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Impact of proofing and baking parameters on B complex vitamins retention of Arabic flatbread produced from wheat flour with different extraction rates

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Abstract: Extensive research studies worldwide have discussed and analysed the effect of processing conditions on the nutritional aspects of Western types of bread; however, the literature on Arabic bread processing is very limited. This study aims to determine the effect of baking temperature and time on the retention of B vitamins in a pocket-forming Arabic flatbread model system. High-crumb flat Arabic bread (Thick Kmaj) was prepared by the straight dough method from three types of flour (patent, straight grade, and whole wheat) fortified with B vitamins. Doughs were fermented and proofed for 0, 30, 60, and 90 min and baked at five temperatures (250, 300, 350, 400, and 450 °C) for three different baking times (1, 2, and 3 min). Baking at lower temperatures (i.e. < 300 °C) resulted in higher B-complex vitamin retention values (more than 90%). Vitamin B6 showed exceptional retention values (about 100%), though these decreased by increasing the baking temperature. Vitamin retention levels in the produced Arabic bread samples are similar to those found in pan and other high-crumb bread types when baked at lower temperatures. Results are expected to positively impact the output and economics of the flour fortification process, as it can be helpful material for upcoming micronutrient survey studies to assess fortification process outcomes.

Keywords: thermal degradation; micronutrient stability; thick kmaj; fortification efficiency; baking temperature

Arabic flatbread was produced for centuries in households and small hearth ovens using the natural microflora of wheat flour as a leavening agent, which requires a longer proofing time. This practice was replaced by the use of pure commercial baking yeast, which resulted in the loss of the characteristic traditional flavour of Arabic bread and its nutritional value (Al-Khamaiseh et al. 2023). Pocket-forming Arabic flatbread, which is the most common in the Levant region, is proofed for shorter times and baked at very high temperatures (up to 500 °C) for short baking times (60 s or less), in contrast to pan and French types of bread. The higher temperatures result in pocket formation due to steam puffing of the loaves rather than oven rise by fermentation gases. Depending on the thickness of the dough sheets, this bread can have either paper-thin upper and lower layers and be almost crumb-free (known as 'Thin Kmaj'), or thin upper and thick lower layers with some

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crumb composition (known as 'Thick Kmaj'). On the other hand, non-pocket forming types with a single layer are also produced by thin, perforated, sheeted dough, or by immediate baking without final proofing (Ajo et al. 2010). Thus, baking with high temperatures for such types of bread is expected to negatively impact heat-sensitive B-complex vitamins. The vitamins deficiency is a global public health concern in many countries, especially those suffering from poverty, food insecurity, and a lack of health knowledge (Rostami et al. 2007; Hwalla et al. 2017). Furthermore, increasing the consumption of highly refined and ready-to-eat foods would exacerbate the problem (Younis et al. 2015). According to the Food and Agriculture Organisation (FAO 2023), the number of hungry people in the world (between 702 and 828 million) is increasing due to prevailing health, social, and other conditions. Therefore, many governments have passed legislation mandating the fortification of wheat flour with vitamins and minerals at the mill level to mitigate vitamin malnutrition and deficiency (Olivares et al. 2007; WHO 2009). The B vitamins are a group of eight water-soluble organic compounds that serve as coenzymes in numerous metabolic processes, primarily involved in energy production and cell function. Since they are water-soluble, the body generally does not store large reserves (with the exception of vitamin B12), necessitating regular dietary intake. The transformation of dough into bread involves several steps: mixing, fermentation (ripening/leavening), and baking, each influencing the final B-complex vitamin content.

Thiamine (B1) is the most heat-labile of the group; its thiazole ring is susceptible to destruction at high temperatures and neutral/alkaline pH. Riboflavin (B2) is generally heat-stable but highly vulnerable to light (UV) degradation. Niacin (B3) is notably thermally stable, with minimal loss expected during typical baking. Pyridoxine (B6) exhibits moderate heat sensitivity, with losses dependent on baking intensity and water content. Folate (B9) is sensitive to both heat and oxidation. Cyanocobalamin (B12) is moderately stable but can be lost in aqueous solutions under heat (Batifoulier et al. 2005; Mihhalevski et al. 2013; Combs and McClung 2017). Tiong et al. (2015) concluded that there is a relative instability of vitamin B1 during the baking of pan bread, which is subjected to about a 20% loss under normal baking conditions. Conversely, many early studies confirmed the relatively high thermal stability of vitamin B3 during pan bread processing, with almost little or no loss observed upon baking at 250 °C for 30 min (Mahgoub et al. 1999). Maleki and Daghir (1967) showed minimal but high destruction of B1 in white and brown flatbread, especially at high baking temperatures, while B2 was found to be highly stable in both bread types. Furthermore, a study conducted by Rubin et al. (1977); using the sponge and dough method and baking at 212 °C for 20 min; showed that the retention values of B1, B2, B3, B6, and B9 vitamins in pan bread were 101, 105, 100, 100, and 105% respectively. Other researchers (Öhrvik et al. 2010; Mihhalevski 2013; Edelmann et al. 2016; Hrubša et al. 2022) reported different levels of B vitamin loss in pan bread during baking, depending on the baking temperature and time. To reduce micronutrient deficiency, the government of Jordan has passed; through the Ministry of Health (MOH); legislation that mandates the fortification of straight-grade flour with water-soluble B vitamins (B1, B2, B3, B6, B9, and B12) and fat-soluble vitamins A and D. Unfortunately, despite national efforts to fortify wheat flour, deficiencies of these vitamins among Jordanians have been reported (MOH 2011; Qatatsheh et al. 2015), which raises concerns about the efficiency of the flour fortification programme or the efficacy of the vitamin mixes, which might be lost during processing conditions such as fermentation and baking. Accordingly, this work aims to study the effect of proofing time, baking temperature, and baking time on the retention of B vitamins in Arabic flatbread, and to determine the optimal baking conditions for the retention of added vitamins.

MATERIAL AND METHODS

Flour samples and fortification

Unfortified 10 kg samples of patent, straight-grade, and whole-wheat flour, milled from Hard Red Winter (HRW) wheat with 65, 80, and 95% extraction rates, respectively, were obtained from a local commercial mill in Jordan. For fortification, a quantity of 30 g of the vitamin premix (DSM Nutritional Products, France) was mixed thoroughly with 1 kg of each flour type, after which it was mixed with the rest of the flour sample. Samples were stored at ambient temperature (25 °C) under subdued light and dry conditions. An unfortified control sample from each flour type was prepared. The nutrient composition of the vitamin premix is as follows:

Vitamin A (11 000 IU), as dry vitamin A palmitate
Vitamin D3 (58 ppm), as cholecalciferol
Thiamine (B1) (11 592 ppm), as thiamine mononitrate
Riboflavin (B2) (14 400 ppm), as riboflavin

Niacin (B3) (140 000 ppm), as nicotinamide

Pyridoxine (B6) (14 480 ppm), as pyridoxine hydrochloride

Folate (DFE) (B9) (6 064 ppm), as folic acid

Cyanocobalamin (B12) (30.5 ppb) (g·kg^{-1}), as cyanocobalamin

Iron (150 500 ppm), as sodium iron EDTA (NaFeEDTA)

Zinc (81 850 ppm), as zinc oxide

Flour characterisation

Moisture, protein, ash, gluten, falling number, damaged starch, and dough rheology (using the Farinograph test) for each type of flour were analysed following AACC methods 44-15A, 46-16.1, 08-01, 38-12.02, 56-81B, 76-33, and 54-21, respectively.

Arabic bread production

The flatbread formula [comprising flour, salt (1.0%), yeast (1.5%), and water (50%, except in the case of whole-wheat flour, which was 60%)] for Thick Kmaj bread was prepared in a local bakery (Alraya Bakeries, Amman, Jordan) following the straight dough method. Three fermentation times (0, 60, and 90 min), five baking temperatures (250, 300, 350, 400, and 450 °C), and three baking times (1, 2, and 3 min) were used in the production of Arabic bread samples using the three types of flour mentioned above. Baking temperatures were monitored using a hand-held, calibrated digital infrared thermometer (ennoLogic, model eT650D, India). Bread moisture content was determined following AOAC method number 930.15. The produced bread loaves were kept in a freezer at –18 °C for further analysis.

Determination of B vitamins in the flour and bread samples

Vitamin extraction. B vitamin extraction was carried out following the method described by Ekinci and Kadakal (2005), in which slightly acidified deionised water (pH 4.2) was used for the extraction of vitamins B1, B3, B6, and B12, whereas alkaline water (pH 9.8) was used for the extraction of vitamins B2 and B9. A quantity of 2 g of flour and 5 g of each dough or bread sample was mixed with 35 mL of the extraction solvent in 50 mL conical tubes and vortexed thoroughly for 1 min (ZX3 Vortex Mixer, VELP Scientifica, Italy). The tubes were kept in a water bath (Memmert WB 14, Germany) at 50 °C for 15 min in the dark with shaking, followed by centrifugation (Hermle Z 206 A, Germany) at 6 000 rpm for 10 min. The supernatant was collected, and the precipitate was re-extracted with a further 15 mL of solvent. The combined supernatant of each sample was subjected to Solid-Phase Extraction (SPE) with Sep-Pak C18 (500 mg) cartridges (Waters, MZ-Analysentechnik, Germany) and then filtered through a 0.45 mm nylon membrane (Fisherbrand, UK). Finally, an aliquot of 20 µL volume was injected into the HPLC. All extraction steps were performed under subdued light conditions.

Preparation of standards. Analytical grades of thiamine nitrate (B1), riboflavin (B2), nicotinamide (B3), pyridoxine hydrochloride (B6), folic acid (B9), and cyanocobalamin (B12) were obtained from Sigma-Aldrich (USA). HPLC-grade water, methanol, acetonitrile, trifluoroacetic acid (TFA), EDTA, dichloromethane, and sodium hydroxide were obtained from Merck (Geneva, Switzerland). Stock solutions of B1, B3, B6, and B12 were prepared separately at a concentration of 100 ppm by dissolving 5 mg of each vitamin in 50 mL of HPLC-grade acidified water, while 5 mg each of B2 and B9 were dissolved separately in 0.025% sodium hydroxide solution. 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. The levels of each vitamin were obtained from the standard curves.

Vitamin analysis. The HPLC method described by Albawarshi et al. (2022) was followed for the analysis, as follows: A Thermo Scientific Dionex UltiMate® 3000 HPLC system; consisting of an LPG 3400 SD pump, ACC-3000 autosampler, and DAD detector; and reverse-phase HPLC with an ACE C18-AR (250 × 4.6 mm × 5 µm) column were used. The gradient mobile phase consisted of solution A, composed of 0.03% trifluoroacetic acid (TFA) in water (pH 2.6), and acetonitrile as solution B (Table 1). The flow rate was 0.9 mL·min^{-1}, the injection volume was 20µL, and the column temperature was 25 °C. Detection was carried out at UV 210, 265, 280, and 361 nm using a photodiode array detector (DAD). The process and the registration of the chromatograms were controlled by the Chromeleon® 6.80 Chromatography Data System (CDS) software. Injections of the extracts were scanned between 210–361 nm; the wavelength that provided the highest reading was used to calculate the vitamin level from the standard curve at that specific wavelength.

Calculating % retention

Vitamin retention in the dough and bread was calculated as a percentage of the levels found in the original flour and unfermented dough, respectively. Results were expressed on a dry-matter basis after correction

Table 1. The mobile phase used in the determination of B vitamins (gradient elution)

B-vitamins mobile phase gradient elution formula		
Time (min)	% A (0.03% TFA in water)	% B (acetonitrile)
0	100	0
2	100	0
4.5	83	17
9.5	83	17
9.6	100	0
13.5	equilibration	

TFA – trifluoroacetic acid; A – Mobile Phase A; B – Mobile phase B

for the recovery percentage of each vitamin from each dough and bread sample.

Statistical analysis of data

Analysis of variance (ANOVA) of the data was carried out using SPSS statistical analysis software (v. 16, IBM SPSS Statistics, USA), and results were expressed as means standard error of the mean (SEM). The least significant difference (LSD) at the 5% level of probability was used to separate the means. Multiple forward stepwise regression and multivariate analysis were used to construct the model of vitamin retention, with vitamin retention as the dependent variable and extraction rate, baking temperature, and baking time as the independent variables.

RESULTS AND DISCUSSION

Wheat flour characteristics

The chemical composition of the flour samples used in this work is illustrated in Table 2, which discloses that an increase in the extraction ratio is manifested by a rise in protein, ash, and wet and dry gluten contents. Results of the chemical and physical analysis of the flour show that as the extraction rate increases, the protein and the wet and dry gluten levels increase, while the gluten index decreases. A higher value for the Gluten Index indicates high-quality gluten and the suitability of the flour type for bread-making (Ćurić et al. 2001; Dowell et al. 2008). Patent flour contains the highest endosperm-to-bran ratio among the flour grades, whereas it has the lowest ash content due to its low proportion of bran and aleurone layer. Bran is the by-product of mill processing; a wheat grain contains the endosperm, germ, aleurone layer, sub-coat layers (testa), and outer layers (pericarp). The highest alpha-amylase activity, as indicated by the falling number test, was observed in patent flour samples, while the lowest value was recorded for the wholewheat flour samples. Levels of the B-complex vitamins in flour samples after fortification are shown in Table 3.

Effect of baking temperature on vitamin retention

ANOVA results showed that the baking temperature has a highly significant ($P \leq 0.05$) effect on the overall mean retention values of B vitamins in this type

Table 2. 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
Protein (%)	10.20 ± 0.06	11.60 ± 0.88	13.10 ± 0.26
Wet gluten (%)	23.14 ± 0.24	24.13 ± 0.25	25.99 ± 0.25
Dry gluten (%)	19.49 ± 0.02	20.61 ± 0.02	21.91 ± 0.03
Gluten index (%)	97.74 ± 0.53	94.50 ± 0.57	84.50 ± 0.64
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
Falling No. (s)	366.00 ± 2.02	400.34 ± 0.50	254.04 ± 0.07

All values are the means of three replicates ± SEM; all values are on an as-is basis.

Table 3. Concentration of B vitamins in the flour samples after fortification (ppm)

Vitamin	Type of flour		
	patent	straight grade	whole
B1	36.50 ± 1.05	36.80 ± 1.45	37.30 ± 0.24
B2	32.50 ± 0.65	31.60 ± 0.07	27.20 ± 1.08
B3	360.00 ± 1.26	367.00 ± 0.02	366.00 ± 0.05
B6	399.00 ± 0.09	410.00 ± 0.24	540.00 ± 0.19
B9	3.80 ± 0.04	3.80 ± 1.50	7.10 ± 0.65
B12 (ppb)*	10.80 ± 0.88	10.71 ± 0.95	11.32 ± 1.22

*ppb: part per billion (µg·kg^{-1}); all values are the means of three replicates ± SEM

of bread (Table 4). The overall retention of B vitamins ranged from 88.3% in the case of vitamin B12 to 156% in the case of B6 when baked at 250 °C. However, it dropped to 49.3% for vitamin B1 when the baking temperature was increased to 450 °C due to the damaging effect of heat. Consequently, it was concluded that vitamin B1 was the vitamin most affected by high baking temperatures, while vitamin B6 was the least affected. As shown in Table 3, vitamin B6 has the highest content after the fortification process compared to vitamin B12; however, it suffered more significant loss at higher baking temperatures. Conversely, at lower baking temperatures (i.e. 250–300 °C), most vitamins showed good stability, with retentions ranging between 80.9% for vitamin B12 and 156.3% for vitamin B6. Generally, as the baking temperature increased to 300 °C, vitamin retention decreased but remained at reasonable levels, while baking at 450 °C caused low retention (49%) of thiamine. Pan bread is produced at low baking temperatures, typically 250 °C (Ning et al. 2017), while Arabic flatbread is baked at 450–500 °C (Amr 1988). Maleki and Daghir (1967) reported lower retention values of thiamine in brown thin-Kmaj bread than in white bread baked at 450 °C. Our findings emphasise the instability of thiamine at high baking temperatures, especially in whole-wheat bread, which is recommended by many nutritionists (Kourkouta et al. 2017). Figure 1 shows clearly that high baking temperatures cause lower retention values (greater loss) of most vitamins in the bread samples produced from high-extraction rates than in bread samples produced from straight-grade or patent-grade flours. The exception was observed in the case of vitamins B2 and B3, where patent bread samples retained lower; though not significantly; amounts of these two vitamins than whole-wheat bread at a baking temperature of 450 °C. It appears that high temperature has a more damaging effect on most B vitamins in whole-wheat bread than in white varieties. This is probably because wheat bran has a higher thermal conductivity than other flour components, as concluded by Seruga et al. (2005). Vitamin B6 showed higher levels of retention (> 100%), although these levels decreased as fermentation time increased. The increase in its level is most likely due to its ease of extraction from bread rather than synthesis by yeast during fermentation, since unfermented dough was used as the basis for calculating the retention of all vitamins in the bread.

Table 4. The overall mean retention values of B1, B2, B3, B6, B9, and B12 in thick Kmaj bread as affected by baking temperature (%)

Baking temperature (°C)	Vitamins					
	B1	B2	B3	B6	B9	B12
250	96.0^{A} ± 0.83	101.0^{A} ± 1.53	97.1^{A} ± 1.85	156.3^{A} ± 6.50	96.0^{A} ± 1.55	88.9^{A} ± 1.70
300	93.1^{B} ± 1.25	91.8^{B} ± 2.13	92.3^{B} ± 2.40	141.8^{B} ± 7.27	92.3^{B} ± 1.51	80.9^{B} ± 2.06
350	85.4^{C} ± 1.31	86.9^{C} ± 2.20	82.7^{C} ± 2.64	114.1^{C} ± 5.75	89.9^{B} ± 1.87	80.5^{B} ± 1.99
400	64.5^{D} ± 2.22	73.1^{D} ± 2.29	73.4^{D} ± 2.56	82.6^{D} ± 5.17	76.8^{C} ± 2.30	67.7^{C} ± 2.69
450	49.3^{E} ± 2.12	61.3^{E} ± 2.57	62.6^{E} ± 2.91	70.1^{E} ± 5.33	66.4^{D} ± 2.72	58.1^{D} ± 2.93

$^{A–E}$different letters in the same indicate significant difference (P < 0.05) according to the LSD test; readings are in % of retention on a dry matter basis, each value is the mean of (27) readings ± SEM

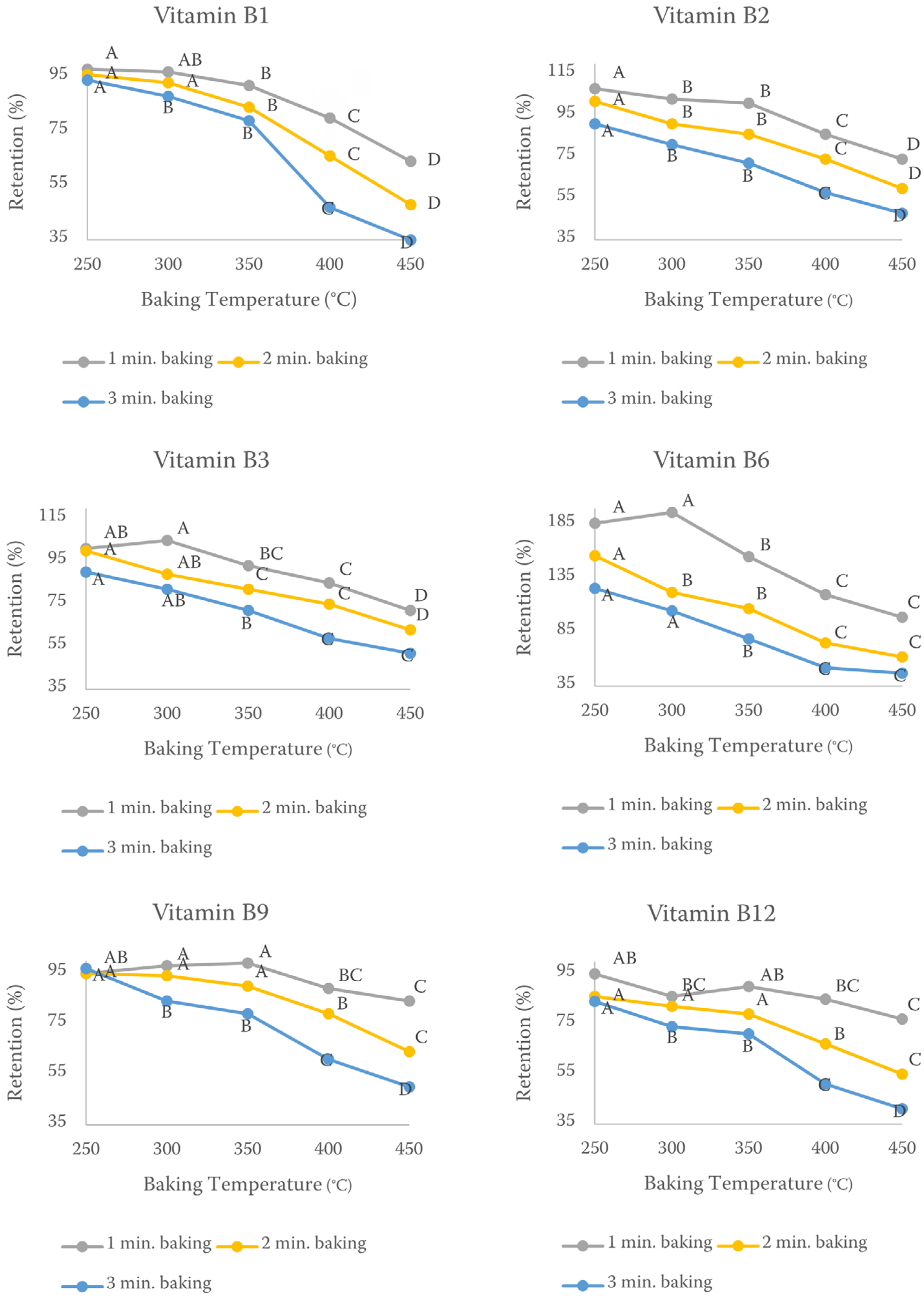

Figure 1. Interaction between baking temperature and time on the retention of the B vitamins in Arabic flat thick Kmaj bread

$^{A–D}$different letters indicate significant difference ($P \leq 0.05$)

Additionally, the kneading process seemed to increase the exposure of B6 to oxygen, which could contribute to the depletion of B6 content in the final products (Batifoulier et al. 2005). Although, increased fermentation time resulted in decreased retention, it led to increased absolute levels of the vitamin when compared to the flour; this is due to the vitamin content released from the yeast. This aligns with the findings reported by Perli et al. (2020), who studied vitamin requirements and biosynthesis in *Saccharomyces cerevisiae*. They stated that strain-to-strain differences occur; *S. cerevisiae* harbours all the necessary genetic information

Table 5. Reduction of retention between the lowest and highest levels of baking temperature and time (%)

Baking condition	Vitamins					
	B1	B2	B3	B6	B9	B12
Temperature (200–450 °C)	48.60	39.30	35.50	55.20	29.60	34.60
Time (1–3 min)	16.27	25.60	21.80	45.70	19.80	25.60

All values are the means of three replicates ± SEM

to synthesise inositol, biotin, thiamine, nicotinic acid, pantothenate, and pyridoxine. Furthermore, some *Saccharomyces* species show higher copy numbers for individual vitamin biosynthesis genes than *S. cerevisiae*, which was reflected in the retention of vitamins in the bread. Vitamin reduction levels were expressed as a percentage difference between the lowest and highest temperatures (Table 5). Accordingly, vitamin B6 suffers the highest reduction percentage of approximately 55% at the highest baking temperature, followed by vitamin B1 at 48%, and vitamin B9 at only 29%.

Effect of baking time

The overall effect of baking time on the retention of B vitamins is shown in Table 6. As with baking temperature, an increase in baking time resulted in a more significant ($P \leq 0.05$) reduction in the % retention of these vitamins in this type of bread across all baking intervals. The greatest % reduction (45.7%) was observed in the case of vitamin B6 when the baking time was increased from 1 to 3 min; all other vitamins suffered retention reductions ranging between 20% and 25%. Based on the reduction results obtained for both baking temperature and time, the percent reduction caused by baking temperature indicates that it contributes significantly more than baking time to the retention or loss of these vitamins.

When the first-order interaction between baking temperature and time was examined (Figure 1), it appeared that the 3 min baking time causes the greatest loss (least retention) at all baking temperatures. Furthermore, baking at lower temperatures resulted in non-significant ($P > 0.05$) reductions in the retention values of some B vitamins. Qarooni (1996) reported that negligible losses of niacin were observed under all baking conditions for Arabic bread samples. Moreover, Batifoulier et al. (2005) found that whole-wheat bread made with longer fermentation times that maintained thiamine levels close to those of the original flour, concluding that a net synthesis of thiamine occurred during fermentation. Capozzi et al. (2011) reported an increase in vitamin B2 content of approximately 2 to 3-fold in pasta and bread samples, which reflects the stability of this vitamin during the baking stages.

Effect of fermentation time on vitamin retention (%)

The ANOVA statistical analysis showed that there are significant ($P \leq 0.05$) differences between the three fermentation times (0, 60, and 90 min) regarding the retention percentage of all vitamins in Thick Kmaj bread. The effect of fermentation time on the retention % of vitamins in bread; regardless of baking temperature, flour type, and baking time; is shown in Table 7.

Thiamine (B1). Significant ($P \leq 0.05$) differences and declines were found in the retention % of vitamin B1, with values of 84.4, 75.9, and 72.7% for 0, 60, and 90 min fermentation times, respectively. The highest retention % of vitamin B1 was recorded at 0 min fermentation compared to those obtained at 60 and 90 min; this may be due to the low porosity and high moisture content of the bread. The highest decline in retention % at 60 min fermentation may be due to the yeast entering the initial

Table 6. The overall retention mean values of B1, B2, B3, B6, B9, and B12 in thick Kmaj bread as affected by baking time (%)

Baking time (min)	Vitamins					
	B1	B2	B3	B6	B9	B12
1	$86.5^A \pm 1.50$	$94.9^A \pm 1.78$	$91.4^A \pm 2.15$	$152.0^A \pm 6.16$	$93.2^A \pm 1.15$	$87.0^A \pm 1.58$
2	$77.7^B \pm 2.09$	$82.9^B \pm 2.05$	$82.0^B \pm 2.17$	$104.5^B \pm 4.42$	$84.9^B \pm 1.81$	$74.0^B \pm 1.91$
3	$68.9^C \pm 2.70$	$70.6^C \pm 2.08$	$71.5^C \pm 2.21$	$82.4^C \pm 4.06$	$74.7^C \pm 2.25$	$64.7^C \pm 2.16$

$^{A–C}$different letters in the same column indicate significant difference ($P < 0.05$) according to the LSD test; readings are in % retention on a dry matter basis, each value is the mean of (45) readings ± SEM; the calculation of the retention of vitamins in bread was based on unfermented dough vitamin content

Table 7. The overall mean % retention values of B1, B2, B3, B6, B9, and B12 in thick Kmaj bread as influenced by fermentation time regardless of baking temperature, the flour type, and baking time

Fermentation time (min)	Vitamins					
	B1	B2	B3	B6	B9	B12
40	84.4^{A} ± 2.24	90.2^{A} ± 1.68	93.5^{A} ± 1.76	118.9^{A} ± 5.81	91.0^{A} ± 1.67	78.8^{A} ± 1.64
60	75.9^{B} ± 2.15	79.9^{B} ± 2.30	77.1^{B} ± 2.16	111.5^{B} ± 5.84	82.0^{B} ± 1.91	75.5^{B} ± 2.25
90	72.7^{C} ± 2.28	78.3^{B} ± 2.44	74.2^{C} ± 2.51	108.5^{B} ± 5.81	79.8^{C} ± 2.09	71.3^{C} ± 2.36

$^{\mathrm{A-C}}$different letters in the same column indicate significant difference ($P < 0.05$) according to the LSD test; readings are in % retention on a dry matter basis, each value is the mean of (45) readings ± SEM; the calculation of the retention of vitamins in bread was based on unfermented dough vitamin content

adaptation phase (0–30 min), followed by a short but vital fermentation phase (30–60 min). In the adaptation phase, yeast cells first consume the preferred sugars (glucose and sucrose) using a constitutive enzyme. Following this, the yeast cells begin a second adaptation phase (a lag phase of 10–15 min), releasing the maltase enzyme responsible for maltose metabolism (Figure 2) (Struyf et al. 2017). It was observed that the retained vitamin declined significantly ($P \leq 0.05$) during the short fermentation time when using yeast as the main fermentation organism. Thiamine loss was facilitated during fermentation due to its utilisation by yeast; this agrees with Praekelt et al. (1994), who ascribed the decline in B1 to its presence in the external growth medium, which leads to the uptake of B1 by the yeast and the maximal inhibition of biosynthesis. The reduction in B1 may also relate to the increase in reducing sugar concentration, mainly maltose, during the adaptive period, which agrees with the work of Doyon and Smyrl (1983) who reported destruction effects of reducing sugars against B1.

In contrast, Certel et al. (2007) reported an increase in thiamine when using both baker's yeast and lactic acid bacteria (from yoghurt) for fermentation, which may be related to the synergistic effect between lactic acid bacteria and baker's yeast during Tarhana dough-making. Moreover, Ranhotra and Gelroth (1986) concluded that there was no significant effect of fermentation on the B1 content of bread; thus, they considered vitamin B1 to be stable during fermentation.

Riboflavin (B2). Retention percentages showed a decline in this vitamin as fermentation time increased. The difference was significant ($P \leq 0.05$) between 0 and 60 min, with values of 90.2, 79.9 and 78.3% for 0, 60 and 90 min fermentation times, respectively. This significant decline may be related to the utilisation of B2 vitamers in redox reactions (the TCA cycle, electron transport chain, and beta-oxidation) during the early stages of the fermentation process, after which the utilisation of B2 and flavin mononucleotide (FMN) is minimised during yeast fermentation (Hucker et al. 2016). In contrast, Batifoulier et al. (2005) concluded that pan bread contained

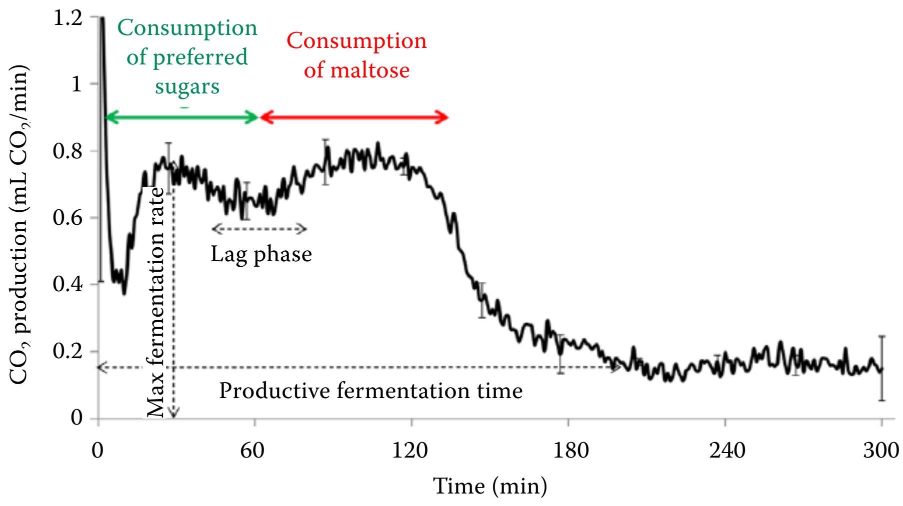

Figure 2. A standard CO_2 profile of *Saccharomyces cerevisiae* bakery strain in dough (Struyf et al. 2017)

more B2 than the original flour, which could be ascribed to the longer fermentation period compared to the shorter fermentation time in our investigation.

Niacin (B3). Niacin levels increased insignificantly during the first 60 min of fermentation, after which they showed a significant ($P \leq 0.05$) decrease. This behaviour may be explained by the mechanism of B3 biosynthesis from B6, in which B3 is produced as a by-product and is later consumed to produce nicotinamide adenine dinucleotide (NAD) (Minami et al. 1982). Moreover, heat treatment will change bound niacin into its free form (Okmen and Bayindirli 1999). Nicotinamide and nicotinic acid are relatively stable during baking. A small portion of chemically bound nicotinic acid (i.e. NAD) can be hydrolysed and converted into bioavailable forms (Mihhalevski et al. 2013).

Pyridoxine (B6). Our results agree with the work of Perera et al. (1979), who noted a decrease in B6 during fermentation and baking, whereas longer fermentation times caused an increase in this vitamin. Lebiedzińska et al. (2008) indicated that the major variables affecting the loss of total vitamin B6 during baking are temperature processing, time, and the relative stability of the B6 vitamers present.

Folic acid (B9). Our work showed a significant decrease ($P \leq 0.05$) in B9 with fermentation time. This decrease may be ascribed to the exposure of yeast to saline and osmotic stress, as well as cation toxicity; consequently, yeast cells accumulate compatible solutes including trehalose, proline, and glycerol; which require B9 as a co-factor; as a strategy for adapting to osmotic stress induced by salt (Tsoi et al. 2009). Hjortmo et al. (2008) observed a rapid decline in the level of H_4 folate due to yeast growth and bud formation during the early period of fermentation, after which the decline slowed as the number of yeasts did not increase and, consequently, the requirements for folate decreased. Results obtained in our work contradict those of other researchers; Ekinci (2005) and Certel et al. (2007) observed an increase in the B9 content in Tarhana bread (a traditional Turkish bread prepared from wheat flour and a variety of cooked vegetables) during fermentation lasting more than one day using lactic acid bacteria and yeast. Gujska and Majewska (2005) examined the effect of fermentation on the stability of added and endogenous B9 during the bread-making of rye and wheat pan bread, confirming a significant increase in B9 during the leavening of dough with baker's yeast. Our fermentation times are much shorter than those for pan bread, which undergoes proofing and fermentation periods that can last up to three hours. Moreover, a higher quantity of yeast is usually used in the production of pan bread.

Cyanocobalamin (B12). Our results for B12 showed no significant difference ($P > 0.05$), despite a declining pattern in the retained vitamin across fermentation times. This decline may be a result of the photodegradation of the chemically modified form of cyanocobalamin (CNCbl) into the natural form, hydroxocobalamin (OHCbl); this process is promoted by vitamins B1, B2, B3, and B6, especially at low pH (Ahmad et al. 2003; Monajjemzadeh et al. 2014). These results agree with Edelmann et al. (2016), who investigated the stability of CNCbl and OHCbl in pan bread and found that proofing time did not affect the levels of either form.

Bread quality

Unofficial sensory evaluation of the bread produced at the different baking temperature/time combinations revealed that the bread samples baked at 350 °C for two or three min possessed the same flavour characteristics as those baked at 450 °C, while retaining a higher proportion of the vitamins.

CONCLUSION

The amount of vitamin retention in this type of flatbread; characterised by a thin upper layer and a thick, crumb-rich bottom layer; was significantly influenced by baking temperature and time. Nonetheless, lower baking temperatures did not significantly affect the retention of most B vitamins and caused only minor loss. At higher baking temperatures (> 350 °C), almost all vitamins suffered significant losses. Vitamin B1 was the most affected by baking temperature in absolute terms, while B6 was the least. However, the highest baking temperature caused the greatest reduction in % retention compared to the lowest temperature, whereas B9 showed the lowest reduction. Vitamin B6 levels in the bread were found to increase compared to the levels in the dough, but these levels decreased as baking time and temperature increased.

Under the conditions of this study, baking temperature had a more deleterious effect on the retention of all vitamins, followed by baking time. In light of these findings, it is recommended to modify baking regimes at the bakery level to a lower temperature and longer duration, moving away from the current high-temperature, short-time method. Such a change would reduce the quantity and cost of the fortification process while improving the final vitamin content of the produced bread. It is worth stressing the importance of this study, as it serves as valuable material for the upcoming micronutrient survey conducted by the FAO to assess nutritional trends among Jordanians.

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