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MODELLING FOR THERMAL DEGRADATION OF THIAMIN (B1) IN FLAT ARABIC BREAD

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ABSTRACT

Flat Arabic bread was prepared from 40.4 ppm thiamin- fortified patent, straight and whole wheat flours, by the straight dough method. Doughs were fermented for three fermentation times and baked at five temperatures for three different times each. The so-obtained bread was analyzed for its thiamin content using HPLC method.A predictive mathematical model using multivariate stepwise regression analysis with % thiamine retention as dependent variable, and baking conditions of fermentation time, baking temperature and time as independent variables, was constructed. Results indicated that regardless of the flour type, the process of baking resulted in about 21-24% loss of the vitamin, with baking temperature as the most significant variable affecting its degradation in this type of bread, followed by baking time. Fermentation time, on the other hand, had no significant effect on the retention of this vitamin. The model also showed that baking temperature is responsible for as much as 76% of variation in the thiamin change during baking.

KEYWORDS: *Arabic bread, Multivariate stepwise regression, Thermal degradation, Thiamin*

Article History

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INTRODUCTION

Wheat bread is the staple of most peoples of the Middle East as it contributes up to about 70% of the total calorie intake of many of these peoples. Health concerns related to dietary intake of vitamins range from mild acute health issues to core life threatening physiological disorders (Health Link BC, 2014).Water soluble vitamins are rapidly depleted, and an individual may start exhibiting deficiency symptoms unless repletion takes place. In order to reduce micronutrient malnutrition/deficiency, the Jordanian Ministry of health (MOH, 2010) has passed legislation that mandates the fortification of straight grade flour with thiamin and other. However, despite national efforts to fortify wheat flour, deficiencies of vitamins among Jordanians including thiamin have been reported (MOH, 2010; Qatatsheh, *et al.*,2015), which raises concerns about efficiency of flour fortification or the efficacy of the vitamin mixes which might be lost during the processing conditions of fermentation and baking.

Thiamin is a water-soluble vitamin that is known to be stable in the dry state and can sustain high temperatures up to 100°C (Ball, 2004), as its requirements has been influenced by the composition of the diet, climate, body weight, physical activity, age, and pathological conditions (Gallagber, 2008).Thiamin is known of being unstable to environmental conditions including high pH, extreme heat, oxygen, ultraviolet light, oxidation, and ionizing radiations (Dwivedi and

Arnold, 1973; Butterworth, 2003).The aim of this work is to elucidate the effect of the various baking conditions on thiamin retention of the flat bread and construct a prediction model for its levels in bread. The ultimate objective is to evaluate the fortification process and decide whether other staples should be fortified to meet the vitamin requirements of the Jordanian population.

MATERIALS AND METHODS

Flour Samples

Unfortified whole wheat, straight grade and patent flours with70-73, 77-80, and 99-100% extraction rates respectively, were obtained from a local commercial mill and stored at room temperature in the cereal technology laboratory of the department of Nutrition and Food Technology of the University of Jordan.

Flour Fortification

Calculated amounts of the vitamin premix from DSM Nutritional Products, France, were added to provide a level of 40.4 ppm of thiamin. The fortified flour was kept under subdued light and dry condition to prevent vitamins from degradation.

Flour Characterization

Moisture, ash, gluten, damaged starch and falling number were determined by official methods of the AACC # 930.15, 08-01, 38-12.02,76-33 and 56-81, respectively.

Bread Production

Lean formula, thick Kmaj bread was prepared from the three flour grades in a local bakery, by the straight dough method. Three fermentation times (0, 60 and 90 minutes), five baking temperatures (250, 300, 350, 400 and 450°C) and three baking times (1, 2 and 3 minutes) were used (Table 1).Baking temperature was measured in an oven equipped with a fixed probe in order to control of temperature, which should be maintained during the baking process.

Table 1: Treatment Structure of the Study

Flour Type	Fermentation Time	Baking Temp.	Baking Time					
			1 Minute		2 Minutes		3 Minutes	
			R1	R2	R1	R2	R1	R2
Patent, Straight, and Whole wheat Flour	0 time	250°C	X	X	X	X	X	X
		300°C	X	X	X	X	X	X
		350°C	X	X	X	X	X	X
		400°C	X	X	X	X	X	X
		450°C	X	X	X	X	X	X
	60 minutes	250°C	X	X	X	X	X	X
		300°C	X	X	X	X	X	X
		350°C	X	X	X	X	X	X
		400°C	X	X	X	X	X	X
		450°C	X	X	X	X	X	X
	90 minutes	250°C	X	X	X	X	X	X
		300°C	X	X	X	X	X	X
		350°C	X	X	X	X	X	X
		400°C	X	X	X	X	X	X
		450°C	X	X	X	X	X	X

Vitamin Analysis

Preparation of Standards

Thiamin in the form of thiamin nitrate (B1) was obtained from Sigma-Aldrich (USA).HPLC grade water, methanol, acetonitrile, trifluoro acetic acid (TFA), EDTA, dichloromethane was obtained from Merck (Geneva, Switzerland).Stock individual solutions of B1 were prepared at a concentration of 100 ppm by dissolving 5 mg of vitamin in 50 ml HPLC grade water.The stock solutions were kept in amber vials and stored at -18°C to avoid degradation. Working solutions of vitamin standards were prepared daily by mixing and diluting individual stock solutions in water to the desired concentrations.

Chromatographic Conditions

The concentrations of vitamin in the extracts were determined using Thermo Scientific Dionex UltiMate® 3000 HPLC system consisting of a LPG 3400 SD pump, ACC- 3000 auto-sampler, and DAD detector. Reverse phase-HPLC with ACE C18-AR (250 x 4.6 mm; 5μm) column was used. Gradient mobile phase consists of 0.03% TFA in water (pH 2.6)(A) and acetonitrile (B) was employed as shown in Table (2).The injection volume was 20 μl, the flow rate was 0.9 ml/min and the column temperature was 25 °C. The signal (peak area) of vitamin was obtained using photo diode array detector (DAD) at four wavelengths 361, 280, 265 and 210 nm. The process and the registration of the chromatogram were controlled by the Chromeleon® 6.80 Chromatography Data System (CDS) software.

Table 2: Mobile Phase used in Determination of Thiamin (Gradient Elution)

Time	% A (0.03% TFA in Deionized Water)	% B (Acetonitrile)
0	100	0
2	100	0
4.5	83	17
9.5	83	17
9.6	100	0
13.5	Equilibration	

Determination of Thiamin in Bread

The bread samples were extracted by simple direct solvent rather than enzymatic, alkaline or acid hydrolysis. According to Ekinci and Kadakal, (2005) slightly acidified water (prepared by adding drops of 0.005M HCL solution to the deionised water until the pH reached 4.2 was reported to be a satisfactory method for extraction of B vitamins. The bread samples were chopped and homogenized then 5.00 g of bread was weighed into 25 ml conical plastic centrifuge tube and mixed with 10 ml deionized water. The mixture was vortexed to mix thoroughly for 1 min, followed by shaking for 15 min in the water bath shaker (Memmert WB 14, Germany) at 50° C in the dark and then centrifuged (Hermle Z 206 A, Germany) at 6000 rpm for 10 min.The supernatant was collected, and the precipitate was re-extracted with 5 ml deionised water, vortexed to mix thoroughly for 1 min and then centrifuged at 6000 rpm for 10 min.The supernatant was combined to those of the first extraction and finally filtered through a 0.45μm nylon membrane and delivered to HPLC analysis.The concentration of vitamin in the bread extract was calculated from the calibration curve. Then the amount of vitamin per dry bread (mg/g) and the retention % of vitamin in bread were calculated (Table 4) depending on the equation 1 (eqn.1) and equation 2 (eqn.2), respectively.

$$mg vitamin per g dry bread =$$

$$Conc. of vitamin in bread extract X \left(\frac{mg}{L}\right) X \frac{Vol. extraction solution (L)}{sample wt g of wet bread} X \frac{100\ g wet bread}{(100 - mositure content) g dry bread} \quad (1)$$

$$\%\ \text{Retention of vitamin} = \left(\frac{mg vitamin per g dry bread measured}{mg vitamin per g dry bread theoritical} X Correction Factor\right) X\ 100\% \quad (2)$$

Statistical Analysis and Modelling

A Randomized Complete Block Design (RCBD) was followed with blocking on the two replicates.Analysis of variance (ANOVA) of the data and stepwise multiple regression and multivariate analysis was used to construct the degradation model of the vitamin was carried out using Statistical Analysis System SAS software (2003).The experiment was conducted in two replicates.

RESULTS AND DISCUSSIONS

Flour Characterization

The flour and dough chemical and physical analysis (Table 3) showed that the gluten index was highest in case of patent flour which contain highest amount of endosperm so the gluten too, where ash content is the highest for whole wheat flour which contains the bran and aleurone layer. The highest α- amylase activity was in patent flour and the lowest in whole flour.

Table 3: Chemical and Physical Properties of the Flour Types used in the Experiment

Properties	Type of Flour		
	Patent	Straight	Whole
Moisture content %	11.85 ± 0.06	12.51 ± 0.05	11.12 ± 0.06
Ash %	0.48 ± 0.02	0.65 ± 0.02	1.53 ± 0.03
Damaged starch%	6.81 ± 0.01	6.72 ± 0.01	6.57 ± 0.01
Wet gluten %	23.14 ± 0.24	24.13 ± 0.25	25.99 ± 0.25
Dry gluten%	19.49 ± 0.02	20.61±v 0.02	21.91 ± 0.03
Gluten index %	97.74 ± 0.53	94.5 ± 0.57	84.50 ± 0.64
Falling no.	366 ± 2.02	400 ± 1.34	428 ± 2.54

The values are mean ± SEM.of determinations made in triplicates

Retention of Thiamin in Flat Arabic Bread

As shown in tables (4 &5), B1 retention % was found as 76.8 ± 1.89, 77.0 ± 2.07, and 79.2 ± 2.78 in patent, straight and whole wheat bread, respectively. There was a significant difference ($p \leq 0.05$) in retention % between whole and both straight and patent bread but not significant ($p \leq 0.05$) between patent and straight bread. Thiamin retention was highest in whole wheat bread and least in bread prepared from patent flour. This is natural as whole wheat contains more of the vitamins and minerals due to its high content of aleurone and bran particles. Refining of flour results in significant loss of vitamins and minerals (NAS, 1998; Aziz, *et al.*2006).In addition, whole wheat bread contains vitamin E, phenolic compounds and phytate which act as antioxidants and chelating agents respectively (Garcia-Estepaet al., 1999; Steiner et al., 2007; Pham, 2014).The low retention of thiamin in patent bread may due to the ability of thiamin to react with cystein or cystine residues that form thiamin-protein Complex, patent flour has the highest gluten index(Table 3)and gluten is known to have high cysteine residues (Okmen, 1999;Rombouts, *et al.*2009).

Table 4: Retention % of Thiamin in Flat Arabic Bread Produced from Different Flour Grades and Produced Under Different Baking Conditions

Baking Temp.ºC	Fermentation Time	Patent			Straight			Whole		
		Baking time			Baking time			Baking time		
		1 min	2 min	3 min	1 min	2 min	3 min	1 min	2 min	3 min
250	0	99.0	99.0	100.0	107.5	106.5	101.0	104.0	101.5	102.0
	60	96.0	95.0	89.5	98.5	91.5	88.5	99.0	97.0	96.5
	90	93.5	91.0	89.0	89.0	89.0	84.0	97.0	96.5	92.0
300	0	100.0	92.0	92.0	101.0	103.5	102.0	113.0	109.0	104.0
	60	96.0	87.0	81.5	90.5	87.0	82.5	95.5	95.5	90.5
	90	91.0	84.5	79.0	92.0	84.5	79.5	98.0	97.0	86.0
350	0	89.0	88.0	86.5	90.0	84.0	79.0	102.5	101.5	98.5
	60	91.5	79.0	79.0	93.0	80.5	73.0	94.5	87.0	82.0
	90	92.0	76.5	70.0	87.0	75.5	62.5	93.0	86.5	83.5
400	0	77.0	72.0	67.0	79.0	75.0	66.5	93.0	66.5	25.5
	60	70.5	58.5	53.5	77.0	68.5	43.0	85.5	74.0	44.5
	90	72.0	57.0	52.0	77.5	64.0	37.0	87.0	58.5	41.0
450	0	68.0	58.0	57.5	72.0	68.5	51.0	67.0	54.5	25.0
	60	61.0	46.0	42.5	68.0	46.0	34.5	61.0	44.0	22.0
	90	60.5	43.0	33.5	61.5	41.5	33.5	60.5	33.5	17.0

Influence of Individual Main Effects on Thiamin Retention

As indicated in table (5), it was found that the retention % of B1 was 84.4 ± 2.24, 75.9 ± 2.15 and 72.7 ± 2.28 % for 0, 60, and 90 min fermentation time, respectively. There were significant ($p \leq 0.05$) differences in retention % of vitamin B1 between 0-60, 0-90 and 60-90 min the lowest degradation was notice dat zero fermentation, this may be because young bread has the highest moisture content at all baking temperatures; here pocketing did not occur and steam remained in the bread raising the specific heat (Budžaki and Šeruga, 2015).The lowest retention % was observed at 60 min fermentation time, this may be due to the improved porosity and more losses of water and decreased specific heat with the increased effect of temperature (Gupta, 1990).

Table 5: The Overall Mean Values of Thiamin in Flat Arabic Bread as Influenced by the Main Effects of Flour Type, Fermentation Time, Baking Temperature and Baking Time

Flour Type				
Patent	Straight	Whole		
76.8^{B} ±1.89	77.0^{B} ± 2.07	79.2^{A} ± 2.78		
Fermentation Time				
0 Min	**60 Min**	**90 Min**		
84.4^{A} ± 2.24	75.9^{B} ± 2.15	72.7^{C} ± 2.28		
Baking Temperature				
250 ºC	**300 ºC**	**350 ºC**	**400 ºC**	**450 ºC**
96.0^{A} ± 0.83	93.1^{B} ± 1.25	85.4^{C} ± 1.31	64.5^{D}± 2.22	49.3^{E} ± 2.12
Baking Time				
1Min	**2 Min**	**3 Min**		
86.5^{A} ± 1.50	77.7^{B} ± 2.09	68.9^{C} ± 2.70		

Readings are in %of retention of the vitamin in the flour on dry matter basis, values are the mean of (135) readings ± SEM.

Means within same row with the same superscripts are not significant (*P*<0.05) different according to LSD test.

There were significant (p≤0.05) difference in retention % of vitamins B1 between the baking temperatures of 250, 300, 350, 400, and 450 ºC.A number of researchers (Bottomley and Nobile, 1962) agreed on the relative instability of B1 during baking.20% of the vitamin is destroyed during normal baking (Hoffman et al., 1940; Dawson and Martin, 1941).Tiong*et al.*, (2015) reported that baking causes a loss ofB1 ranges from 20 to 56%., and this is in agreement with our results. At high temperature (450 ºC) the B1 showed the least retention (49.3%), this may be due to its high sensitivity to heat. The results (Table 5)also indicate that thiamin suffered significant loss (p≤0.05) loss with baking time, Lebiedzińska*et al.*(2007) noted that the degradation of vitamins will increase as baking time increase.

Prediction Model for Degradation of Thiamin in Flat Arabic Bread

Stepwise regression technique was used to elucidate the effect of the fermentation time, baking temperature and baking time on the retention of the vitamin in this bread. In order to separate the effect of flour type which cannot be numerically represented, each flour type was treated separately. The results are as shown in these equations.

- Patent:$\% \ B1 = 165.90556 - 0.21944T - 6.15000B$
- Straight:$\% \ B1 = 172.00000 - 0.22067T - 8.86667B$
- Whole:$\% \ B1 = 204.15556 - 0.29222T - 11.35000B$

Whereas, T (Baking temperature) and B (baking time)

Table 6: R^2 Values of the Different Independent Variables as Obtained from the Regression Equations

Type of Flour	R^2	Variable Interred
Patent	0.7541	Baking temperature
	0.8331	Baking temperature + baking time
Straight	0.6356	Baking temperature
	0.7725	Baking temperature + baking time
Whole	0.6202	Baking temperature
	0.7450	Baking temperature + baking time

The results are shown below, which indicate that T and B contribute to decreasing the % retention of vitamins. Fermentation time has no significant effect on % retention of vitamin when each type of flour was treated individually. The model shows that % retention of the vitamin increases by the increasing the extraction rate of the flour from which bread is prepared, i.e.79% in case of whole wheat bread compared to77 and 76%in case of straight and patent breads respectively. These values were obtained by substitution in the regression equitation for baking temperature (T = 350 °C) and baking time (B = 2 min).The highest retention of B1 in whole bread this is likely due to the higher vitamin B1 content of the whole meal flour compared to that of patent flour (4.6 vs.1.0 ppm)((Paul and Southgate, 1980) or possibly due to the protective effects of other constituent on the vitamin from the heat. From the R^2 value it seems that heat contributes to vitamin loss in case of whole wheat bread less than in case of patent and straight bread (Table 6) this probably is due to the patent composition effect of other constituents like reducing sugar which present in higher amount in patent which has higher α-amylase activity (Table 4)as thiamin reacts strongly with cystein or cystine which increases its degradation, patent flour also has higher amount of gluten in endosperm (Table 4) (Doyon and Smyrl, 1983).

From the regression equations and R^2 (Table 6) values, the baking time contributes more to the B1 degradation in case of patent bread compared with whole and straight bread.

CONCLUSIONS

Thiamin in flat Arabic bread undergoes thermal degradation during baking under the conditions of high temperature and short time. The % retention of the vitamin in bread is influenced by the type of flour used in the preparation of bread, with the highest extraction flour produces bread with highest vitamin retention and vise versa. Among all baking conditions, the baking temperature had the highest effect on vitamin retention in this bread type with its effect most pronounced in case of the patent flour as indicated by the mathematical model. On the other hand fermentation time had no significant effect on retention of this vitamin. Baking time, while has a significant effect on its retention, it is less responsible for the changes in its level than baking temperature.

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