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THE ROLE OF STARCH GRANULES IN THE BALADY BREAD STRUCTURE FORMATION

Abstract

The microstructure of fresh and three days-stored Balady bread was studied using two different microscopic methods (LM and SEM). These both techniques revealed a great differences in the starch microstructure as well as protein distribution between two layers of fresh Balady bread. The lower layer of Balady bread is characterized by the greater extent of starch geletinization as compared to the upper one. The upper layer of Balady bread is formed by continuous protein matrix with embedded lenticular-shaped starch granules.

The microstructure of Balady bread after 3 days of baking differs from that of the fresh one mainly in the starch granules structure. The visible differences seem to be connected with different degree of starch gelatinization in each of the layers as well as free water (released from granules) redistribution between the layers. It was found that mainly these changes in stored bread allowed rapid retrogradation of the main soluble starch component – amylose.

Introduction

Dough ingredients combined with the old traditional Egyptian methods of the Balady bread production determine a unique and unusual form of this kind of bread. Balady flat bread differs significantly in appearance, microstructure, texture, nutritional value and taste from traditional European cereal baking products. The microstructure of bread and/or dough is highly variable and very often depends on the processing parameters and methods. Especially the methods of baking may vary widely from one type of bread to another [1]. Due to high temperatures (350–450°C), and short time of baking (2–4 min), the piece of dough of Balady bread raises in the oven and separates into two thin layers. The loaf is characterized by an open space between the top and bottom layer. The time and especially high temperature have also a significant influ-

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ence on deterioration of protein quality as well as the degree of starch gelatinization during baking [4, 5, 15]. The swollen and partially solubilized starch granules are the essential structural elements of bread. Cooling and staling of bread are responsible for transformation of both starch polymers (amylose, amylopectin), including gelatinization and crystallization.

The SEM and LM microscope techniques have been used to examine the distribution of the specific components within two layers of Balady bread. Moreover, much attention was paid to the studies on the role of starch granules in the structure formation of the fresh and stored bread.

Materials and methods

Balady bread was prepared using a commonly known baking procedure [4, 5, 8, 15]. The dough was fermented at 28°C for 40 min and divided into 180-g pieces. The dough pieces were placed on a wooden tray, which had been previously covered with a thin layer of bran. The dough pieces were placed in a fermentation cabinet for 15 min. After the first step of dough proofing, the pieces were flattened by rolling to a 20-cm diameter and 1.25-cm thickness, and left to a final fermentation for 50–60 min at 75–85% relative humidity [3]. The baking temperature reached 300–400°C for 2–4 min.

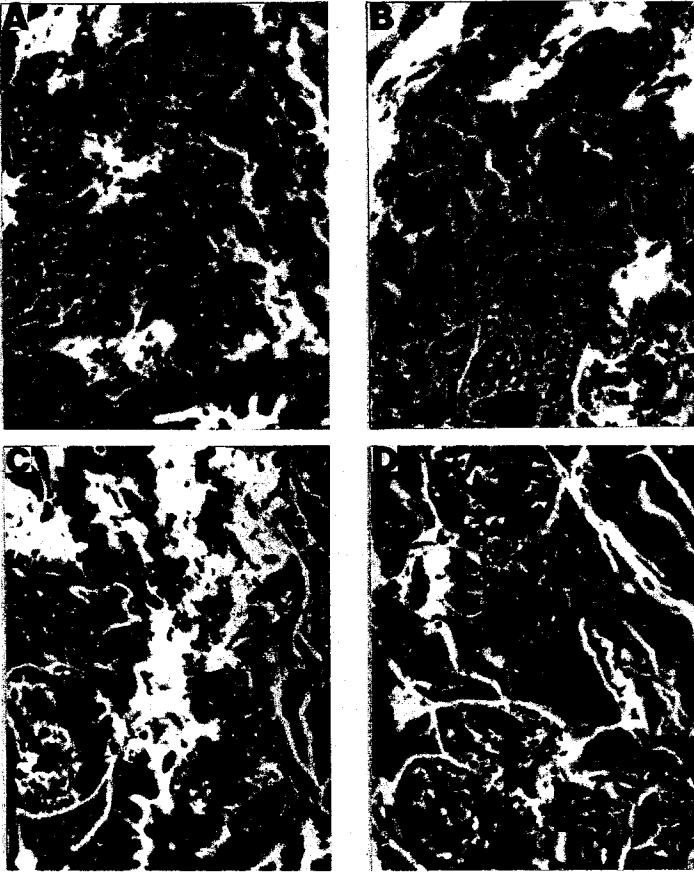
Moisture, ash and protein levels were determined by the ACC Methods: 44–15, 08–01, 46–10, respectively [16]. Fat content, crude fibres as well as sugars level were analyzed as recommended by the AOAC methods [1].

Small pieces of bread (lower and upper layer) have been prepared for the SEM studies by the freeze-drying method prior freezing in a liquid nitrogen. The dried samples were coated with gold and examined in a JSM 5200 microscope.

For the LM study, the small samples of bread were fixed in glutaraldehyde (dehydrated with ethanol series, polymerized and embeded in Historesin Kit medium according to producent data (Reichert-Jung, Germany). Sections, 2.5 μm , were cut with a microtome (Reichert-Jung, Germany) and stained with 0.1% Light Green and Lugol's solution. The sections were examined and photographed with an Olympus BX 60 microscope.

Results and discussion

The major structural change that takes place during baking process is starch gelatinization [2]. Light microscopy after iodine and light green staining provided considerable information about microstructure of both layers of Balady bread, which was different concerning the starch granules structure. In opposite to the lower layer of Balady bread, the upper one is rich in gluten proteins (Phot. 1a). The internal baking temperature reaches a point high enough to develop steam that 'puffs' the bread with



Phot. 1. LM pictures of Balady bread;

A/ upper layer of fresh bread; B/ lower layer of fresh bread; C/ upper layer after 48 hours of storage; D/ lower layer after 48 hours of storage.

almost explosive rapidity [6, 16]. Thus, the baking conditions as well as moisture content can affect significantly the changes in the protein structure resulting in a greater extent in protein redistribution mainly to the upper layer of bread. The structure of upper layer is created mostly by the continuous protein matrix with swollen starch granules embedded in it. The lower layer of bread (Phot. 1b) seems to be poorer in gluten proteins and consists mainly of gelatinized starch granules being in the higher stage of destruction. There is also observed an evident leakage of solubilized amylose through the equatorial groove to the intergranular space. The swollen starch granules together with solubilized amylose and amylopectin phase form a tightly-packed gel-like system. An interesting phenomenon, consisting in the different mechanism of starch gelatinization between the two bread layers could be also observed. The upper layer consists

of much less swollen starch granules at the first stage of swelling (elongated, lens shape A-type granules), while amylopectin-rich granules from the lower one are characterized by tangential deformation. These strongly pronounced microstructural changes in lower layer of Balady bread could be visible probably due to an easier access of starch to water in this part of bread as well as to a direct contact with the hot metal part in the oven. According to Faridi&Rubenthaler [6] the extent of starch gelatinization (under similar conditions) was generally higher for granules from center-most part of the loaf (85% for the lower crust), than that of granules from the exterior one (83.8% for the upper crust).

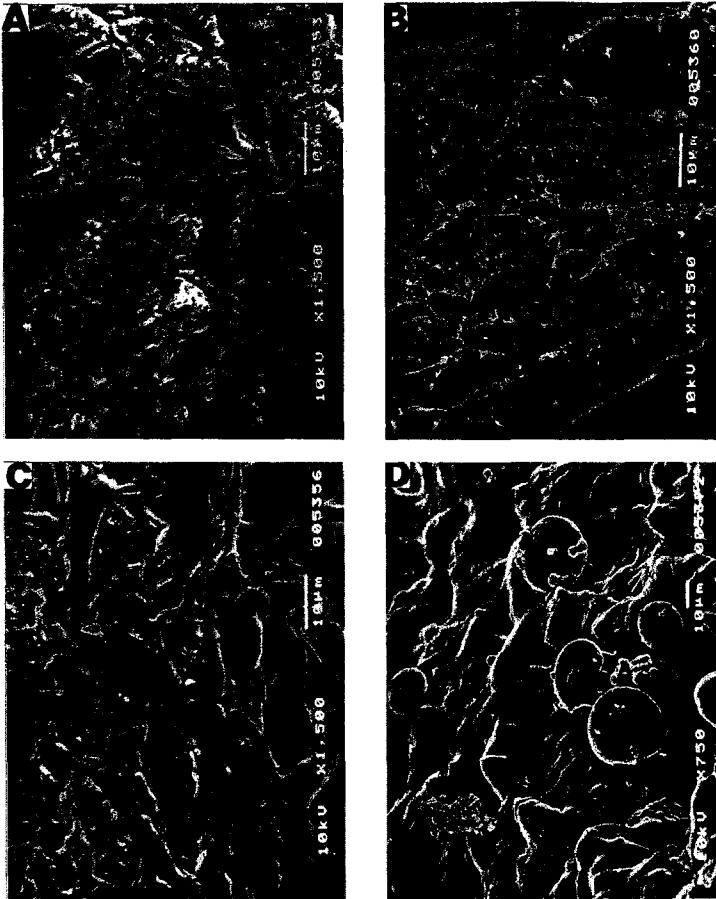
In order to obtain some additional details on starch and protein microstructure from layers of fresh Balady bread, the SEM technique was also performed. The continuous phase of the upper layer of Balady bread (Phot. 2a) seems to be composed of gelatinized starch granules mounted in a gluten matrix. The characteristic filaments belonging to the solubilized and free amylose phase were visible in the lower layer of Balady bread (Phot. 2b).

Changes that occur during cooling and storage of starchy products are mainly related to retrogradation. Bread staling affects the starch microstructure as well as can influence gluten protein, what results from release of free water from retrograding starch granules. Redistribution, i.e. mobility of the free water in the whole crumb of bread, seems to be strongly related to the quality of gluten protein as well as starch properties [9, 18]. Water diffusion in staling bread was explained by Hosney [10], as displacements of the molecule toward next-neighbouring binding sites, like -OH groups of the glucose units of polysaccharide molecules able to form hydrogen bonds. It is well known that during storage time water mobility decreases and the crumb structure becomes more firmer. In case of Balady bread the moisture content is not so high (Table 1). It is probably due to the moisture loss by evaporation during and immediately after oven baking as well as fast water redistribution between both layers.

Table 1

Chemical composition of fresh Balady bread.

	content % (dwb)
ash	1.01
fiber	1.16
lipids	1.32
protein	15.5
moisture	30.0
total carbohydrates	81.08



Phot. 2. SEM pictures of Balady bread;

A/ upper layer of fresh bread; B/ lower layer of fresh bread; C/ upper layer after 48 hours of storage; D/ lower layer after 48 hours of storage.

The microstructure of Balady bread after three days of storage differs from that of the fresh one mainly in the starch granules structure. The amylose, which was observed in fresh bread as a gel-like particles, now it seems to be strongly associated with amylopectin remnants and gluten protein matrix (Phot. 1c,d). The microstructural changes of amylose released (fresh bread) into insoluble crystallites are possible due to redistribution of free water as well as dehydration of soluble starch during bread storage. The aggregated amylose can be expected to have a stabilizing effect on the gluten protein matrix (Phot. 1c) and probably it is responsible for the firmness of bread. [2, 12, 13, 19]. Photo 1d shows the field of the lower layer of stored Balady bread. The

changes in appearance of granule remnants (more shrunken, darker) seem to be a reason of rapid reorganization of the starch biopolymers within their structure [7]. Also the lack of solubilized amylose, observed in the lower layer of bread, can be connected with stronger dehydration caused by ageing, as compared to the upper layer. According to these changes, it can be stated that the higher degree of granules gelatinization as well as presence of solubilized amylose strongly affected the starch retrogradation, during bread storage, especially in its lower layer.

In opposite to the above-mentioned observations, Faridi&Rubenthaler [6] reported that the main soluble starch material leached from crumb of freshly baked (415°C, 2 min) bread was predominantly amylopectin 1.62%. At the total amount of soluble starch material (2.42%), the amylose content was only 0.80%. They reported progressively decrease in amount of soluble starch (1,18%) as well as marked decrease in pasting properties after 48 hours of bread storage. Rapid and significant decrease in the amylose content (0.16%), obtained by these authors after 48 hours of storage, confirms our microscopic observations that amylose underwent retrogradation at a more rapid rate as compared to amylopectin (1.02%).

However, the mechanism of retrogradation affects not only changes in the amylose structure [7]. Several authors suggested that during bread staling also some changes in the amylopectin structure, i.e. its recrystallization, take place, being the main cause of bread firming [6, 17]. Inagaki and Seib [11] clearly proved that the amylopectin recrystallization is stronger associated with crumb firming than amylose. The SEM photo 2c, seems to confirm this hypothesis, due to a bigger surface area of highly swollen and elongated amylopectin-rich granules they probably could easier interact with gluten protein matrix. In contrary, the presence of solubilized amylose (the gel-like phase) surrounding the swollen granules results in the formation of a strong gel on cooling as was shown on SEM photo 2d. These observations are in accordance with Martin et al., [14] suggestions about passive role of swollen starch granules in the crumb firming.

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STRUKTUROTWÓRCZA ROLA GAŁECZEK SKROBIOWYCH W CHLEBIE BALADY

Streszczenie

Składniki ciasta odgrywają niezwykle ważną rolę w produkcji tradycyjnego egipskiego chleba Balady. Są to płaskie chlebki wypiekane w temperaturze 350–450°C. Przy krótkim, 3–4 minut trwającym wypieku kawałki ciasta, rosną rozpoławiając się na dwie cienkie warstwy. W ten sposób w bochenku pomiędzy warstwą górną i dolną znajduje się pusta przestrzeń.

Za pomocą technik mikroskopowych SEM i LM zbadano rozkład specyficznych składników ciasta pomiędzy obie warstwy. Szczególną uwagę zwrócono na rolę gałeczek skrobiowych w tworzeniu struktury bochenków.

Okazało się, że w przeciwieństwie do dolnej warstwy, górna warstwa chleba Balady była bogata w białko, tworzące własną matrycę. Matryca glutenowa, zawierająca kleikowane gałeczki skrobiowe, tworzyła ciągłą fazę warstwy górnej. Warstwa dolna w ogóle nie zawierała fazy glutenowej i składała się głównie ze skleikowanych gałeczek skrobiowych.

Z powodu bardziej zaawansowanego kleikowania gałeczki skrobiowe w dolnej warstwie bochenka wykazywały istotne zmiany mikrostrukturalne. Gałeczki były bardzo zniszczone i wykazywały wyciek amylozy. Zdjęcia SEM wykazywały bardziej subtelne zmiany. W dolnej warstwie widać było charakterystyczne pasemka amylozy, natomiast w górnej warstwie widać było skleikowane gałeczki wbudowane w matrycę białkową. Wydaje się, że bezpośredni kontakt dolnej części bochenka z gorącymi elementami pieca ma istotne znaczenie dla stopnia skleikowania skrobi i zachowania się białka. ☒