Original article

Effect of antimicrobial agents and dough conditioners on the shelf-life extension and quality of flat bread, as determined by near-infrared spectroscopy

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Summary   Middle Eastern Countries are experiencing the emergence of high volume production and retail marketing over traditional unit baking and retailing. This phenomenon has revealed a shortcoming in quality issues in Arabic flat bread (AFB) manufacture. Therefore, shelf-life extension and quality improvement are in demand to limit economic loss. Five improvers and a control (without any improver) were selected for improving shelf-life and quality of AFB. The improver treatments included: (1) sodium 9 stearoyl-2-lactylate (SSL), (2) monoglycreides (MG), (3) hydroxy propyl methyl cellulose gum (HPMC), (4) high-fructose corn syrup (HFCS), and (5) a combination of all the aforementioned improvers. The texture analysis data indicated that the presence of HFCS may act as a plasticizer through contributing to the longer tearing time. The other treatments exhibited a significant decline in tearing time throughout storage. The NIRS data indicated that after 3 days, the control was less fresh than AFB formulated with HFCS or improver combinations. The sensory evaluation of AFB exhibited that the improver combination significantly improved the quality attributes.

Introduction

Flat breads contribute approximately 85% of the caloric value to the diet of Middle Eastern populations (Mousa et al., 1979; Qarooni, 1996). Arabic flat bread (AFB), like other breads, may exhibit a limited shelf-life due to mould growth and staling. Many Middle Eastern countries lack either appropriate packaging or storage facilities to extend the shelf-life and quality of the AFB. The economic loss contributed to spoilage and waste is estimated to be over one billion dollars per year (Baik & Chinachoti, 2000). The water activity of AFB ranges between 0.9 and 0.95 (Quail et al., 1996), which facilitates mould growth. The most prominent type of moulds associated with AFB are *Aspergillus*, *Rhizopus* and *Penicillium* (Grundy, 1996). Quail (1996) suggested several methods to inhibit microbial growth in AFB, including modified atmospheric packaging (Co_2, NO_2), irradiation, freezing and preservatives.

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The Arabic flat bread starts to lose strength and becomes firm after 24 h. Therefore, the bread is considered optimum quality during the first few hours after baking (Quail, 1996). These changes represent the initial stages of the staling process. Bread staling is a complex process that involves a set of physiochemical changes (moisture distribution, firmness, tearing properties and opacity) in the crumb, not those resulting from the action of microorganisms (Zobel & Kulp, 1996; Toufeili et al., 1998).

Many improvers have been used for several years to enhance and extend the quality attributes of white pan bread (Van Eijk & Hille, 1996; Huglten et al., 2001). These improvers have provided the necessary quality attributes that consumer desires in 'fresh bread'. The literature on transferring the use of these improvers into an AFB formulation is scarce. However, improvers have been used to improve the sensory qualities in tortillas. Improvers have been cited to increase the shelf-life of tortillas by 16 days (Wijaya, 2003).

The present study hypothesised that the addition of improvers in the white pan bread industry may be

applied in AFB formulations to reduce staling and extend shelf-life. Thus, the researchers manufactured five AFB with selected improvers in addition to the control (no improver was used) and measured the physical and sensory properties over time.

Materials and methods

Wheat collection and milling

Jagger wheat was collected from a local farm in Manhattan KS. The wheat samples were cleaned and tempered (16% grain moisture content) at ambient temperature for approximately 24 h prior to milling. The wheat was milled in the Department of Grain Science and Industry (KSU Pilot mill, Kansas State University, Manhattan, KS, USA). A target of 74–75% extraction was set. The flour was collected in 50 lb-bags and stored in a freezer (−10 °C). Proximate analyses were done on the wheat and flour milled in the Analytical Laboratory in the Department of Grain Science and Industry (Kansas State University). The proximate analyses methods were selected from American Association of Cereal Chemist. (1995). These approved methods included the moisture determination (44–15 A), crude protein (46–16) and ash (08–01). Additional American Association of Cereal Chemist. (1995) methods used to characterize the flour included falling number method 56–81B, starch damage method 76–30A and flour colour (*L*, *a*, and *b* values) which were determined using a colorimeter (Mini Scan Minolta CR-210, Minolta Corporation, Ramsey, NJ, USA).

Arabic bread production

Six different AFB formulae were prepared by mixing 1000 g flour (14% moisture basis), 1% yeast and 1% salt and an improver either singly or in combination (sodium stearoyl-2-lactylate (0.25%; Paniplex® SK from ADM, IL, USA), α- monoglycerides (MG) (0.25%; Panalite® 90-DK from ADM, IL, USA), hydroxy propylmethyl cellulose gum (HPMC) (0.75% K-90 from TIC gums, IL, USA), high-fructose corn syrup (HFCS) (4.2%/42% DE), and an improver combination (all these were added at the same level as mentioned above). All formulations included a preservative (0.3% sodium propionate and 0.2% fumaric acid).

A farinograph was used to determine the amount of water absorption and mixing time. Qarooni *et al.* (1987) reported that the dough development peak of 850 BU was the most reliable prediction of water absorption for AFB production. The water absorption levels varied with the improver(s) used. The moisture added to the control was 53%, 52.4% for SSL, 56.7% for HPMC,

53.75% for MG, 53% for HFCS, and 53% for the improver combination treatment. The optimum dough mixing time was defined as the maximum dough development time (time taken to reach 850 BU) plus 1 min. The dough was mixed to optimum development using a Hobart planetary mixer (model C-100, Hobart, Troy, OH, USA) at two speed setting stages. At the first stage, the ingredients were mixed thoroughly at very low speed for 3 min. At the second stage, further mixing at higher speed setting was done to develop the dough (The optimum dough mixing time was used) for the following times depends on the improver(s) used: 3 min for the improver combination, 3.5 min for either SSL or MG, and 4.5 min HPMC or HFCS. After mixing, the dough was transferred to a covered plastic bowl, placed into a proofing cabinet (National Manufacturing Co., Lincoln, NE, USA) and allowed to ferment for 60 min at 30/C and 70 ± 5% RH.

After fermentation, the dough was scaled off into 24 pieces each weighing 60 g. The pieces were rolled by hand and covered with Saran Wrap™ to prevent skin formation. The dough was kept for 10 min and then lightly dusted with wheat flour and flattened by gentle hand pressure. The dough was passed through a two-stage roll sheeter. The gap between the rolls were set at 10 and 3 mm, for the first and second stage, respectively. The flattened dough was transferred to a stainless board and covered (to minimize moisture loss). The dough was transferred back to the proofer for 30 min under the same conditions.

The bread was baked at 400/C for 90 s, on a pre-heated (1 h) aluminium tray (215–450 mm) that accommodates two full-sized loaves. To maintain consistency, only one loaf was baked each time. After baking, the bread was cooled for 10 min and immediately packaged in clear polyethylene bags to maintain freshness. The 24 loaves were randomly subdivided into two groups, 12 loaves each. The first 12 loaves were used to understand moisture, colour, water activity, and TA. The remaining 12 loaves were used for NIRS.

Day 0 testing was initiated within 2 h of baking. The loaves designated for shelf-life studies (days 1, 2 or 3) were stored at 23 °C/50% RH. Literature reports that the critical period during which staling and moulding occurs in AFB is within the first 72 h of manufacture of bread (Qarooni, 1996; Quail, 1996).

Arabic flat bread analyses

Moisture content and water activity
The moisture content of AFB was measured using AACC approved method 44-15A (American Association of Cereal Chemist, 1995) and water activity (AOAC, 1980) was determined using an AQUA LAB CX-2 (Decagon CO., Pullman, WA, USA). *Tensile Testing*. All the specimens were tested according to the

standard D 882-91. Nine specimens from each treatment were examined. A dump-bell shaped template was constructed of heat-treated stainless steel Type 17-4. The template was placed on the centre of the top portion of the AFB to cut a uniform section; this section had dimensions of 115 mm × 20 mm × 6 mm. Each end of the dump-bell shaped AFB was placed in a special clamp designed for tensile testing (A/AT Tensile Grips) with a texture analyzer (TA.XT2 Texture Analyzer, Texture Technologies Corp., Scarsdale, NY, USA). The return distance between the two arms was set to 85 mm. The tensile test with grip probe was used with the following settings: pretest speed: 1 mm s^{-1}, test speed: 1.7 mm s^{-1}, post-test speed: 10 mm s^{-1}, 40% strain force: 10 g, trigger: mode auto with acquisition rate 250 pps. Cross-sectional area (thickness and width) of the specimens were measured to the nearest 10 μm using a hand micrometer.

In preliminary studies, a comparison between tearing force at rupture and tearing time at rupture was evaluated. The time taken to rupture or tear each specimen was more reproducible than the force required to tear each specimen as determined by coefficient of variance (CV). Thus, only the time required to tearing each specimen was reported for this study.

Near-infrared spectroscopy

A diode-array NIR spectrometer (DA7000 Perten Instruments, Springfield, IL, USA) was used to collect the spectra. The wavelength range was 400–1700 nm. Data were recorded as $(1/R)$, where R is the relative reflectance. A reference standard, Spectralon®, was used to collect baseline data. Each spectrum was recorded as an average of 15 scans/s. NIR spectra were taken daily on 12 loaves for each treatment (top and bottom section separately) until mould appeared on the bread. The mould was used as an indication of the end of shelf-life.

Data analysis

A cross-validation method was employed to determine the applicability of NIRS to distinguish freshness in AFB within each treatment. The NIR spectra of the 12 loaves for the top and bottom sections were combined for the cross-validation analysis.

Spectra were analysed with partial least squares (PLS) regression (Martens & Naes, 1989) using a commercial software (PLSPlus/IQ for Grama/32, Galactic Industries, Salem, NH, USA) at 550–1700 nm wavelength range. The optimum number of PLS factors was determined by cross-validation. The number of PLS factors for the treatments control, SSL, HPMC, MG, HFCS and the improver combination treatment was 8. This factor was judged by the following criteria: beta coefficient (the spectra with

the least amount of noise), higher R^2, and the minimum partial residual error sum of squares (PRESS). Cross validation was employed to create the calibration models. The critical wavelengths were identified as those with large beta coefficients values. The cross-validation involved removing one sample from the data set (n) and performing the calibration with the rest of the data set ($n-1$). All samples in the data set were left out and measured once in turn ($n-a$, $n-b$). The first step in creation of the calibration model was to determine the PRESS value.

A validation model was used to predict the loss of freshness among treatments compared to the control. Development of the validation method to create the calibration model involved the entire data set (n) from the control. The loss of freshness for the, SSL, HPMC, MG, HFCS and improver combination was determined based on the calibration model of the control for days 0, 1, 2 and 3. The NIRS spectra prediction of storage days for all treatments correlated with actual storage time. The AFB loss of freshness was analysed by plotting NIRS-measured storage time against actual storage time. The predicted storage time and the raw spectra absorbance differences were used to characterise the freshness of the AFB.

The RPD (ratio of performance to deviation) is calculated by dividing the standard deviation of the reference data by the standard error of performance. The RPD is an indicator of the usefulness of calibration used in the validation or prediction by the standard error of performance (Williams, 1997).

Sensory analysis

Sensory evaluation was conducted in the Food Science Laboratory at Call Hall, Kansas State University. Sensory evaluation was performed only on the control and improver combination treatment. As this treatment exhibited potential as a commercially viable product. Seven panelists (five male and two female) ageing 22–50 were trained in descriptive analysis of AFB properties. The panelists were consumers of AFB and trained for 2 h (1-h session/day) in quality attribute profiling (rollability, cracking and separation) as described by Qarooni *et al.*, 1987 and Toufeili *et al.*, 1998. Anchors were prepared to provide using AFB (fresh and 4-days old) for the panelists. During each training session, the panelists were familiarised with AFB quality attribute profile to be evaluated using a nine-point hedonic scale (1 – fresh and 9 – 4-day-old bread). At the end of the training session the differences in the ranking scores amongst panelists for each of the attributes did not exceed score 1.

The AFB treatments and anchors were baked and stored in polyethylene Ziploc™ sandwich bags and sealed. A random three-digit code was assigned to each

sample prior to presenting to the panelists. Samples were served to the panelists monodically.

Statistical analysis

The order of serving was determined by random permutation. A nine-point hedonic scale was used to evaluate each attribute (e.g. 1 – complete separation, 9 – no separation).

A two-way factorial classification in complete randomized design (CRD). Data were analysed using statistical analysis software (version 8.2, SAS Institute Inc., Cary, NC, USA). Three batches of bread were produced for each treatment. Analysis of variance (ANOVA) and means separations were calculated by the general linear model procedure (Proc GLM). Comparisons among treatments were analysed using Fisher least significant difference (LSD). Treatment means were considered significant at $P < 0.05$. Correlations were performed to determine the importance of using the tensile test on the texture analyzer as a tool to determine the AFB's loss of freshness during storage.

Results and discussion

Flour quality

The flour quality profile of the Jagger wheat used in the AFB production is presented in Table 1. These are consistent with other reported values for Jagger wheat flour under similar extraction conditions (Pomeranz, 1971).
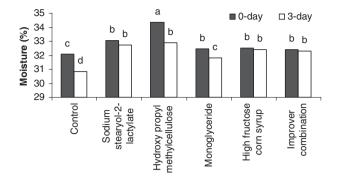
Arabic flat bread quality

Moisture content and water activity
The HPMC treatment exhibited a significantly higher moisture content at day 0 compare to all treatments (Fig 1). Whereas, the control exhibited a significantly lower moisture content compared to all other treatments. The higher water absorption (3.5% higher) of

Table 1 Profile of Jagger wheat that was 75% extracted[a]

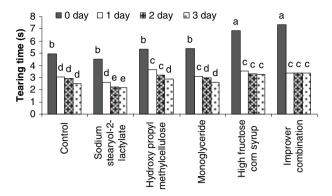| Properties	Average
Ash (%)	0.51 ± 0.01
Falling number	482 ± 2
Moisture (%)	12.63 ± 0.01
Protein (%)	10.89 ± 0.04
Starch damage (%)	2.89 ± 0.05
Flour colour	
L-value	90.93 ± 0.08
a–value	2.44 ± 0.03
b-value	8.89 ± 0.04

[a]*n* = 4.

Figure 1 A comparison moisture changes of Arabic flat bread formulated with control (C), sodium stearoyl-2-lactylate (SSL), monoglycerides (MG), hydroxy propyl methylcellulose (HPMC), high-fructose corn syrup (HFCS), or improver combination during storage at 23 °C/ 50% RH.

HPMC may be the reason for the higher moisture content. The moisture content of the SSL, HFCS and improver combination treatments were not significantly different from each other. After 3 days, the moisture content of HPMC treatment significantly declines, as did the control, and MG treatments. The control exhibited the lowest moisture content of all treatments after day 3. The HFCS, SSL and improver combination treatments did not show a significant change in moisture content compared to day 0. This observation suggests that these treatments may possess better moisture retention than the other treatments. Also, the changes in the moisture content of this type of bread during storage could be due to the redistribution between bread crumb and crust rather than moisture losses by evaporation. In staling studies on bread (Yasunga *et al.*, 1968; Mahmoud & Abou-Arab, 1989) it was found that the loss of crispness of fresh bread was largely caused by moisture redistribution.

There was no significant difference in the water activity among all the treatments. The water activity for AFB formulated with the different additives ranged between 0.945 (control) and 0.955 (HPMC). The water activity of each treatment did not significantly change from day 0 to day 3. These results are consistent with the results obtained by Czuchajowska *et al.* (1989) who found that there was little change in a_w (0.951–0.961) between the fresh crumb and the older crumb.

Tearing time

At day 0 a significantly longer tearing time (approximately 30%) was needed to rupture the AFB formulated with HFCS or improver combination treatments (Fig. 2). There were no significant differences in tearing time at day 0 amongst the other treatments. All treatments exhibited a significant decline (approximately 50%) in tearing time from day 0 to day 1. The MG,

Figure 2 The tearing time at rupture of Arabic flat bread formulated with control (C), sodium stearoyl-2-lactylate (SSL), monoglycerides (MG), hydroxy propyl methylcellulose 27 (HPMC), high-fructose corn syrup (HFCS), or improver combination during storage at 23 °C/ 50% RH.

HPMC and SSL showed further a significant decline in tearing time during the remainder of the storage period. The improver combination, HFCS and control did not decline significantly after day 1. Albeit, the control showed the lowest tearing time compared to all treatments. The presence of HFCS may act as a plasticizer in the formulation contributing to the longer tearing time which reduced the rate of freshness losses. It seems that HFCS, and improvers combinations can interact with dough system and affects the rate of firmness, starch crystallinity, tearing properties, and better moisture retention. Therefore, these additives can be used as conditioners/strengtheners, crumb softener and antistaling agents (Armero & Collart, 1996). These results were consistent with the results obtained by Toufeili *et al.* (1998) who reported that probing extensibility (indicating the time of tearing) decreased significantly as aging progressed. Rogers *et al.* (1988) found that the moisture content was inversely related to the rate of staling. The researcher further reported that there was no significant difference in the rate of firming between white pan breads with a 2% difference in moisture content. Hallberg & Chinachoti (2002) found that standard white bread (43% moisture content) showed a more rapid increase in firmness during storage than ready to eat bread (Military long shelf-life bread containing 23% moisture plus humectants) mainly due to higher loss of moisture to the crust and surrounding environment. Quail (1996) reported that AFB staling is due, in part, to moisture loss, but is mainly due to a chemical change in the starch–protein interaction.

The determination of freshness of Arabic flat bread using NIRS

The cross-validation model of the AFB was evaluated within each treatment. The coefficient of estimation (R^2)

for the cross-validation model for each treatment ranged from 0.87 (control) to 0.99 (improver combination) (Table 2). The validity of the cross-validation model was determined by the ratio of standard error of performance to standard deviation (RPD), also referred to as the ratio of reference data in the prediction sample set to the standard error. The RPD for the treatments ranged from 3.32 (HFCS) to 6.2 (improver combination). These results were similar to Xie (2002) for white pan bread, where the RPD ranged between 3.7 and 5.0. The high R^2 and RPD suggested the importance of using NIRS as a tool to study the loss of freshness of AFB formulated with different improvers.

The validation model was used to evaluate the effectiveness of NIRS to measure the loss of freshness of each AFB treatment. The R^2 ranged from 0.89 (SSL) to 0.95 (HFCS and improver combination) (Table 3). The high R^2 and standard error of determination (SEE) indicated that the validation model was a reliable method to predict loss of freshness in AFB as a function of improver addition.

The validation model was applied to all the AFB treatments (top and bottom sections combined) to differentiate loss of freshness (Fig. 3). The improver combination treatment exhibited significantly less loss of freshness than all other treatments throughout the storage period. These results indicate that improver combination exerts a synergistic effect with the crumb antifirming action, unlike using each improver singly. The HFCS treatment showed a significantly higher freshness than all treatments except the improver combination.

The raw absorption spectra was analysed to determine if these data may be used as another means of differentiating improvers and their subsequent effect on loss of freshness (Fig. 4). The HPMC and the HFCS

Table 2 Cross-validation model for different treatments

Treatments	$R^{2\ a}$	SEE[b]	RPD[c]
Control	0.87	0.3	4.36
Sodium stearoyl -2- lactylate[d]	0.92	0.29	4.55
Hydroxy propyl methylcellulose [e]	0.93	0.27	4.96
Monoglyceride[f]	0.92	0.34	3.75
High-fructose corn syrup[g]	0.98	0.39	3.32
Improver combination[h]	0.99	0.21	6.2

[a]All correlations are with actual storage time.
[b]SEE refers to standard error of estimation.
[c]RPD = ratio of standard error of performance to the standard error deviation of the reference data.
[d]0.25% Sodium stearyol-2- lactylate.
[e]0.75%Hydroxy propylmethyl cellulose.
[f]0.25% Monoglycerides.
[g]4.2% High-fructose corn syrup.
[h]All improvers combined.

Table 3 Summary of NIRS for Different Treatments Using the Control as a Calibration Model

Treatments	$R^{2\ a}$	SEE^b
Control	0.94	0.30
Sodium stearoyl -2Lactylate [c]	0.89	0.34
Hydroxy propyl methylcellulose[d]	0.90	0.32
Monoglyceride[e]	0.91	0.33
High-fructose corn syrup[f]	0.95	0.32
Improver combination[g]	0.95	0.4

[a] All correlations are with actual storage time.
[b] SEE refers to standard error of estimation.
[c] 0.25% Sodium stearyol-2- lactylate.
[d] 0.75%Hydroxy propylmethyl cellulose.
[e] 0.25% Monoglycerides.
[f] 4.2% High-fructose corn syrup.
[g] All improvers combined.

treatment exhibited the highest raw absorption spectra, whereas the improver combination showed the lowest raw absorption spectra.

Spectra used to show the difference between the control and individual improver treatments may determine differences among treatments (Fig. 4). The greatest difference in absorbance spectra was observed at 1430 to 1495 nm wavelength. This peak might relate to the second overtone of C-H, $C-H_2$, $CONH_2$, ROH, and H_2O. This means that moisture loss, starch retrogradation, and protein interaction might affect bread loss of freshness. Xie (2002) reported that two important wavelengths, 550 and 1465 nm, were keys for the NIRS to successfully classify the starch–starch and starch–protein interaction in white pan bread. Wilson *et al.* (1991) reported that the maximum absorption for bread occurred at 1414 and 1465 nm.
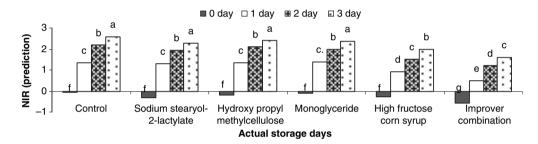

Figure 3 The NIR results of staling rate of Arabic flat bread formulated with control (C), sodium stearoyl-2-lactylate (SSL), monoglycerides (MG), hydroxy propyl methylcellulose (HPMC), high-fructose corn syrup (HFCS), or improver combination during storage at 23 °C/ 50% RH using a validation model.
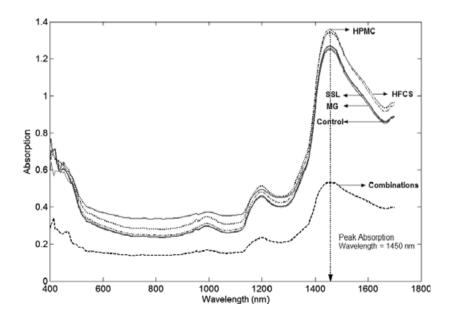

Figure 4 Raw spectra absorption of Arabic flat bread formulated with control (C), sodium stearoyl-2-lactylate (SSL), monoglycerides (MG), hydroxy propyl methylcellulose (HPMC), high-fructose corn syrup (HFCS), or improver combination after storage 3 days at 23 °C/ 50% RH.

Table 4 Sensory attributes of Arabic flat bread prepared with commercial chemical bread improvers

Treatments	Rolling and folding	Separation	Tearing
Sodium propionate-fumaric acid (PF-0 day)	1.71 ± 0.48 c	1.71 ± 0.47 a	2.5 ± 0.67 d
Sodium propionate-fumaric acid (PF-4 day)	8 ± 1 a	1.42 ± 0.78 b	4.14 ± 1.0 a
Control (3 day)	8 ± 1. 15 a	1.71 ± 0.5 a	3.42 ± 0.9 b
Improver combination (3 day)	7 ± 0.75 b	1.28 ± 0.76 c	2.85 ± 0.78 c

Based on the NIRS data (Fig. 3), the improver combination was expected to have the highest raw spectra absorption. The improver combination decreased the raw spectra absorption while the HFCS treatment exhibited the highest raw spectra absorption. The lower raw absorption for the improver combination in the AFB system may be attributed to larger amount of scatter back to the sensor, which would be seen as lower absorption. Thus, raw spectra absorption may not be sensitive enough to evaluate the loss of freshness when evaluating addition of multiple improvers.

Sensory analysis

'The control at 0 day exhibited significantly lower rollability scores, which may suggest higher quality compared to the other treatments (Table 4). Although, at day 3 the improver combination treatment exhibited a significantly lower rollability score compared to the control at day 3, indicating a product that may be more preferrred by consumers. A significantly higher rolling quality was observed in the AFB formulated with the improver combination except for fresh bread. These results indicate that the improver combination treatment slowed the loss of freshness. The lowest quality rolling and folding was observed in the 4-day-old bread. Wijaya (2003) reported significantly higher rollability scores in tortilla formulated with SSL, MG and gums. The maximum quality of separation (lowest sensory scores) was observed for the AFB with improver combination. Significantly higher tearing scores were observed in the improver combination compared to the control at day 3. These results were consistent with the texture analysis data, which showed that adding the improver combination to the flour formula significantly improved the sensory quality attributes of AFB.

Conclusions

NIR was used to predict the loss of freshness among treatments compared to the control. Improvers used in white pan bread may be incorporated into AFB formulations for shelf-life extension and to maintain sensory qualities as the bread ages. This has potential to limit economic loss where transportation is a limiting factor. The texture analysis, near-infrared spectroscopy and sensory evaluation results indicated that the improver combination addition significantly can improve the quality attributes and extend the shelf-life of AFB.

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