Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2024-178

Research Article

Open Access

# Bread Staling Measurement Techniques: A Review

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**Received:** May 11, 2024
**Accepted:** May 27, 2024
**Published:** May 31, 2024

**Citation:** Mohammadi-Moghaddam T, Morshedi M, Ansari RM, Morshedi A, Golmohamadi A, et al. 2024. Bread Staling Measurement Techniques: A Review. *J Food Chem Nanotechnol* 10(2): 85-93.



Published by United Scientific Group

## Abstract

The global production volume of wheat production is almost 785 million metric tons during 2023. Bread is a staple food produced with flour, water, or milk, with or without yeast. It is an important part of people's worldwide people diet and provides their daily energy. One of the most significant problems in bread production and consumption is its staleness, which begins immediately after baking. Staling is divided into two categories: texture and microbial changes. Texture changes occur first due to moisture migration, followed by microbial changes. Ultimately, the aroma, flavor, and texture of bread become undesirable to consumers. There are many methods for evaluation staling: chemical, macroscopic, rheological, microscopic, structural features, and molecular features methods. All these methods are effective and can be used according to the facilities and conditions of the producers.

## Keywords

Bread, Staling, Measurement methods

## Introduction

The increased demand for food is a significant challenge for global food security [1]. The top three major food industries are fruits and vegetables, meat, and bakery products [2]. Wheat, as a 28 worldwide staple food, plays a critical role in ensuring food security [3]. The global production volume of wheat is almost 785 million metric tons, and most people consume wheat bread as an important part of their worldwide diet [4-7]. Usually, "bread" is a staple food whose basic constituents are flour (mostly wheat), with milk or water; with or without yeast [2, 8, 9]. It contains fibers, carbohydrates, lipids, proteins, vitamins, and minerals, providing a significant amount of energy [9-12]. In some parts of the world, bakery products provide approximately 75% of total caloric intake. Consumption of bakery products not only fulfills people's need for calories, but also provides more than 90% of their needed protein. The bakery market expanded from 207.81 billion dollars in 2021 to 227.94 billion dollars in 2022 and has increased 9.7%. With the increasing population, the demand for bakery products and bread will also increase annually, with food demand projected to increase by 70% by 2050, reaching almost ten billion tons [2, 13, 14] Two viable solutions to meet the increasing demand for bakery 40 products are 1) improving the wheat grain yield using classic and molecular breeding techniques 41 [5, 15], and 2) enhancing the quality and durability of the bakery products, which is the focus of 42 this study.

The most significant problem with bread and bakery products is their short shelf-life, primarily due to spoilage and stealing. Staling is the main reason of bread waste, causing undesirable changes such as loss of freshness, aroma, flavor, and bread softness [16-18]. Bread staleness begins immediately after production with changes in texture, flavor, freshness, and aroma. During the staling process, moisture redistributes from crumb to crust, leading to some chemical changes in gelatinized starch and retrogradation (re-crystallization) [10, 12, 19, 20]. The entire mechanism of physical stalling is not fully understood yet.

Several theories related to staling, with one of the most important stating that staling involves interactions among bread components, especially moisture, with other compounds, referred to as moisture migration [10]. This physico-chemical reaction typically results in lower acceptance by consumers and occurs when gelatinized starch cools to ambient temperature or lower. The gelatinized starch gradually hardens, resulting in firmness in the bread crumb [21]. Staling usually occurs alongside microbiological spoilage due to moisture migration in the bread texture [11].

Stalking affects two main parts of bread, the crumb, and the crust. While staling mainly refers to the crumb part of bread, which constitutes the majority, crust contains a smaller portion, with exceptions such as different flat breads [22]. Bread chemical compounds, such as starch, protein, sugars, and additives, play important roles in the staling process [18, 23].

## Bread starch

### Physico-chemical properties

Starch in cereals like wheat, commonly used in bread formulation, is an important and dominant part, consisting of glucose units and in plant cellules [24, 25]. Granules' shape and size vary among different cereals (Figure 1). For example: potatoes have elliptical and the largest starch granules, while rice has the smallest multi-dimensional ones. Corn is the most important source of starch globally, followed by potato, wheat, rice, and cassava [26].

Starch has two subdivisions: amylose (17 - 30%) and amylopectin (70%). Amylose is a linear molecule composed of glucose with α (1 - 4) glucose bonds, while amylopectin has α (1 - 6) branches besides α (1 - 4) glucose bonds (Figure 2). Starch cannot be soluble in cold water but can attract water. Amylose is water soluble and amylopectin is insoluble, actually [27].

### Gelatinization

Gelatinization occurs when starch and water mix and warm up, breaking down hydrogenic bonds in granules and forming new hydrogen bonds, resulting in increased solution viscosity [28, 29]. During this process, amylose molecules migrate out of starch granules, becoming soluble. Gelatinization typically occurs at a specific temperature, usually around 95 °C. When cold water is added to undamaged starch granules, amylose molecules attract water, increasing their volume. This volume increase is reversible, below the 65 °C; at higher temperatures, the volume change is irreversible [27-29].

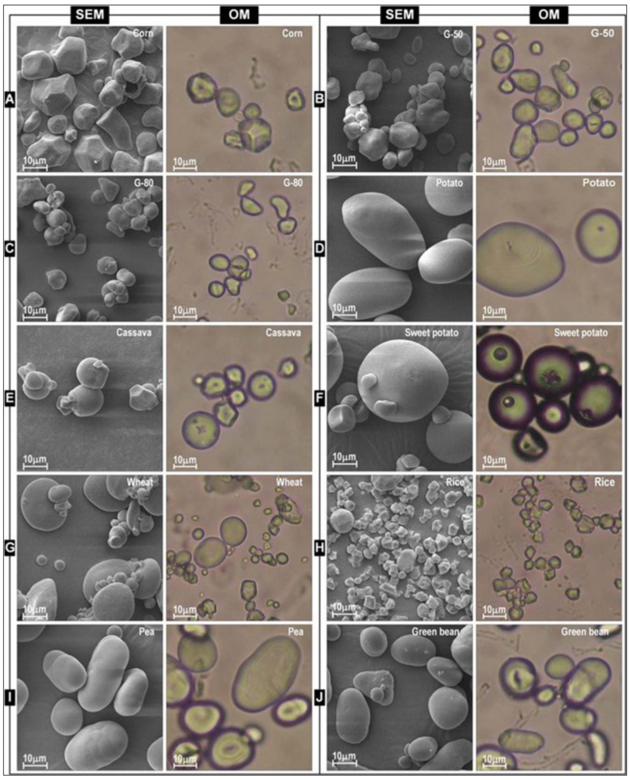


**Figure 1:** The effect of black plum peel extract and storage time on the peroxide value of sunflower seed oil.

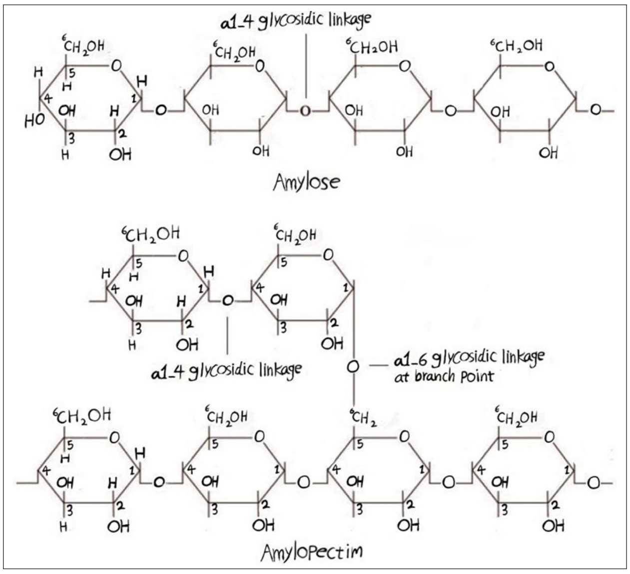


**Figure 2:** Amylose and amylopectin chemical structure.

### Retrogradation

Retrogradation occurs when warm gelatinized soluble cools rapidly, forming a starch gel. When the cooling process occurs slowly, amylose molecules have the chance to migrate from granules to the soluble, bending together via hydrogen bonds, leading to retrogradation. Retrogradation decreases starch increases and increases viscosity. This chemical reaction has its maximum rate at 4 ℃, with the speed of depending on amylose molecules length. Waxed starch contains large amounts of amylopectin and retrogrades rarely [30, 31].

## Bread staling

Bread staling happens immediately after baking and is very complicated. One of the most important factors is amylopectin retrogradation. Retrogradation starts with the formation of starch gel and progresses with the expansion of this structure, and after that, the moisture content of bread's internal texture decreases, the color changes and the bread harden. This process happens in two main steps [5, 32-34].

### Bread staling steps

**Amylose retrogradation:** The first step occurs after baking, with amylose molecules attempting to join and slowly getting closer, forming new hydrogen bonds, and initiating retrogradation. After forming the new hydrogenic bonds, the bread texture changes and becomes elastic [35, 36].

**Amylopectin retrogradation:** Over time, the amylopectin molecules gradually come closer, forming hydrogen bonds. During the formation of these hydrogen bonds, water molecules are produced, resulting in bread hardening as water migrates to the bread crust, causing the crumb color to change to white and become hard [35, 37].

## Staling measurement

Many researchers studied bread stalls and their measurements (Table 1). Bread staling is an important subject over time, with the moisture content of bread decreasing and the bread texture hardening [10, 35, 38]. Various methods exist for measuring bread staling to explain the phenomenon according to consumer preferences [32].

### Chemical methods

There are different chemical methods for measuring bread staling.

### Sedimentation evaluation method

One method is sedimentation evaluation, where sedimentation decreases over storage time, with Zeleny test evaluating protein quality and swelling properties [39-41]. Soluble starch measurement, although time-consuming, checks starch retrogradation by sedimentation using alcohol, with the percentage of soluble starch decreasing after staling. Bread swelling measurement observes bread crumb's water absorption and swelling, which decreases during storage time. Moisture content measurement tracks in moisture content of bread crust and crumb, with more significant changes in the bread core during staling [40, 42, 43].

### Soluble starch measurement

This method is very time-consuming and therefore its use is very limited. It can be said that it is almost obsolete. In this method starch retrogradation will be checked by starch sedimentation using alcohol. The percentage of soluble starch decreases after staling [44-46].

### Bread swelling measurement

Breadcrumbs absorb water and get swallowed. After the baking process, this swelling will decrease during storage time. For this experiment, first the bread sample gets wet by adding water and after that, it will be centrifuged in low rpm. Finally, the sedimented phase volume would be measured [47-49].

**Table 1:** Bread staling researchers.

| Name | Research topic in brief | Ref. | Year |
|---|---|---|---|
| Amigo et al. | Using maltogenic amylase effect on bread staling | [50] | 2021 |
| Han et al. | Olive leaf micro powder effect on bread staling | [51] | 2024 |
| Zhang et al. | Effect of lactulose on wheat bread staling | [52] | 2022 |
| Chen et al. | Effect of amylase addition on bread staling | [36] | 2021 |
| Fillipcev et al. | Psyllium addition on gluten free bread staling | [53] | 2021 |
| Ju et al. | Effect of adding potato flour on bread staling | [54] | 2020 |
| Fadda et al. | Bread staling | [32] | 2014 |
| Abd-El-Khalek et al. | Texture analyzer usage in bread staling determination | [55] | 2019 |
| Ribotta et al. | X-ray diffraction method usage in bread staling | [56] | 2004 |
| Sehn and Steel | Staling kinetics of bread | [38] | 2020 |
| Arp et al. | Staling kinetic of high-amylose resistance starch bread | [35] | 2020 |
| Eliasson | Wheat starch structure during staling | [30] | 2015 |
| Ma et al. | Different physico-chemical changes in staled bread | [37] | 2022 |
| Alfaris et al. | Effect of gluten in bread staling | [57] | 2022 |
| Marinopoulou et al. | Physicochemical and sensory changes during bread staling | [58] | 2020 |
| Kou et al. | Inulin effect on bread staling | [59] | 2019 |
| Curti et al. | NMR usage in bread staling evaluation | [60] | 2017 |
| Choi et al. | Analytical methodology of bread staling | [61] | 2010 |
| Nawaz et al. | Modified starch effect on staling | [62] | 2020 |
| He et al. | NIR usage in starch retrogradation evaluation | [63] | 2023 |
| Correa et al. | Hydrocolloids effects on bread staling | [64] | 2021 |
| Curti et al. | NMR usage in bread staling evaluation | [65] | 2011 |
| Pourfarzad et al. | Staling kinetics in barbari bread | [66] | 2012 |
| Sheikholeslami et al. | Texture, physicochemical and sensory changes during bread staling | [67] | 2019 |
| Santos et al. | Effect of chickpea flour, psyllium, and their combination on bread staling | [68] | 2021 |
| Schiraldi and Fessas | Bread staling mechanism | [48] | 2001 |

### Moisture content measurement

During the storage time, the moisture content of bread crust and crumb will change. These changes in bread core are more and more impressive. During staling process, the crumb of bread would be lost its moisture during time and measurement of this loss could be a sign of staling [32, 49, 69].

## Macroscopic methods

Gluten is crucial in dough formation and bread production, with its viscoelastic behavior impacting various chemical and physical reactions. In a moist environment, proteins can undergo several reactions: between different parts of a protein, among different proteins, and among proteins with other compounds. Bread flour is a homogenous substance that has two phases. The first phase is containing swallowed proteins in water and the second phase is water that is surrounded the protein net and contains starch granolas [70-72].

## Sensory methods

Sensory methods evaluating different sensory features based on consumers preferences, including bread core hardness, softness, texture, flavor, overall acceptability, cohesiveness, adhesiveness, moistness, and density [32, 73-74]. Sensory evaluation is categorized into two main parts: consumers preferences, and product preferences. Finally, the sensory evaluations should be analyzed statistically.

## Consumer preferences

In this method, a volunteering group of consumers group would be formed of 100 - 500 people. They will test the products in marketplaces, malls, and shopping centers. They will not be thought professionally or do not have any sensitive taste. This method needs time and is expensive. This method usually is known as total acceptance [75-81].

## Product preferences

Trained panelists in small groups of 5 - 10 people are usually used for this type of sensory evaluation. They should evaluate each feature of products after training and consider a point within a specified range of points. This method is routine in grand manufacturing companies and research centers [78, 82, 83].

## Rheological methods

Most of researchers believe that the best way for bread staling recognition is texture analysis because a good bread texture has a direct impact on bread total acceptance and taste panel [32, 84]. Mechanical features of bread crump like firmness (or hardness), moistness, and compressibility are very important factors which could be effect on customers preferences, as well [84]. The routine test of texture is compression/deformation tests. The distance through which a bread texture is compressed by a standard force could be consider as a rheological method. There is another way for doing compression test: the bread could be compressed through a standard distance and the needed force would be considered as a bread texture staling [84]. The most common device to perform this test is instron. In this test, the bread resistance against compression would be measured. It is possible to perform different tests with instron like tension, density, piercing, and shear. During storage time, the shear, density, pierce, and tension forces would be increases [32, 59, 85].

Farinograph and amylograph are another instrument for staling evaluation that works based on dough rheological features. The dough and bread viscoelasticity could be checked by farinograph. There are some research that indicated during storage the bread viscoelasticity decreased [38, 86, 87]. The bread staling could be assessed by amylograph by monitoring the starch viscosity changes. Different researchers believed that during time and staling, the starch viscosity decreased [66, 88].

## Molecular based methods

Water molecules play a significant role in bread staling by increasing molecular motions among polymer filaments besides coordinating among polymers [5, 65]. Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) are used to study molecular structure specially monitoring the water molecules [10, 89, 90].

## NMR

In NMR method, permanently two magnetic poles and a core are formed. The core attracts different electromagnetic waves that depend on magnetic field (Figure 3). NMR is a very accurate method to investigate food materials dynamics, especially water at a molecular level. Two types of NMR are used in bread staling evaluation: $^1$H NMR spectroscopy and $^1$H FID (measured at 20 - 23 Hz). $^1$H FID uses mobility changes of bread and bakery products during storage; that increases during the time, while $^1$H NMR spectroscopy is used for highlighting proton population. The bread molecular motion changes during the staling process that can be monitored by NMR. Staling causes reducing water molecules mobility [65, 60].

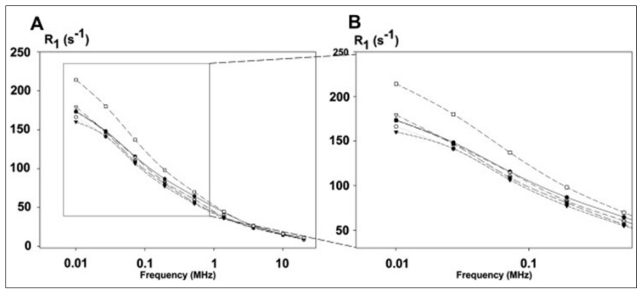


**Figure 3:** $^1$H $T_1$ NMRD profile of bread crumb samples during storage at 0.01 – 20 MHz. **(A)** Day 0 - ●; Day 1 - ○; Day 3 - ▼; Day 5 - ▿; Day 7 - ■; Day 14 - □; and **(B)** $^1$H $T_1$ NMRD profile zoom of the data in the 0.01 - 0.52 MHz frequency range [49].

## MRI

MRI provides 3D information. Using MRI, researchers can study image contrast based on molecular mobility, water physicochemical features, and food structures for example gas cells with small diameters (Figure 4). It is very useful for monitoring moisture transfer and water distribution in bread. The MRI information has shown that during storage time, water-gluten combination mobility decreases[65, 91].

## Microscopic methods

Microscopic methods like scanning electron microscopic methods (SEM) and optical and polarized microscopic method are used to study bread and its stalling.

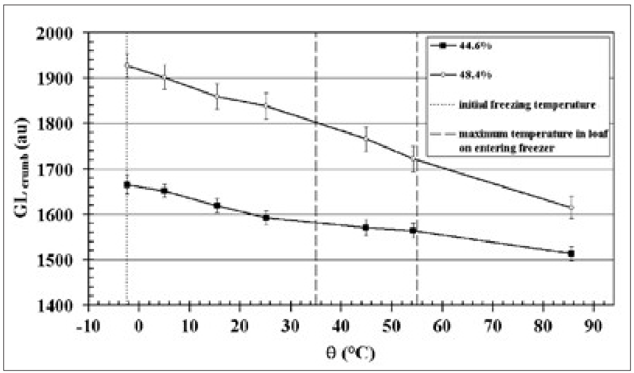


**Figure 4:** MRI relaxation signal for non-yeasted (44.6% and 48.4% water) bread dough baked for 120 min at 90 °C [91].

## SEM

With SEM determining gas cavities and size and cryo-SEM offering higher accuracy by freezing samples (Figure 5). In cryo-SEM method, the samples should be frozen, therefore water and oil are frozen and will not be lost. Cryo-SEM is accurate method for gluten net monitoring and bread staling [92-95].

## Optical and polarized microscopic method

This method is used with 200 - 500 nm resolution for monitoring bread and bakery products. Optical microscopes are routine instruments for different dough monitoring and recognition of their flour qualities. Optical and polarized microscopes observe starch retrogradation and bread staling, identifying an independent phase during storage in a gluten-starch net [33, 61, 96].

## Structural features measurement

## Differential scanning calorimetry (DSC)

DSC monitors bread stalling thermodynamically by evaluating thermal properties, with starch retrogradation causing the sample to reduce more heat than the blank material (Figure 6). DSC is a method that the amount of input thermal energy between the sample and a reference material could be calculated at a controlled temperature program. In this method, thermal features of bread crumb could be evaluated. These properties are temperature dependent like bread staling and starch retrogradation. During staling process, the sample produces more heat than the blank material [97, 98].

## Dynamic mechanical analysis (DMA)

DMA utilizes in evaluating the viscoelastic behavior of polymers, that facilitates the simultaneous determination of mechanical and thermal properties of polymeric materials over a broad range of frequencies (0.01 - 200 Hz) and temperature (-150 - 600 °C) [99]. DMA calculates tension for samples, monitoring gluten and starch dynamic tension (Figure 7). The tension has two categories: inter phase (Elastic), and outer phase (Viscose) [99, 64].

## Image processing method

In this method, hyperspectral imaging (HSI) is used. HSI, chemical imaging or spectroscopic imaging is an analytical

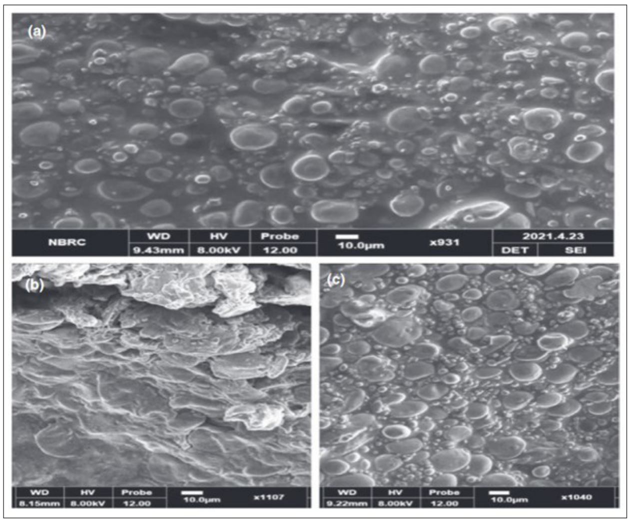


**Figure 5: (a)** SEM image of control bread (100% wheat); **(b)** bread (90% wheat and 10% rye); and **(c)** bread (80% wheat and 20% rye).

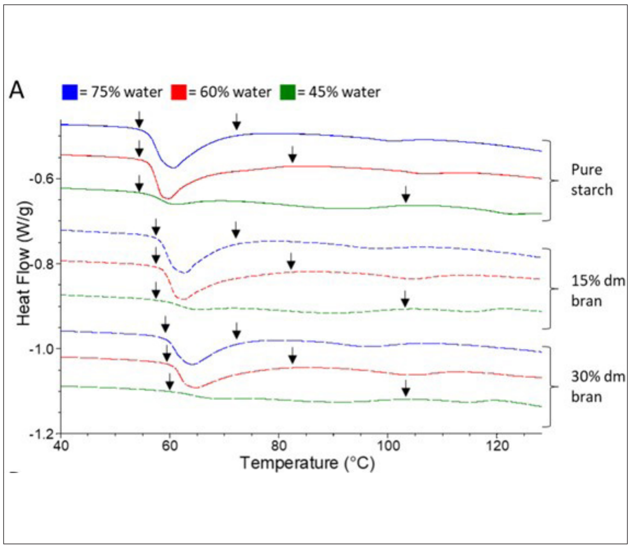


**Figure 6:** DSC profiles of mixtures with different starch-bran-water ratios: solid lines (pure starch), short dashes (starch with 15% bran), and long dashes (starch with 30% bran); blue (moisture content 75%), red (moisture content 60%), green (moisture content 45%).

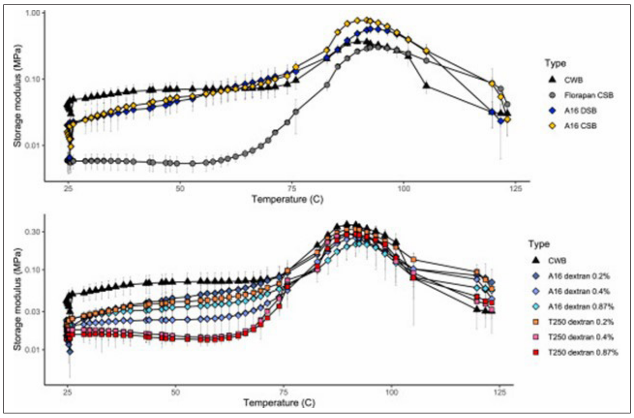

**Figure 7:** Storage modulus of bread dough under baking conditions in DMA [99].

technique that combines the chemical information obtained by spectroscopy with the spatial information obtained by spectroscopy with spatial information of the surface measured (Figure 8). This method is became as a standard technique in pharmaceutical production, environmental monitoring, and food science [50, 100]. Data obtained from scanned sample by HSI is a three-dimensional data cube, which contains a vast amount of spatial and spectral-chemical information [63, 101]. In this technique, a slice of bread scans and by using special software, its pictures particles dimension, color and color intensity will be analyzed [63].

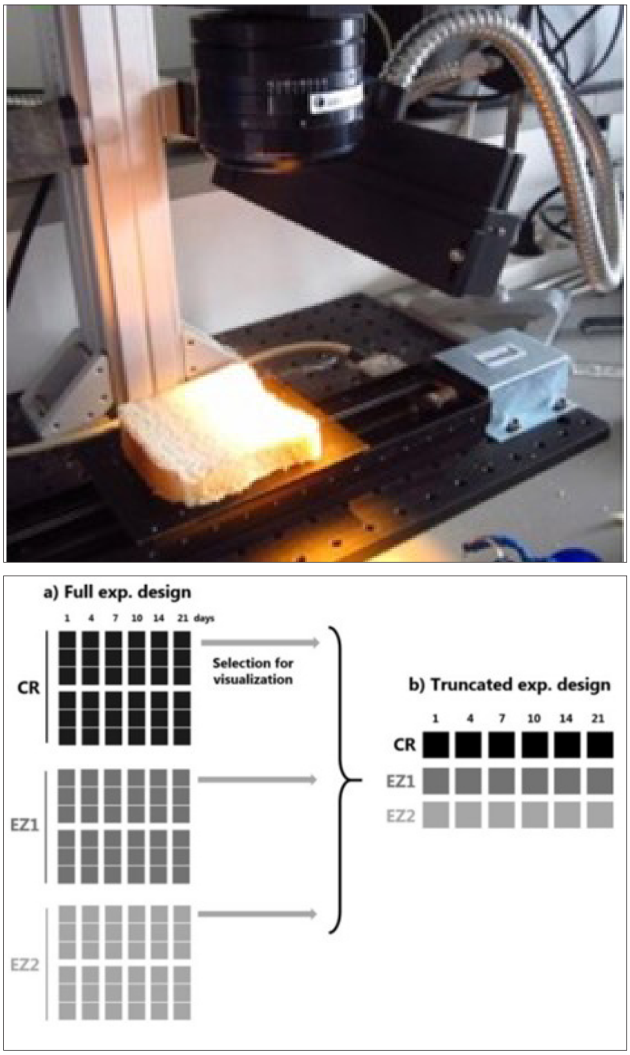


**Figure 8: (a)** A bread loaf scanning with a HSI-NIR camera; and **(b)** Arrangement of the different bread loaves and their replicates in the final hyperspectral image [50].

## Conclusion

Bread is known as the dominant food worldwide. Besides preparing energy, they supply protein and minerals in different diets. Usually, cereals are consumed as bread and one of the most problem in bakery is bread staling. Bread stalling happens after baking through complicated physico-chemical reactions. There are different methods for evaluating bread stalling, but they are different in answering time, cost, user skill, accuracy, and investment. Each company or producer can choose a proper method based on its potential. But taste panel and rheological experiments are routine methods in the factories with mass production. Methods based on evaluation the structural features, molecular properties, and microscopic methods are suitable ones for research. Finally, chemical methods are usually used in the laboratories.

## Acknowledgements

None.

## Conflict of Interest

None.

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