluten to starch. On the other hand, Whistler and Daniel (17) suggested that water was expelled from the starch matrix to neighboring molecules due to starch retrogradation. Changes in gluten and starch lead to moisture redistribution and alter the degree of binding between water molecules and these macromolecules. Thus, the physical states of water may change during bread staling (18).

Leung *et al.* (19) studied water mobility during bread staling by pulsed $^{1}H$ nuclear magnetic resonance (NMR). They found an overall decrease in water mobility and an increase in water binding. They suggested that some water became more bound as retrograded starch entrapped some water molecules in a starch crystalline lattice. Wynne-Jones and Blanshard (20) also supported this. Kim-Shin *et al.* (21), however, found the opposite using $^{17}O$ NMR. While they observed a significant decrease in water mobility as measured by $T_2$, this was not due to the incorporation of the water molecules into the starch crystallization structure that develops upon staling. They suggested more change was occurred within the amorphous domains of gluten matrix. Recently, Chen *et al.* (18) studied the water mobility in bread during staling by using pulsed $^{1}H$ NMR. They suggested that more than two kinds of water states existed, indicating a dynamic structural transformation of macromolecules and microscopic migration of moisture in the staling bread.

## Molecular Interaction of Water with Starch and Gluten

Water mobility has an important effect on the overall mobility and structural properties of metastable food polymer systems (22). Although they interact with one another by hydrogen bonding, water molecules are in a dynamic state and show very rapid translational and rotational movements. A water molecule can rotate about its own axis once every 3 picosecond ($10^{-12}$ sec). In addition, it can exchange position with another molecule in the water matrix, on a scale of femtosecond ($10^{-15}$ sec) (23).

While pure water has a dielectric relaxation correlation time ($\tau$) of about 10 psec, water bound to macromolecules have a residence time in the sub-picosecond range (24). Furthermore, when all possible factors affecting $^{1}H$, $^{2}H$, and $^{17}O$ relaxation times in NMR experiments were evaluated including chemical exchange, short-range pertubation of water tumbling rate and its anisotropy are both affected by interactions with macromolecules (25). This short-range interaction involves water in 2 molecular layers on solid surfaces. This interacted water population may be associated with macromolecules by a variety of possible mechanisms such that it behaves thermodynamically or kinetically different from bulk water. So-named 'bound' water reflects a microenvironment, on a pico- to micro-second time frame, that has a characteristic density, viscosity, and rotational and translational motions that are different from those of pure water (24).

Since starch and gluten are the main structural components of bread and dough, their hydration behaviors are of great interest. The effects of water on starch and gluten are manifested by the complex chemical nature of both polymers and hence they have complex interaction with water (26). Plasticization by water can lead to an increase in intermolecular space or free volume, a decrease in viscosity, and an increase in polymer mobility (27). Not all water is intimately mixed with starch or gluten as these two polymers are poorly water-soluble. Although starch and gluten seem plasticized by water, water is not necessarily in homogeneously mixed with these two polymers. Thus, a marked discrepancy may be observed between the mobility of water and starch. For instance, water in a glassy starch, which has been earlier proposed to be immobile and rigid like the polymer, was reported to be highly mobile (26). This was supported by Umbach *et al.* (28) who found that a small amount of water in dry starch was very tightly associated, but additional water did not interact with starch and thus remained quite mobile. They also reported that, once further hydrated, there was more water-gluten interaction. Li *et al.* (26) applied solid state NMR proton relaxation to investigate mobility in starch and gluten. It was obvious that the molecular mechanism of water-gluten interaction was distinct from that of water-starch interaction. At <2% moisture content, water in gluten was found to be in a relatively immobile phase (26). Changes in gluten and starch during staling lead to moisture redistribution and alter the degree of binding between water molecules and these macromolecules. Thus, the physical states of water may change during bread staling. However, water-starch and water-gluten interactions are still controversial and need more investigation.

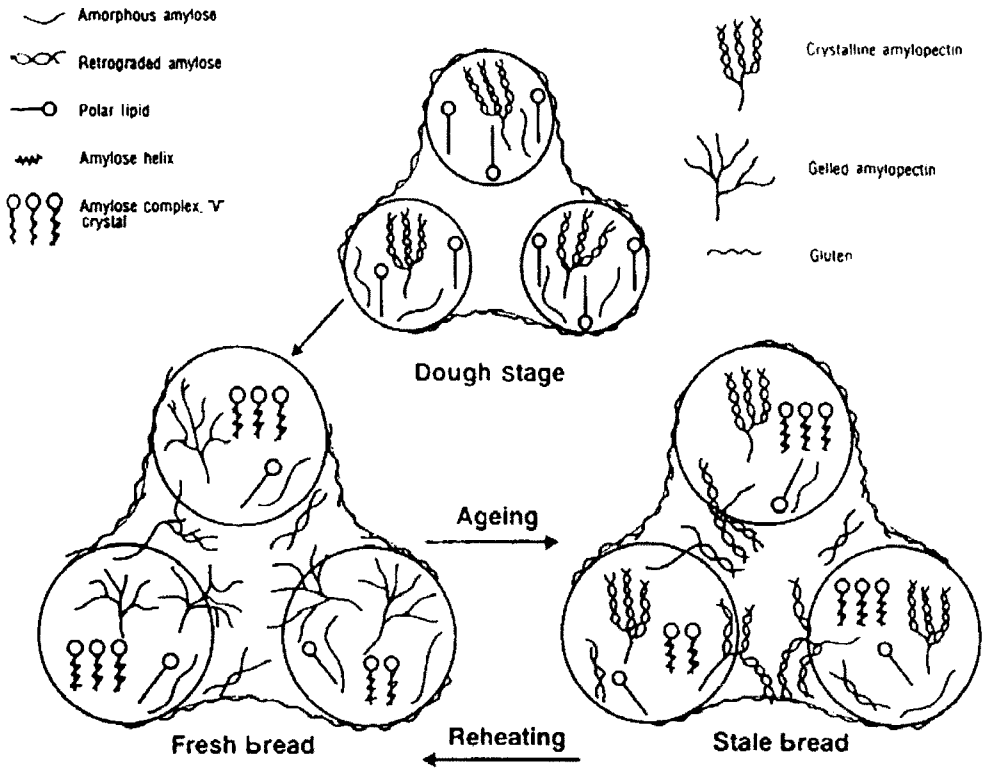


**Fig. 1. A proposed model for breadcrumb staling showing the molecular structures present in the dough stage, fresh bread, stale bread, and bread refreshed by heating [Zobel and Kulp (29)].**

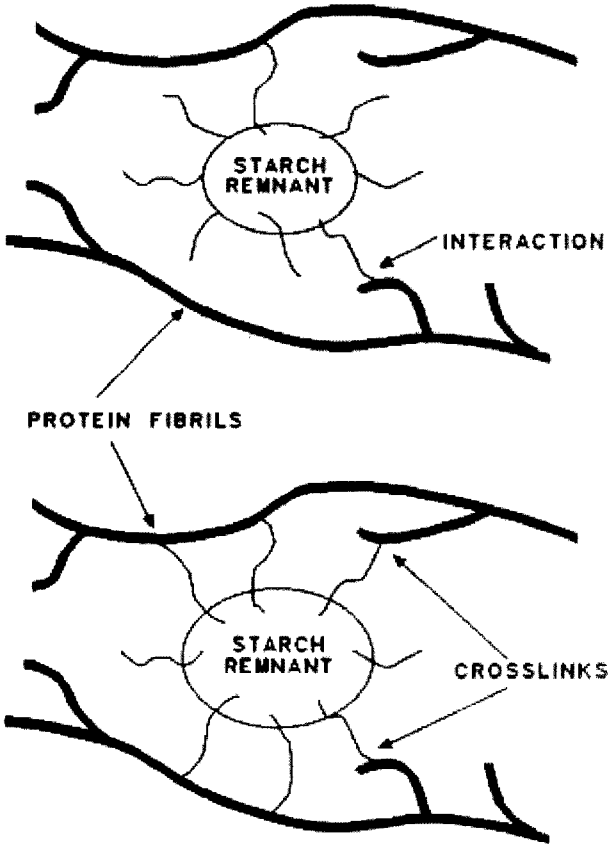


**Fig. 2. A proposed model for breadcrumb firming showing the role of starch swelling and cross-links between starch remnants and gluten [Martin *et al.*(13)].**

## Proposed Bread Staling Models

Bread staling can be defined as loss of freshness, such as loss of aroma, loss of crumb softness, and development of crumbliness during storage, which makes bread less acceptable to consumers. Reported bread staling mechanisms are very complex. Many factors, such as water migration and redistribution, starch retrogradation, and gluten transformation are believed to be related to bread staling.

Zobel and Kulp (29) proposed a bread staling model, which mainly focused on the role of starch in bread staling (Fig. 1). In the dough stage, the amylopectin fraction exists as crystalline. During baking, amylopectin crystallinity is disrupted, permitting granule gelatinization and swelling to occur. With these changes in the granule, portions of the amylopectin molecules have the freedom to expand into the intergranular space. In fresh bread, amylopectin molecules lose their crystalline structure and some amylose and amylopectin molecules leach out from granule. At this point, some amyloses form complexes with lipid and/or aggregate with each other. The stale bread shows amylopectin reorganization into a more crystalline structure during aging. This amylopectin reorganization imparts rigidity to both the swollen granule and intergranular material by acting as physical cross-links in the overall gel structure (29).

On the other hand, Martin *et al.* (13) proposed a different bread firming model (Fig. 2). In this model, they emphasize the role of cross-links between swollen starch granule and gluten. Fibrils of gluten serve as the continuous phase and the discontinuous phase is the starch remnants and partially leached amylose. They suggest that cross-links between starch and gluten reveal breadcrumb firmness. According to this theory, less swollen starch has relatively less surface area exposed to gluten and fewer or weaker cross-links occur with gluten; therefore the firming rate is reduced. Fully swollen starch has more and stronger cross-links with gluten and firms faster (13).

In fresh bread, there is a high moisture gradient between crumb and crust. During storage, moisture migrates from crumb to crust and breadcrumb starts to lose moisture. The loss of moisture greatly increases breadcrumb firmness and starch recrystallization. Breadcrumb firming and starch recrystallization still happen at a relatively slow rate (6,30) even when the moisture of breadcrumb is maintained by storing bread after removing the crust. This indicates that prevention of moisture loss from crumb to crust can only retard bread staling and in addition to drying, water plays an important role on bread staling. Since starch and gluten have different water binding capacity, moisture migration between starch and gluten has been investigated but the theories are still controversial. One group suggested that moisture migrates from gluten to starch (15,19,20,31). They suggested that starch changes from the amorphous state to the crystalline state during staling. Water is needed for the crystallization of starch. As water is incorporated into crystalline structure, water molecules become immobilized. The other group suggested that water migrates from starch to gluten (29,32,33). They suggested that the moisture sorption capacity of starch gel decreased with aging but that of gluten did not change. Therefore, they concluded that starch expelled water to gluten. Another group suggested that water becomes immobilized during staling. However, starches at different degree of crystallization did not show any differences in water mobility so that most of moisture redistribution would have to occur mainly in amorphous region (21).

## Role of Water and Water Redistribution on Bread Staling

The water migration or redistribution during bread storage clearly plays a role in staling. Moisture in bread can affect crumb firming in a number of ways, 1) drying or moisture redistribution, 2) recrystallization of starch, 3) aging or maturing of amorphous network, 4) gluten properties etc.

Most of the work on bread firming has dealt with the role of starch and its retrogradation. The fact that bread becomes firm approximately at the same time as retrogradation suggests that two phenomena might be related (12). Schoch (34) attributed the firming of breadcrumb during staling to physical changes in the amylopectin fraction of the wheat starch granules. However, several researchers have shown that bread firming is not synonymous with starch retrogradation (5,9-14).

An obvious omission from that traditional view of bread staling is the role of gluten. Ponte *et al.* (35) and Maleki *et al.* (36) showed that bread firming was greatly affected by the protein (gluten) content of flours. Maleki *et al.* (36) suggested that the rate of firming was related to the protein quality of the flour. Martin *et al.* (13,14) proposed a bread

firming mechanism featuring interactions (cross-links) occurring between gluten and starch during baking. During staling, as the crumb lost kinetic energy, interactions increased in number and strength. However, bread firming mechanism is multivariate and its relationship to the abovementioned phenomenon of starch retrogradation or starch-protein cross linking does not satisfactorily explain this process.

Longton and LeGrys (37) reported that recrystallization in starch gels is profoundly influenced by gel moisture. They noted that crystallinity reached maximum development in 50% gels and disappeared altogether in very dilute (10%) or concentrated (80%) gels. This differential scanning calorimetry (DSC) data supports the X-ray diffraction studies of Hellman *et al.* (38), who found that 50% gels produced the most intense X-ray pattern but that pattern intensity decreased at higher and lower concentrations. Zeleznak and Hoseney (39) reported similar results with above reports that the greatest recrystallization occurred in the 50-60% starch gels and there was little crystallinity present in the dilute or very concentrated gels. They also suggested that the crystallinity was controlled by the water present during retrogradation and that gelatinization moisture had little or no effect. Baik *et al.* (40) also found the highest crystallinity at 60% starch gels in their study on recrystallization of waxy and non-waxy rice starch gels.

It has been found that the moisture content was inversely proportional to the rate of firming (6,12,41,42). However, water loss is not the only cause of the changes in bread during storage. It has been reported that the crumb moisture during storage can be maintained with an appropriate packaging but the firming of the crumb cannot be prevented (6). This suggests that prevention of moisture redistribution in bread during storage can not stop firming of bread.

A moisture gradient between the center and the edge of a loaf leads to water migration, which affects the kinetics with various contributions. Differences in thermal and moisture history among various locations in bread are inherent factors that are simplistically avoided by studying bread staling only at the crumb center (43). The moisture content, water activity, and enthalpy changes (by DSC) in the crumb center and near crust zones of bread during storage have been determined (30). They suggested that thin crust bread could retard the staling by reducing the moisture transfer from near crust area to crust. Slowing down the dehydration rate was more effective in retarding staling than increasing the initial moisture content in the bread (5). It has been suggested that water acted as a plasticizer for amorphous regions, and may affect the rate and mode of bread staling but the changes in bread during storage could not be explained only by the differences in moisture content (44).

Willhoft (45) suggested that crumb firming involved a loss of moisture from the gluten to the starch phase, whereas Cluskey *et al.* (32) insisted that moisture might migrate from starch to gluten in staling bread. Water has been reported to become more bound upon ageing of bread (19-21). This was earlier explained as a result of an incorporation of water into the crystalline starch structure developed over time. Water in crystallized starch has low mobility and thus is more bound (19). Recrystallization of amylopectin within swollen starch granules leads to the development of a partially crystalline structure with B-type crystalline regions. B-type starch crystal is a higher moisture crystalline type, which has 36 water molecules/

**Table 1. List of instrumental analysis for bread staling used in this review**

| Textural analysis | Reference No. | Thermal and thermomechanical analysis | Reference No. | X-ray diffraction analysis | Reference No. | NMR analysis | Reference No. |
|---|---|---|---|---|---|---|---|
| Scoch and French (1947) | 8 | Longton and LeGrys (1981) | 37 | Zobel and Senti (1959) | 9 | Leung *et al.* (1983) | 19 |
| Cluskey *et al.* (1959) | 32 | Zeleznak and Hoseney (1986) | 39 | Dragsdorf and Varriano-Marston (1980) | 10 | Wynne-Jones and Blanshard (1986) | 20 |
| Ponte *et al.* (1962) | 35 | Czuchajowska and Pomeranz (1989) | 30 | Zobel (1988) | 46 | Umbach *et al.* (1992) | 28 |
| Maleki *et al.* (1980) | 36 | Le Meste *et al.* (1992) | 51 | | | Taub *et al.* (1994) | 58 |
| Ghiasi *et al.* (1984) | 11 | Hallberg and Chinachoti (1992) | 57 | | | Kim-Shin *et al.* (1996) | 21 |
| Rogers *et al.* (1988) | 12 | Slade and Levine (1993) | 27 | | | Li *et al.* (1996) | 26 |
| He and Hoseney (1990) | 6 | Aynie *et al.* (1994) | 52 | | | Ruan *et al.* (1996) | 47 |
| Martin *et al.* (1991) | 13 | Chinachoti (1994) | 53 | | | Chen *et al.* (1997) | 18 |
| Martin and Hoseney (1991) | 14 | Taub *et al.* (1994) | 58 | | | Li *et al.* (1998) | 55 |
| Larsen and Greenwood (1991) | 43 | Vodovotz *et al.* (1996) | 7 | | | Gomi *et al.* (1998) | 59 |
| Xu *et al.* (1992) | 41, 42 | Davidou *et al.* (1996) | 44 | | | Baik and Chinachoti (2003) | 63 |
| Rao *et al.* (1992) | 2 | Schiraldi *et al.* (1996) | 50 | | | | |
| Piazza and Masi (1995) | 5 | Baik and Chinachoti (2000) | 60 | | | | |
| Ruan *et al.* (1996) | 47 | Baik and Chinachoti (2001) | 61 | | | | |
| Baik and Chinachoti (2002) | 62 | | | | | | |

unit cell (12 glucose residues), while A-type starch requires an incorporation of 4 water molecules/unit cell (46). Thus, this recrystallization is partly responsible for moisture migration from the amorphous to crystalline regions. Once incorporated, water is less available as a plasticizer.

Kim-Shin *et al.* (21) found a slight decrease in the average $^{17}O$ NMR molecular mobility of the relatively free fraction of water during bread staling. About 20% of the mobile water became more bound (i.e., became undetected by NMR). While, this finding supported earlier works (19, 20,31), it was found that only a small part could be accounted for as water of crystallization in the starch (21). They also found that bread with varying degrees of amylopectin crystallization did not show differences in the degree of change in water mobility. Thus, they concluded that the redistribution took place mostly in the amorphous regions.

Ruan *et al.* (47) considered separate fractions of water molecules in bread during staling based on distinguishable $^1H$ NMR mobility by using a 2-component model, consisting of $T_2$ in msec range and $T_2$ in μsec range. They reported that as storage time increased, the mobility of the less mobile water fraction decreased whereas the mobility of more mobile water fraction increased upon staling, suggesting that water molecules with different mobility may contribute to the firming process in different ways, depending upon their interactions with the macromolecules. Macromolecules may expel some water molecules as a result of molecular structural changes, causing an increase in the water fraction with higher mobility. At the same time, macromolecules may incorporate some water molecules into the crystalline structure resulting in a decrease in the water fraction with lower mobility (47). It has been reported that wheat flour which contain 0.53 g water/g solid presents 2 distinct $T_2$ relaxation time (54 and 12 msec) by using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (48). Additionally, D'Avigon *et al.* (49) measured the $T_2$ relaxation time of work free wheat flour dough (45% moisture) using CPMG pulse sequence in $^1H$ and $^2H$ NMR. They obtained three $T_2$s in the range of 0.4, 5.6-6.0, and 12.7-20.6 msec. However, a single $T_2$ value (8.0 msec for 0.57 g water/g solid) for breadcrumb has been reported (19). Chen *et al.* (18) suggested that $T_2$ of the so-called bound-water in the food matrix may be in the range of a few to tens of μsec and an one pulse experiment is possible to detect the very short $T_2$ that may be associated with macromolecules. Therefore, they measured the various range of $T_2$s using an one pulse experiment and CPMG pulse sequence and obtained three $T_2$s in the range of 10 and 300 μsec from one pulse experiment and 2.5 msec from CPMG pulse sequences. However, interpretation of NMR relaxation data is complicated and need more investigations.

Starch retrogradation (due to amylopectin crystallization) could be affected by the local presence of water resulting in heterogeneously variable firming rate (12). Rate of firming was reported to be more rapid under conditions where starch did not retrograde suggesting that starch retrogradation may not be directly responsible for crumb firming. It has been suggested that water molecules would be displaced along polymer chains acting as sliders of an interchain zipper and bread firmness would depend on the formation of crosslinked network rather than amylopectin crystals (50). Other researchers insisted that nucleation and propagation modes of starch crystals might be different in a breadcrumb and in a starch gel (44). Therefore, bread firming during storage cannot be explained by starch retrogradation or by the moisture content alone.

Le Meste *et al.* (51) and Aynie *et al.* (52) described the influence of water on the viscoelastic behavior of white bread using thermal mechanical analyzer (TMA) and dynamic mechanical analyzer (DMA), respectively. Depending upon temperature and moisture content, different regions in the products exhibited different textural characteristics, i.e., the glassy state, the glass transition region, and the rubbery plateau. Above 25% moisture content, freezable water might be present and frozen sample begins to melt at the same range of temperature as the glass transition temperature of the amorphous matrix. Thus, the overall change in mechanical properties was a result of both glass transition of the amorphous regions of bread and melting of ice (7,53). Therefore, increasing moisture above Wg' will have no further plasticizing effect on the Tg of polymer-water system. Wg' is the amount of unfrozen water in a highly viscous solid. This water in the freeze concentrated solute-water glass is rendered unfreezable, on a practical time-scale (54). Unfreezable water in glassy starch is highly mobile on NMR time scale and not vitrified in viscous glassy state of starch. Thus, starch chain may be molecularly rigid in a glassy state, water in the surrounding is mobile (55,56).

Hallberg and Chinachoti (57) and Taub *et al.* (58) investigated the effect of glycerol on glass transition temperature (Tg) of bread by using DMA. They observed 2 major peaks in DMA thermograms indicating distinct immiscible phases (glycerol and gluten phases) which exhibit some interaction. The presence of glycerol affects the starch and gluten Tg leading to a change of water-glycerol interaction thereby altering the glycerol Tg (58).

Gomi *et al.* (59) reported that, based on the the rate of starch gelatinization using pulsed field gradient (PFG) -NMR, water diffusivity decreased as starch gelatinization progressed, possibly due to the increase in the amount of carbohydrate polymer dissolved in the aqueous phase during heating. They also suggested that the change of water self-diffusivity is due to the change in local moisture content in the aqueous phase generated by starch gelatinization.

Recently, Baik and Chinachoti (60-63) investigated the role of glycerol and moisture redistribution on phase transition, thermomechanical properties, mechanical properties, and water mobility during bread staling. They reported that moisture redistribution from crumb to crust played a significant role. Moisture migration from crumb to crust greatly reduced the total and freezable water in the crumb region resulting in a significant increase in storage modulus (60). Additionally, domain-to-domain (amorphous) and crumb-to-crust moisture migrations are two critical phenomenological changes associated with aging and could lead to significant local dehydration of some amorphous regions contributing to mechanical firming during storage (61). When they added the glycerol to bread, higher firming was observed compared to normal bread. They suggested that hardening of aged bread by glycerol might be explained by local dehydration of bread polymer due to

osmotic dehydration or competition for water, which in turn promote more rapid amorphous network formation but less amylopectin recrystallization (62). Competition of water may be a key influencing factor in this case. Glycerol and loss of moisture (according to crumb-crust moisture gradient) triggered a shift in moisture redistribution from starch and gluten to glycerol. This could have contributed to the increased structural rigidity and more rapid firming of the glycerol-added bread. As a result, a greater firming rate was observed in glycerol-added bread even with less amylopectin recrystallization as compared with the control (63). Therefore, bread staling mechanism can not be explained only single phenomena such as moisture redistribution, starch retrogradation, local dehydration, and water-biopolymer interactions. Future investigation of complex inter-relationships among polymers, solutes and water in such system would be valuable for future understanding of the bread staling mechanism.

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