Effect of preservatives addition on the shelf-life extensions and quality of flat bread as determined by near-infrared spectroscopy and texture analysis

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Summary

Many people do not have refrigeration or frozen storage to inhibit mould growth and keep the Arabic bread fresh for more than a few days. Therefore, shelf-life extension is necessary for this type of bread. The present study hypothesised that the addition of preservatives may be done in Arabic flat bread (AFB) to extend shelf-life. Thus, objectives of this study were to evaluate selected preservatives to inhibit mould growth and to employ physical techniques, to monitor bread aging. Three preservatives, fumaric acid (0.2%, F), sodium propionate (0.3%, P), and sodium propionate-fumaric acid mixture (PF) were used. Tensile tests, and near-infrared reflectance spectroscopy (NIRS) were used to monitor bread ageing. The addition of PF in the AFB formula significantly increased the time of tearing at 0 day. For all treatments, the NIRS results showed high $R^2$-values between the actual storage days and NIRS predictions. The NIRS and texture analysis are valuable tools to detect the effect of the preservatives on AFB shelf-life and quality.

Keywords

Antimicrobial agents, flat bread, shelf-life, near-infrared spectroscopy, texture analysis.

Introduction

Flat bread is the oldest and most popular bread in the world (Qarooni, 1990). Over 1.8 billion consume flat breads daily (Qarooni, 1996). Flat breads contribute approximately 85% of the caloric value to the diets of Middle Eastern populations (Mousa et al., 1979). Arabic flat bread (AFB) not unlike other breads has a limited shelf-life. The two major considerations of shelf-life are spoilage and staling. Many 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 over 1 billion dollars per year (Baik & Chinachoti, 2000). The water activity of AFB ranges between 0.9 and 0.96 (Quail et al., 1990) which facilitates mold growth. The most prominent types of moulds associated with AFB are Aspergillus, Rhizopus and Penicillium families (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 (acetic, fumaric, sorbic and propionic acids or their potassium and calcium salts).

The AFB is considered to be at optimum quality during the first few hours after baking. The bread starts to lose strength and become firm after 24 h (Quail, 1996). These changes represent the initial stages of the staling process. Bread staling is a complex process that involves a set of physicochemical 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).

Several methods have been used to specifically evaluate the firmness of AFB. These methods include instron universal testing machine (Gurjal & Gaur, 2002), differential scanning calorimetry (Sidhu et al., 1997), and dynamic rheological testing (Toufeili et al., 1994). These are very diverse analytical methods that range from destructive to nondestructive, each with their inherent advantages and disadvantages.

Xie et al. (2003) used NIRS to follow the progress of staling. Using the NIRS, the researchers detected changes in white pan bread structure during storage. They compared the NIRS results to texture analysis (TA). They found that there was a high correlation...
between NIRS and TA measurements although NIRS can follow the staling during storage better than TA.

The objectives of this study were to identify one or more preservatives that may be included in an AFB formulation to extend shelf-life. Secondly, to investigate if TA and NIRS could be used to detect the effect of preservatives addition on the changes associated with staling.

Materials and methods

Wheat collection and milling

Jagger wheat from crop year June 2002 was collected from a local farm in Manhattan KS. This Jagger wheat was used to obtain flour with approximately 74–75% extraction rate after the milling (milled in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA). The flour was collected in 50 lb-bags (22.7 kg) and stored in a –10 °C freezer. Proximate analysis of the wheat and the flour used in the bread production were conducted by the Analytical Laboratory of the Department of Grain Science and Industry (Kansas State University, Manhattan, KS, USA). Moisture content was determined by using AACC approved method 44–15 A. Crude protein was determined according to AACC approved method 46–16. Ash was determined according to AACC approved method 08–01. Falling number was conducted according to AACC approved method 56–81B (1995). Starch damage was determined according to approved method 76–30A using Chopin SD4. Flour colour was determined using a colorimeter (Mini Scan Minolta CR-210, Minolta Corporation, Konica Minolta Germany, Munich, Germany).

Arabic bread production

Sodium propionate (0.3%) and fumaric acid (0.2%) were used as preservatives in four different Arabic bread formulas. These preservatives were either used alone or in combination. A mixture containing 1000 g flour (14% moisture basis), 1% yeast and 1% salt were mixed with and without preservatives (sodium propionate 0.3% and/or 0.2% fumaric acid). Water (53%; for a consistency of 850 BU was used). This consistency was found by experimentation to give the most reliable prediction of baking absorption (Qarouni et al., 1987; Quail et al., 1990) when mixed with the dry ingredients at different speeds. The dough was mixed at: low speed for 3 min. and then at medium speed for another 3 min (1 min beyond development time) by using Hobart planetary mixer (model C-100, Hobart, Troy, OH, USA). After mixing, the dough was transferred into a covered plastic bowl, placed into a proofing cabinet, allowed to ferment for 60 min at 86 °F (30 °C) and 70 ± 5% RH.

After fermentation, the dough was scaled off into 24–60 g pieces. The pieces were rounded by hand into balls and covered with plastic cover to prevent skin formation. The dough rested for 10 min. The dough was dusted lightly with flour and flattened by gentle hand pressure after which the dough was passed through a two stages roll sheeter. In the first stage, the gap between the rolls was 10 mm, and in the second stage, the gap was set to 3 mm. The sheeted dough was transferred to a stainless board and covered with a piece of cloth to minimize moisture loss. Then, the dough was transferred for final proofing for 30 min at 86 °F (30 °C) and 70 ± 5% RH.

The bread was baked at 400 °C (752 °F) for 90 s, on a preheated aluminium tray (215–450 mm) that accommodates two full size loaves. However, for consistent results, one loaf was baked each time. Three batches of bread, 24 loaves per batch were produced for each treatment. After baking, the bread was cooled for 10 min. Twelve loaves were stored in high density polyethylene bags at 23°C/ 50% RH and used for near-infrared spectroscopy at days 0, 1, 2, 3 for control (C) and fumaric acid (F) treatments, and 0, 1, 2, 3, and 7 days for propionate (P) and fumaric acid combined with propionate treatments (P–F) (These days were selected according to the mould appearance and bad bread quality which indicate ending loaves shelf-life). The other twelve loaves were stored in high-density polyethylene bags and used for texture, moisture, water activity and colour evaluation for the same storage periods and conditions and stored at 23 °C/ 50% RH. After 2 h, the loaves were assessed with the quality parameters for day 0 quality evaluation.

Arabic bread analysis

Shelf-life

Mould growth on the AFB is an indicator of length of shelf-life. Every AFB surface was checked every day for the appearance of mould.

Moisture content, water activity and (pH)

The moisture content of AFB was measured using AACC method 44–15A (American Association of Cereal Chemist., 1995). The AFB moisture content was measured at 0 day for all the treatments and after 3 and 7 days of storage at 23°C and 50% RH. Water activity was determined using an AQUA LAB CX-2 (Decagon CO, Pullman, WA, USA) after calibration with standard salt (AOAC, 1980). The pH was measured with a pH meter (Accumet® portable AP63, Fisher Scientific, Denver, Colorado, USA) with automatic temperature compensation following the AACC method 02–05 (American Association of Cereal Chemist., 1995)-Electrometric method. The pH meter was calibrated with buffer solutions of 4 and 7. The pH measurements were made at ambient temperature.
Texture analysis.
The change in the texture of AFB due to staling was measured using the tensile test. Measurements of time for tearing in tension (hold until time) was carried out. Uniform AFB strips shaped like a bar bell (taken from the center of the top 115 mm ‘long’, 20 mm wide at the ends, and 6 mm wide at the centre) were gripped with a special clamp, one end was attached to a TA.XT2 Texture Analyzer plate form, and the other end was attached to the texture analyzer arm. The distance between the two arms was set to 85 mm. A tensile grip probe was used with the following settings: pretest speed: 1 mm/s, test speed: 1.7 mm/s, posttest speed: 10 mm/s, 40% strain force: 10 g, trigger: mode auto with acquisition rate 250 points per second (pps).

Near-infrared spectroscopy – spectra measurements
A diode-array NIR spectrometer (DA7000 Perten Instruments, Springfield, IL, USA) was used to collect 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 the baseline. Each spectrum was recorded as an average of 15 scans/s.

Twelve loaves from each treatment were measured daily until the bread reached end of shelf-life as determined by the presence of mould. NIRS Measurements was taken on the top and the bottom of each loaf. The average of 15 scans were taken on each section every second and was recorded as one spectrum. Twenty four NIR spectra were taken for each treatment/day.

Data analysis
A cross validation method was employed to determine the applicability of NIRS to distinguish staling in AFB within each treatment. Spectra were analysed with partial least squares (PLS) regression (Martens and Naes, 1989) using a commercial software (PLS Plus/IQ for Grama/32, Galactic Industries, Salem, NH, USA) at 550–1700 nm wave length range. The optimum number of PLS factors was determined by cross validation. The number of PLS factors for the treatments, C, F, P, and PF was 12, 7, 10, and 10, respectively. These factors were judged by the following criteria: beta coefficient (the spectra with the least amount of noise), higher R², and the minimum partial residual error sum of squares (PRESS). Cross validation was employed to create the calibration models. The critical wavelengths identified (those with the maximum absorbance) were used to measure the beta coefficients for the calibration models. 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 dataset were left out and measured once in turn (n–a, n–b). The first step in creating of the calibration model was to determine the PRESS value.

A validation model was used to predict the staling among treatments compared to control. The control data was used to predict the change in staling for 0, 1, 2, and 3 days for all treatments. The control was used only for the first 3 days because shelf-life expired after 3 days because of mould growth. Staling at day 7 for PF treatments was predicted using a model developed using all P data. The NIRS prediction of storage days for all treatments correlated with actual storage time. The AFB staling rate was analysed by plotting NIRS measured storage time against actual storage time. The predicted storage time and the raw spectra absorbance were used to characterize the staling of the AFB. These raw spectra were prepared by averaging the scanned spectra of all 24 loaves of the three replicates for all of the treatments.

The RPD (Ratio of performance to deviation) and statistical analysis

The statistical model was a two-way factorial classification in complete randomised design. Data were analysed using statistical analysis software (version 8.2, SAS Institute Inc., Cary, NC, USA). 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.

Results and discussion
Moisture content, water activity and pH
Moisture content did not differ significantly among treatments at 0 (Fig. 1), although, there was a significant decrease in the moisture content within treatments from day 0 to day 3. Zobel & Kulp (1996) reported that water is an effective plasticizer in bread. They noted that after 3 days, the water content significantly decreased and the bread was firmer. Therefore, they concluded that less water was available to act as a plasticizer to keep the bread soft. Quail (1996) reported that staling is due, in part, to moisture loss but is mainly because of a chemical change to the starch–protein interaction. It was found that the baked breads at low water levels (during moisture redistribution) bread begins of mechanical firming, and losses of softness. This could be due to the starch recrystallisation and starch–protein interactions which are the major component of bread firming.
3 days
7 days
360

there was little change in the 

a

b

PF] at 0, 3, and 7 days storage.

different preservatives [control (C ), fumaric acid (F), sodium-

obtained by Czuchajowska

storage. These results are consistent with the results

preservatives ranged between 0.945 and 0.955 and was

crust and surrounding environment.

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crust and surrounding environment.

Rogers et al. (1988) reported that moisture content was

found to be inversely proportional to the rate of staling. Hallberg & Chinachoti (2002) found that standard white

dough showed a more rapid increase in firmness during

storage mainly because of the loss of moisture to the

crust and surrounding environment.

The water activity for AFB formulated with the

preservatives ranged between 0.945 and 0.955 and was

not significantly different among treatments or during

storage. These results are consistent with the results

obtained by Czuchajowska et al. (1989) who found that

there was little change in the aw between the fresh crumb

and the older crumb.

The pH of the bread for the control, F, P, and P–F

were 5.51, 4.27, 5.60, and 4.77, respectively. These

differences in the pH play an important role in determin-

ing the taste, colour, and texture of the finished

product (Maselli & Pomper, 1960).

Shelf-life of AFB formulated with preservatives

After 3 days, the AFB control and F treatments

exhibited mould growth (Rhizopus nigricans - bread

mould) indicating end of shelf-life (Fraizer & Westhoff,

1978). The P treatment extended shelf-life of the AFB an

additional 5 days compared to the control and F

treatments. A 16 day shelf-life was obtained for AFB

formulated through the synergetic action of PF in the

combination preservative treatment.

The addition of fumaric acid to the bread formula

increased acidity (pH 4.27) but did not extend the shelf-

life of AFB compared to other preservatives. The

moulds can grow at this pH range (Fraizer & Westhoff,

1978), but fumaric acid increased the effectiveness of

preservative agents (Maselli & Pomper, 1960).

Addition of P to the bread formula did not change the

pH (pH 5.60), but the shelf-life of AFB was extended to

8 days. Luck & Jager (1997) reported that the propion-

ate inhibitory action may be as a result of the accumu-

lation of the preservative in the cells of the mould.

Within the cells of mould, sodium propionate dissoci-

ates in water to form sodium ions and propionate ions.

The propionate ions react with water to form propionic

acid (three carbon skeleton). The toxicity of propionic

acid is related to inability of the moulds to metabolize

the three carbon skeleton (Fennema, 1996). This action

inhibits the growth of the mould.

Adding sodium propionate combined with fumaric

acid to the bread formula changed the pH ( pH 4.77).

Essentially, the fumaric acid increased the effectiveness

of sodium propionate. These results are consistent with

those obtained by Wijaya (2003) in tortilla bread.

Tearing force

Just because preservatives can inhibit microbial growth

does not mean that the quality of the product is

extended or improved.

Figure 2 shows that no significant difference in tearing

forces among the treatments at day 0. After 1 day, P and

PF treatments exhibited the same tearing force as day 0,

indicating that there was no change in bread quality.

However, a significantly lower tearing force was

observed for the control and the F treatment at day 1.

At day 2, both the P and PF treatments showed a

significant decrease in tearing force. At day 3, there was

no significant difference between the control and PF

treatment in tearing force. These treatments showed

significantly higher tearing force compared to F and P

treatments, suggesting that adding PF treatment to the

flour did not affect the AFB tearing force after 3 days.

However, adding F or P separately affected the AFB

tearing force after 3 days. These results may indicate

that PF treatment slowed staling; the AFB was not as

brittle as for either the F or P treatment. Thus, the

preservatives do inhibit mould growth but do not help

with maintaining freshness.

Overall, the force required to tear the bread decreased

significantly with storage time for all the treatments,

suggesting that the bread quality of different treatments

decreased over time. These results are consistent with

the results obtained by Rao et al. (1986) in Chapati

bread. They found that the tearing strength (indicative

of brittleness) decreased markedly during storage.

Tou-

felli et al. (1998) reported that the force required to

initiate tearing in AFB and probing extensibility
decreased significantly as the bread aged. The sample

lost resilience and became brittle after 3 days of storage.

These changes may be as a result of the retrogradation

of amylopectin fractions, protein–starch interactions,

and the redistribution of moisture between starch and
protein systems (Toufeili et al., 1993; Sidhu et al., 1997). These changes as well as the differences in the pH between different treatments may be responsible for the changes in the firming of AFB during storage.

**Tearing time**

The AFB-PF treatment exhibited a significantly higher tearing time compared to the other treatments at day 0 (Fig. 3). After day 1, the PF treatment exhibited a 67% reduction in tearing time compared to day 0. Additionally, all other treatments exhibited a significant decline in tearing time from day 0 to day 1. The reduction in tearing time may indicate loss of freshness. At day 3, there was no significant difference between the control and PF treatment in tearing time, although there was a significant difference between the control, PF from one side and F, and P from another, suggesting that adding the PF combination to the flour did not affect the AFB tearing time after storage compared to the control while adding F and P separately affected the AFB tearing time after storage.

The measure of tearing time vs. tearing force is ostensibly a more sensitive indicator of a change in freshness. The tearing time results obtained were more consistent than the tearing force results. These results were consistent with the results obtained by Toufeili et al. (1998) who found that probing extensibility (indicating the time of tearing) decreased significantly ($P < 0.05$) as aging progressed. These changes could be attributed to the retrogradation theories mentioned above (Toufeili et al., 1993; Sidhu et al., 1997). Bread staling is a complex process that involves a set of physicochemical chemical changes (moisture distribution, firmness, tearing properties). These changes could be responsible for the significant reduction in the bread tearing force and time during storage.

The $R^2$ for the tearing force ranged between 0.61 and 0.98, whereas with standard error of determinations ranged between 5.1 and 9.1. However, the $R^2$ for the tearing time ranged between 0.84 and 0.99, with standard error of determination between 0.28 and 0.65. These results indicate the importance of using the tensile test by using texture analyzer to study the changes in Arabic bread over time.

**Staling evaluation of Arabic flat bread using NIRS**

The cross-validation model was used to evaluate the effectiveness of the NIRS to measure staling within each
treatment. The top-bottom models of the AFB were evaluated. The \( R^2 \), SEE, RPD were shown in Table 1. These high correlations and good range of RPD indicate the importance of using NIRS as a tool to study the staling of AFB produced by different treatments. It means that they can be used to predict the effect of different additives on the staling. These results were consistent with the results obtained by Xie (2002) in studying the staling of white pan bread by using NIR. Researcher reported that RPD for different batches ranged between 3.7 and 5.0.

The validation model was used to evaluate the effectiveness of the NIRS to measure staling amongst treatments. The top-bottom models of the AFB were evaluated. The \( R^2 \) range between 0.88 and 0.92 (Table 2) with SEE range between 0.25 and 0.52 for all the different treatments. These results indicate the importance of using NIRS validation to distinguish between staling of different treatments.

The top and bottom sections of the AFB-F treatment exhibited a significantly higher freshness loss than treatments using other preservatives at day 0, 1, 2, and 3 of storage (Fig 4). However, the top and bottom AFB-P treatment exhibited a significantly lower staling than treatments using other preservatives at day 0, 1, 2, and 3 of storage. There was no significant difference in the freshness loss between the control and PF after 3 days of storage. So the highest freshness loss was in bread produced using F alone. This may be as a result of the active double bond within the fumaric acid structure. Sidhu et al. (1980) reported that fumaric acid has a reducing effect. This mechanism appears to be a free radical that stabilizes the sulphhydryl protein groups, resulting in a dough structure that has fewer disulphide cross-links. So the final product was \( S \)-succinyl-L-cysteine with additional two carboxyl groups. This compound may promote interactions between the amylose and amylopectin fractions or may result in an increased interaction between protein and the starch during aging, which would directly affect the staling rate. As discussed previously, F decreased the pH (4.27) of the bread when compared to the control (5.52). Thus, fumaric acid may change the ionic status of the gluten and subsequent aggregation (Friend et al., 1995). The lowest freshness loss was observed for the bread produced with sodium propionate alone (pH 5.62). However, Luck & Jager (1997) reported that propionic acid may interact with some amino acids in the gluten chain and affect protein functionality.

The absorption spectral differences offer another way to evaluate freshness loss. These spectral differences are shown in Fig 5. The absorbance spectral differences were highest between C and F treatments and the absorbance spectral differences (Fig. 5) were lowest between C and PF treatments. The absorbance for the control was lower than the absorbance for the bread with either sodium propionate or the combination of sodium propionate and fumaric acid. These results were consistent with the results of freshness loss mentioned above. The bread with F had highest freshness loss with lowest absorption while the bread formulated with the PF combination had the same freshness loss as control and nearly the same absorption. These results may be explained by the starch retrogradation and crystallinity. Starch reverts to an insoluble state during staling (Zobel

Table 1 Summary of the NIRS for different treatments using cross validation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Loaf section</th>
<th>( R^2 )</th>
<th>SEE</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Top + bottom</td>
<td>0.82</td>
<td>0.25</td>
<td>5.1</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>Top + bottom</td>
<td>0.88</td>
<td>0.18</td>
<td>3.9</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>Top + bottom</td>
<td>0.93</td>
<td>0.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium propionate + fumaric acid</td>
<td>Top + bottom</td>
<td>0.93</td>
<td>0.54</td>
<td>2.39</td>
</tr>
</tbody>
</table>

*All correlation are with actual storage time.

Table 2 Summary of the NIRS for different treatments using validation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Loaf section</th>
<th>( R^2 )</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Top + bottom</td>
<td>0.95</td>
<td>0.25</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>Top + bottom</td>
<td>0.88</td>
<td>0.69</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>Top + bottom</td>
<td>0.97</td>
<td>0.48</td>
</tr>
<tr>
<td>Sodium propionate + fumaric acid</td>
<td>Top + bottom</td>
<td>0.92</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*All correlation are with actual storage time.

SEE refers to standard error of estimation.

0.2% Fumaric acid.

0.3% Sodium propionate.

0.2% Fumaric acid + 0.3% Sodium propionate.

Figure 4 The NIRS results of the staling of Arabic flat bread with different preservatives (control (C), fumaric acid (F), sodium-propionate (P), and sodium propionate–fumaric acid combination (PF)) using validation. Bars with the same letters are not significantly different at \( P < 0.5 \).
& Kulp, 1996). This crystal form of starch may scatter more light back to the sensor, which is seen as lower absorption in the bread with fumaric acid and higher absorption in bread with sodium propionate. Although there was no change in absorbance spectral differences for all of the treatments were observed from 550 to 1150 nm except the Control-Fumaric spectral differences. The greatest difference in absorbance spectra was observed from 1430 to 1495. This peak might relate to the second overtone of C–H, C–H₂, CONH₂, ROH, and H₂O.

This means that moisture loss, starch retrogradation, and protein interaction might affect bread staling. Xie (2002) reported that two important wavelengths 550 and 1465 nm, were key for the NIRS to successfully classify the starch–starch and starch–protein bread. However, three wavelengths 970, 1155 and 1395 nm, were associated with amylopectin retrogradation. Wilson et al. (1991) reported that the maximum absorption for bread occurred at 1414 and 1465. These wavelengths were used to study the states of water in the foods with respect to hydrogen bonding.

There was a good relationship between NIRS and texture analysis data for the control and PF treatments. In both methods, the control and PF treatments showed no differences in the freshness loss.

Conclusions

The F–P inhibited mould growth 320% longer compared to the control. Tearing time proved to be a better indicator of loss of freshness compared to the tearing force. NIRS proved to be very valuable tool in indicating the changes in freshness of AFB during the storage with high correlations and low standard deviations.

References


