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Investigation of the effect of different storage conditions on vitamin content of enriched pasta product

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RESEARCH ARTICLE

Abstract

In this study, thiamine, riboflavin and niacin contents of enriched pasta product were analysed during eight months of storage at ambient conditions under daylight, ambient conditions in the dark, and in a refrigerator for investigating the effects of different storage conditions. Thiamine content did not change significantly at different storage periods and conditions. Riboflavin content halved starting from the first month and decreased gradually under daylight conditions. However, in the dark and refrigerated conditions riboflavin content did not change significantly during the storage. Niacin content did not change due to storage under different conditions but gradually decreased during storage. Cooking water analysis indicated that the highest vitamin loss to cooking water was observed in niacin, so that niacin was more easily leached out from the pasta product as compared to other vitamins. Validation studies confirmed that the equipment, method and system performance were suitable for the purposes of the study.

Keywords: enriched pasta, thiamine, riboflavin, niacin, HPLC, storage, validation

1. Introduction

Micronutrients such as vitamins are essential substances for health. The lack of vitamins leads to serious public health problems. Vitamin contents of daily consumed foods related to the mental and physical development and health (Papadoyannis *et al.*, 1997). Vitamin deficiencies tend to be symptomatic in high risk groups such as pregnant women, infants and young children, elderly people, vegetarians, food-insecure people, individuals with intestinal parasites and infections, dieters and smokers (Funk, 2016). Fortification of foods by micronutrients can be good solution for the vitamin deficiency problems. Currently, food fortification is carried out in a number of countries, the main foods being margarine, milk and derivatives, cereal flours, beverages, fats/oils and sugar (Liberato and Pinheiro-Santana, 2006; Melini and Carcea, 2016). Fortification of cereal products plays an important role in terms of public health (Ranum, 2000). Due to the increasing consumption of pasta products, fortification of pasta products might be a good choice in the solution of vitamin deficiency problem.

Extraction of water-soluble vitamins from food matrices is a challenging stage in analysis and involves some difficulties. One of them is the interaction of B vitamins with other components in food matrices. Furthermore, low levels of vitamins naturally present in some foods make the analysis more difficult (Finglas and Faulks, 1984).

There are standard vitamin analysis methods based on microbiological and fluorometric principles (AACC, 2000; AOAC, 1990; ICC, 1995). In recent years, B vitamins are commonly analysed by high performance liquid chromatography (HPLC), which has high sensitivity and reproducibility and results in rapid and reliable results (Eitenmiller and Landen, 1999; Hollman *et al.*, 1993; Nouri *et al.*, 2018; Olds *et al.*, 1993; Ollilainen *et al.*, 2001). Although there are some studies on vitamin analyses of foods using HPLC methods, the studies on the determination of B vitamins in pasta products are limited (Bui and Small, 2008; Kamman *et al.*, 1981; Watanabe and Ciacco, 1990; Woodcock *et al.*, 1982).

UV and fluorescence detectors combined with HPLC are used for determination of water-soluble vitamins in foods. The use of UV detectors often does not provide sufficient sensitivity and specificity. Fluorescence detector based HPLC methods uses natural fluorescence of vitamin, but derivatisation provides better sensitivity and specificity (Finglas, 1993). Furthermore, a number of vitamins can be identified at the same time by HPLC (Eitenmiller and Landen, 1999; Esteve *et al.*, 2001). For example; there are some methods in which thiamine and riboflavin can be conveniently analysed from the same extract (Arella *et al.*, 1996; Finglas and Faulks, 1984; Ndaw *et al.*, 2000; Ollilainen *et al.*, 2001; Van Den Berg *et al.*, 1996; Wimalasiri and Wills, 1985).

Chromatographic methods used to determine the vitamin content of foods can be listed as normal and reverse phase chromatography, ion exchange chromatography, ion exclusion chromatography and reverse phase ion pair chromatography. The extraction and purification procedures and the properties of the vitamin are the main factors in selecting the HPLC based chromatographic method (Ball, 2006). On the other hand, the chromatographic analysis should be optimised for various foods (Eitenmiller and Landen, 1999; Hollman *et al.*, 1993; Olds *et al.*, 1993; Ollilainen *et al.*, 2001).

The durability of thiamine depends on the severity and duration of heating and the properties of the food matrix. The loss of thiamine during storage also depends on the pH and there is no thiamine degradation in a strongly acidic medium such as lemon juice (Belitz *et al.*, 2009). Thiamine is not stable at alkaline pH and it is degraded by heat at weak acidic conditions (Eitenmiller and Landen, 1999). Thiamine is a water soluble vitamin; hence the removal of cooking water from pasta products causes significant thiamine losses (Lešková *et al.*, 2006).

Riboflavin is quite stable in food processing, so the riboflavin losses usually range from 10-15%. Exposure to light (especially in the visible spectrum at 420-560 nm) cleaves the ribitol part of the riboflavin molecule, and converts it photolytically to lumiflavin form (Belitz *et al.*, 2009). Riboflavin is used in various cereal enrichment programs.

Niacin is a highly resistant vitamin; therefore, its loss during cooking or food processing is lower than other vitamins. It is resistant to temperature and oxidants, and is hydrolysed only in strong acidic and basic solutions. Food processing and cooking procedures do not cause deactivation of niacin. Removal of the cooking water is the primary reason for the niacin losses (Eitenmiller and Landen, 1995). On the other hand, niacin is not affected by light (Eitenmiller and Landen, 1999; Gregory, 1997).

The objective of this research was to investigate the effects of different storage conditions on vitamin B (thiamine, riboflavin and niacin) contents of enriched pasta product. Enriched pasta products were stored at three different conditions: in the dark, under daylight and in a refrigerator. Samples stored in the dark and under daylight were kept in ambient conditions. The vitamin contents were determined by HPLC. The vitamin B contents of enriched pasta products were determined once a month during the storage period of 8 months. At the end of the storage period, vitamin B contents were also determined in the cooking water. Quality characteristics of the pasta samples were also determined to investigate the effects of storage on pasta quality. In addition, validation studies have been carried out to evaluate the equipment, analysis method and system performance.

2. Materials and methods

Materials

The vitamin and mineral enriched pasta products used in the study were supplied by Filiz Food Industry Co. Bolu, Turkey (Barilla G. e R. Fratelli S.p.A., Parma, Italy).

Reagents and chemicals

Thiamine hydrochloride, riboflavin, nicotinic acid and nicotinamide (Labor Dr. Ehrenstorfer-Schlosser, Augsburg, Germany) were used as vitamin standards. Takadiastase (Fluka, Buchs, Switzerland) and alpha-amylase (*Aspergillus oryzae*, Sigma-Aldrich, St. Louis, MO, USA) enzymes were used for B vitamin extractions.

For B vitamin analyses; certified reference materials; whole wheat flour (BCR® 121) and milk powder (BCR® 421) (BCR: Community Bureau of Reference, the former reference materials programme of the European Commission, Belgium) were used. Methanol (HPLC grade, Merck, Darmstadt, Germany), potassium ferricyanide (Sigma-Aldrich, Steinheim, Germany), distilled water (HPLC grade), sodium hydroxide (Merck), sodium acetate trihydrate, potassium dihydrogen phosphate, copper (II) sulphate pentahydrate, hydrogen peroxide, hydrochloric acid (Riedel-de Haën, Seelze, Germany), sulphuric acid (J.T. Baker, Deventer, the Netherlands), dipotassium hydrogen phosphate (Fluka, Madrid, Spain), Whatman filter paper No.541 (Whatman, Maidstone, UK), syringe and syringe filter (0.2 μm) (Alltech, Lexington, KY, USA) were used.

Equipment

All experiments were carried out using a HPLC system (Agilent 1200 series, Basel, Switzerland) equipped with a degasser (Agilent, G1322A), a quaternary pump system (Agilent, G1311A), an auto sampler (Agilent, G1329A), a

temperature control unit (Agilent, G1330B), a column oven (Agilent, G1316A), and a fluorescence detector (Agilent, G1321A). The analytical columns used in vitamin analyses are µ-Bondapak C18 (3.9 × 300 mm, 10 µm, Waters, Milford, MA, USA) and Lichrospher RP 18-5 end capped (4.0 × 250 mm, 5µm, Hichrom, Reading, UK). RP18 (4 × 4 mm, 5 µm, Hichrom) was integrated to the system as a guard column.

Other equipment used in the study are shaking water bath (Kotterman Labortechnik, Uetze, Germany and Şimşek Laborteknik, Ankara, Turkey), ultrasonic water bath (FALC, Treviglio, Italy), centrifuge (Sigma 3-18K, Darmstadt, Germany), deep freezer (Sanyo, Osaka, Japan), pH meter (Schott CG840, Mainz, Germany), magnetic stirrer (VELP Scientifica, Usmate Velate, Italy), vortex stirrer (VELP Scientifica), incubator (Şimşek Laborteknik), food processor (Raks MR 1001, Istanbul, Turkey), black light lamp (Phillips, Amsterdam, the Netherlands).

Validation studies

Within the scope of validation studies; linearity, repeatability of the equipment, repeatability of the method, recovery, accuracy, limit of detection (LOD) and limit of quantification (LOQ) values were calculated in order to evaluate method and system performance.

Linearity

Linearity is the method's ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. It is defined by the coefficient of determination (R^2) (Ball, 2006; Holcombe, 1998; Huber, 1998; Şengül *et al.*, 2016; Sharifuldin *et al.*, 2016). In order to evaluate linearity; two 5-point calibration curves were prepared using vitamin standards, one containing the working concentration and the other containing low concentrations to observe the linearity at different working intervals and the R^2 was determined for both of them.

Repeatability of the equipment

Precision is defined as the change in the accuracy of independent analytical measurements. Repeatability is a measure of precision and is defined by the standard deviation, variance or coefficient of variation (CV). The same matrix, same method, same staff, same laboratory conditions should be used in determination of repeatability (Ball, 2006; Holcombe, 1998; Huber, 1998). In the criterion of repeatability of the equipment; in addition to these conditions, the same extract is being worked on so that only the variation due to the equipment is examined. In order to evaluate this criterion; the extract prepared using vitamin enriched pasta sample was analysed 10 times by HPLC. The reproducibility of the equipment was evaluated by calculating the CV.

Repeatability of the method

For determination of repeatability of the method, the same matrix, same method, same staff, same laboratory conditions are provided, and also it is performed by extracting different samples from the same matrix by using the same method under the same conditions (Ball, 2006; Holcombe, 1998; Huber, 1998). Thus, the total variation from both the extraction and the equipment, namely the whole method, are examined. Therefore, the repeatability of the method includes the repeatability of the equipment. In order to evaluate this criterion; 10 different extracts from vitamin the enriched pasta sample were analysed by HPLC method. The repeatability of the method was evaluated by calculating the CV.

Recovery

Recovery is performed by adding the analyte to a food matrix. The most appropriate recovery work is done using a food matrix that does not contain the substance being analysed. On the other hand, the reference material which contains this substance at a certain level can also be spiked with the substance to be analysed. The standard solutions are usually added to the matrix to obtain low, medium and high concentrations. In the present study, spiking levels were 50, 100 and 150% of the values indicated on the label of the certified reference material for low, medium and high analyte concentrations. For thiamine analyses BCR® 121-whole wheat flour and for riboflavin and niacin analyses BCR® 421-milk powder was used as certified reference material. The thiamine content was 4.63 µg/g in BCR® 121-whole wheat flour; the riboflavin content was 14.50 µg/g and the niacin content was 68.00 µg/g in BCR® 421-milk powder. Recovery experiments were performed at different spiking levels (50, 100 and 150%) for each vitamin, i.e. 2.32, 4.63 and 6.95 µg/g for thiamine; 7.25, 14.50 and 21.75 µg/g for riboflavin and 34.00, 68.00 and 102.00 µg/g for niacin. After the extraction, the analyte concentrations were determined by the method and recovery values were calculated by comparing the values obtained during the analysis with the ones of spiked certified reference materials and the results are given as % recovery (Ball, 2006; Holcombe, 1998; Huber, 1998; Şengül *et al.*, 2016; Sharifuldin *et al.*, 2016).

Accuracy

Accuracy is a criterion which indicates the closeness of the experimental value to the actual amount of the analyte in the matrix. It can be determined by using a certified reference material or comparing the results of the newly developed method with those of a well-defined, validated method (Ball, 2006; Holcombe, 1998; Huber, 1998).

In this study, in order to evaluate accuracy; the B vitamin contents of the certified reference materials were obtained by HPLC and compared the results with the indicated values of the certified reference materials. The result was given as % accuracy.

Limit of detection and limit of quantification

LOD is the lowest analyte concentration that can be detected, but not necessarily quantified. LOQ is the lowest analyte concentration that can be determined with acceptable precision and accuracy (Shrivastava and Gupta, 2011). In this study, in order to determine LOD and LOQ; plain pasta with no vitamin additives was used. Firstly, the vitamin content of plain pasta product was determined. Standard vitamin solutions were added to the ground pasta product at decreasing concentrations and B vitamins were extracted from the matrix. Then, the determinable lowest concentration was obtained and the vitamin value found in plain pasta was subtracted from this concentration. B vitamins were added to the matrix at this determined lowest concentration and it was analysed 10 times by HPLC. Then, the standard deviation of the data was calculated. The LOD and LOQ values were calculated by multiplying the standard deviation with 3 and 10, respectively (Ball, 2006; Holcombe, 1998; Huber, 1998; Şengül *et al.*, 2016; Sharifuldin *et al.*, 2016; Shrivastava and Gupta, 2011).

Quality analyses of pasta products

In order to determine the quality characteristics of pasta samples; cooking loss, water absorption, and total organic matter (TOM) analyses were applied at the beginning of the storage, and after 4 and 8 months of storage (AACC, 2000; D'Egidio *et al.*, 1982; ICC, 1995). The moisture content of pasta was determined by the AACC Method No. 44-01 (AACC, 2000).

Sample preparation

Enriched pasta products were ground using a food processor (Raks MR 1001), and then sieved through 1 mm sieve.

Determination of thiamine and riboflavin content

Thiamine and riboflavin extraction was carried out by modified method of AACC (method 86-70 and 86-80) (AACC, 2000; Bilgi Boyaci *et al.*, 2012). In this procedure; 25 ml 0.1 N H_2SO_4 was added to 1 g ground pasta sample. The solution was heated to 100 °C and incubated for 30 minutes. After cooling to below 40 °C, 2.5 ml 2% (w/v) Takadiastase solution in 1 M acetate buffer was added. The sample solution was then incubated at 37-40 °C for 4 hours in a shaking water bath. After incubation, the solution was cooled down to room temperature. Then it was transferred to a volumetric flask for diluting the volume of the solution to 50 ml with distilled water. The diluted solution was then mixed and filtered through Whatman 541 filter paper into bottles and stored at -20 °C until analysis.

The same extraction procedure was used for thiamine and riboflavin, but their HPLC analysis was different. Chromatographic determination of thiamine and riboflavin was carried out by the method of Finglas and Faulks (1984) as modified by Bilgi Boyaci *et al.* (2012). Due to its fluorescence property, the riboflavin extract was directly used in chromatographic analysis after filtration through syringe filter (0.2 µm). On the other hand, thiamine was converted to its fluorescent derivative, thiochrome, by adding 1 ml 0.18 M potassium ferricyanide solution in 3.75 M aqueous sodium hydroxide to 5 ml filtrated extract. The solution in an amber glass bottle was shaken vigorously for 1 minute and left to stand in the dark for 1 minute. Then it was filtered through a syringe filter (0.2 µm) into vials for HPLC analysis. Standard solutions of thiamine were prepared by using distilled water and also treated with potassium ferricyanide solution as the samples before HPLC analysis. Standard solutions of riboflavin were prepared by using mobile phase and not treated before HPLC analysis.

Thiamine and riboflavin were analysed by HPLC system (Agilent 1200 series). The method comprises isocratic elution by using methanol and water in connection with using the constant mobile phase concentration. The HPLC programme and chromatographic conditions for thiamine and riboflavin analysis are as follows:

- Column: Waters µ-Bondapak C18 reverse phase column (3.9 × 300 mm, 10 µm).
- Column temperature: 25 °C for thiamine, 30 °C for riboflavin.
- Mobile phase: methanol-water (30:70, v/v), isocratic mode.
- Flow rate: 1 ml/minute.
- Injection volume: 20 µl (injected three times).
- Detector: fluorescence detector (excitation and emission wavelengths for thiamine and riboflavin were 365 nm and 435 nm, 450 nm and 510 nm, respectively).

Determination of niacin content

Niacin extraction of pasta products was carried out by the method of Rose-Sallin *et al.* (2001) as modified by Bilgi Boyaci (2008). In this procedure; 70 ml 0.1 M HCl was added to 3 g ground pasta sample. The solution was heated to 100 °C and incubated for 1 hour. After cooling to room temperature, pH of the solution was adjusted to 4.5-4.6. Then the volume was adjusted to 100 ml with distilled water, mixed and filtered through Whatman 541 filter paper into bottles and stored at -20 °C until the analysis.

Niacin was converted to its fluorescent derivative by using post-column derivatisation. The photochemical reaction

was carried out in a Teflon tube (10 m × 0.6 mm i.d.) which was wound around a black light lamp (300-400 nm, 8 W, Phillips). It was installed between column and fluorescence detector to provide UV radiance (Figure 1).

Niacin was analysed by HPLC system (Agilent 1200 series). Nicotinamide and nicotinic acid were identified by their retention times and results were expressed as niacin by summing their concentrations. Standard solutions of niacin were prepared by using distilled water and not treated before HPLC analysis (Figure 1). The gradient programme and chromatographic conditions for niacin analysis are as follows:

- Column: Lichrospher RP 18-5 end capped reverse phase column (4.0 × 250 mm, 5 µm).
- Column temperature: 30 °C.
- Mobile phase: two mobile phases (mobile phase A and B) were used in the HPLC analysis of niacin. Mobile phase A was prepared by dissolving 9.54 g/l potassium dihydrogen phosphate, 2.53 ml/l hydrogen peroxide and 1.25 mg/l copper (II) sulphate pentahydrate in water. Mobile phase B was used as a washing diluent and prepared by mixing 10% (v/v) methanol in mobile phase A. (Run time: between 0 and 34 min: mobile phase A; between 34 and 36.5 min: mobile phase B; between 36.5 and 51 min: mobile phase A).
- Flow rate: 1 ml/minute.
- Injection volume: 20 µl (injected three times).
- Detector: fluorescence detector (excitation and emission wavelengths of 322 nm and 380 nm).

Statistical analyses

The variations on B vitamin contents of pasta samples during storage time, also cooking loss and TOM values were evaluated by using SPSS Data Editor 11.5 with one-way ANOVA (SPSS, IBM, Armonk, NY, USA). When significant differences were found, Duncan test was used to determine the differences among means. A probability level of $P<0.05$ was used throughout the evaluations in this study. In order to compare the average vitamin content of different storage conditions (ambient conditions under daylight, ambient conditions in the dark, and in a refrigerator), t-test was also used.

3. Results and discussion

Validation studies

Within the scope of validation studies; the results of accuracy, recovery, repeatability of the equipment, repeatability of the method, LOD and LOQ values for thiamine, riboflavin and niacin analysis were determined and are given in Table 1.

For thiamine; %CV value for repeatability of the equipment was quite low indicating a high repeatability. On the other hand, %CV value for repeatability of the method is relatively high indicating a low repeatability. The probable reason for this might be quick degradation of thiamine between derivatisation and analysis stages. Furthermore; personal error can also cause deviation in thiamine results (Table 1). For riboflavin; %CV value for repeatability of the equipment

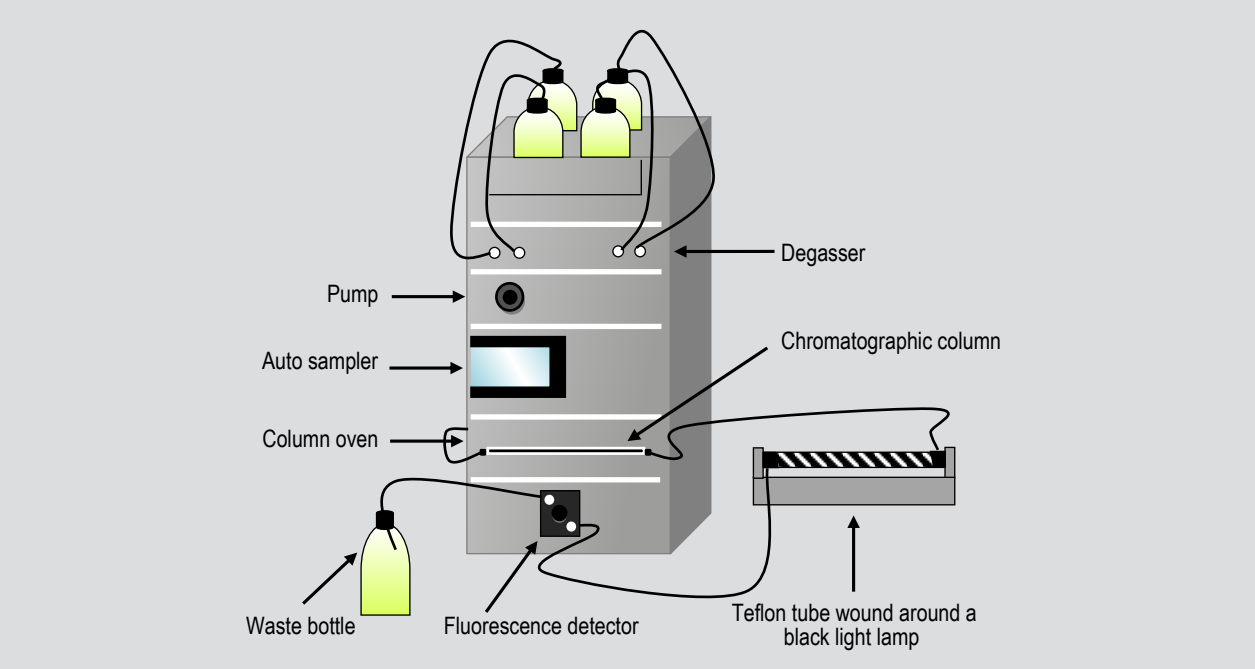

Figure 1. HPLC system for the analysis of niacin (Bilgi Boyaci, 2008).

Table 1. Validation results for vitamin analysis.[1]

Vitamin	Repeatability of the method (%CV)	Repeatability of the equipment (%CV)	LOD / LOQ (µg/g)	Accuracy (%)	Recovery (%)[2]		
					50	100	150
Thiamine	9.37	1.14	0.42 / 1.40	97	97	97	103
Riboflavin	7.21	1.03	25.00 / 82.00	93	91	99	101
Niacin	0.22	0.04	53.00 / 177.00	99	106	105	107

[1] CV = coefficient of variation; LOD = limit of detection; LOQ = limit of quantification.
[2] 50: spiking level is 50% of the one indicated on the label of the certified reference material; 100: spiking level is 100% of the one indicated on the label of the certified reference material; 150: spiking level is 150% of the one indicated on the label of the certified reference material. Certified reference materials: BCR® 121-whole wheat flour for thiamine analyses, BCR® 421-milk powder for riboflavin and niacin analyses.

was quite low indicating a high repeatability and lower than the thiamine results. The %CV value for repeatability of the method is also lower than the thiamine results but not very low probably because of the personal error in riboflavin analysis. The reason for the relatively higher repeatability of the method in riboflavin analysis might be due to the lack of derivatisation stage (Table 1). The riboflavin extract is directly used in HPLC analysis because of its fluorescence behaviour. So the degradation problem of thiamine between derivatisation and analysis stages was not observed in riboflavin analysis. The best repeatability results were obtained in niacin analysis compared with the repeatability results for thiamine and riboflavin analyses. The probable reason for this might be utilisation of black light lamp for conversion of niacin to its fluorescent derivative. By this way; deviation of niacin results arising from personal error during derivatisation was avoided (Table 1).

The LOD and LOQ values were quite low especially for thiamine results. These results indicate that low analyte concentrations can be detected with acceptable precision and accuracy (Table 1). LOD and LOQ results are important for evaluating the analytical performance of a method to understand its capabilities and limitations. Defining the limits of a method at low concentration is directly related to analytical measurement range. Hence, in the present study, the analysis methods are suitable for the determination of low vitamin contents.

At different spiking levels, the recovery values were 97, 97 and 103% for thiamine; 91, 99 and 101% for riboflavin; 105, 106 and 107% for niacin (Table 1). The recovery results were found to be acceptable for all vitamins. The recovery results were also consistent with each other at different spiking levels.

The accuracy values were 97, 93 and 99% for thiamine, riboflavin and niacin, respectively (Table 1). The accuracy results were quite high for all vitamins. High accuracy values indicate that the results obtained by the HPLC method used were quite consistent with the indicated values of the certified reference materials.

For observing linearity values; two calibration graphs were prepared: for low level of vitamin content, for high level of vitamin content. The calibration graphs for thiamine and riboflavin were prepared in the ranges of 0.1-1 ppm and 6.25-100 ppb. The calibration graphs for nicotinamide and nicotinic acid were prepared in the ranges of 1-5 ppm and 0.1-1 ppm. For all calibration graphs; the coefficient of determination (R^2) values were above 0.998. Hence, the results were linear not only in the ranges of the vitamin contents studied in the present study but also for the lower vitamin contents.

Quality analyses

The quality characteristics (cooking loss, water absorption, and TOM) of pasta samples were determined at the beginning of the storage, and after 4 and 8 months of storage and are presented in Table 2.

During storage time; some differences were observed in cooking loss and TOM values, however the increases were not substantial. Optimum cooking time of the samples was determined as 10 min and did not change during storage. One-way ANOVA results indicated that under all storage conditions; there were no significant differences between cooking loss values of the samples after 4 month of storage as compared to the beginning value, however, the cooking loss values significantly increased after 8 month of storage (P<0.05). The same trend was also observed in the TOM values (Table 2). However, according to t-test results; there were no significant differences between average cooking loss values of 4 months stored samples within each storage condition (in the dark, under daylight and refrigerated conditions). The same trend was also observed in 8 months stored samples and the TOM values of the 4 months and

Table 2. Quality characteristics of pasta samples stored under three different conditions.[1]

Storage condition	Storage time (month)	Cooking time (min)	Cooking loss (%)	Water absorption (%)	Total organic matter (%)
-	0	10	5.36 b	121.30	1.31 b
Dark	4	10	5.32 b	124.90	1.32 ab
	8	10	5.49 a	123.60	1.38 a
Daylight	4	10	5.39 b	123.40	1.34 ab
	8	10	5.46 a	122.20	1.39 a
Refrigerator	4	10	5.35 b	124.10	1.34 ab
	8	10	5.48 a	122.40	1.37 a

[1] Values followed by the same letter in the same column are not statistically significant.

8 months stored samples within each storage condition (Table 2). One-way ANOVA results also indicated that water absorption values were not significantly affected from storage time and conditions.

Thiamine analyses

Thiamine contents of vitamin enriched pasta products stored under different conditions and storage periods were determined and the results are presented in Table 3.

One-way ANOVA results indicated that there were no statistically significant differences between the thiamine content of enriched pasta products over 8 months of storage under all storage conditions. According to t-test results; there were no significant differences between average thiamine content of the samples within each storage condition (in the dark, under daylight and refrigerated conditions; Table 3). Therefore; thiamine contents of pasta products were not affected from different storage conditions and 8 months of storage.

The thiamine level was determined in the range of 11.08-11.88 mg/kg in the study. These analysis results were a little higher than the thiamine content indicated on the label (9.5 mg/kg). This might be due to the tendency of the pasta manufacturers to add more thiamine to enriched products to compensate the possible thiamine loses during processing and prevent the thiamine content to decrease below the level indicated on the label.

Watanabe and Ciacco (1990) indicated that 96% of thiamine was retained during 3 months of storage in dark conditions. Kamman *et al.* (1981) found that little or no thiamine loss was observed during storage of pasta for 1 year under moderate temperature and in dark conditions. In another study, storage of dried noodles at room temperature for 4 months was not resulted in any thiamine loss (Bui and Small, 2008). Therefore; it can be concluded that the results in this study are compatible with the literature.

Table 3. Thiamine content of pasta products during 8 months of storage under different conditions.[1]

Storage time (month)	Ambient conditions		Refrigerator (mg/kg)	Label value (mg/kg)
	Dark (mg/kg)	Daylight (mg/kg)		
0	11.85 a	11.85 a	11.85 a	9.50
1	11.29 a	11.37 a	11.22 a	9.50
2	11.74 a	11.25 a	11.88 a	9.50
3	11.11 a	11.44 a	11.76 a	9.50
4	11.37 a	11.88 a	11.08 a	9.50
5	11.42 a	11.27 a	11.49 a	9.50
6	11.84 a	11.67 a	11.08 a	9.50
7	11.14 a	11.60 a	11.13 a	9.50
8	11.31 a	11.31 a	11.37 a	9.50

[1] Average values of three repetitions were given in the table. Values followed by the same letter in the same column are not statistically significant.

Riboflavin analyses

Riboflavin contents of vitamin enriched pasta products stored under different conditions and storage periods were determined and the results are presented in Table 4.

One-way ANOVA results indicated that there were no statistically significant differences between the riboflavin content of enriched pasta products over 8 months of storage in the dark and refrigerated storage conditions. However, riboflavin content of pasta products stored under daylight condition halved starting from the first month and decreased gradually under daylight conditions (Table 4). Approximately 78% reduction was observed on riboflavin content of pasta stored under daylight condition. Riboflavin content of pasta products was not affected from storage in dark conditions and 8 month of storage time however exposure to light was highly affected riboflavin content.

According to t-test results; there were no significant differences between average riboflavin content of the samples with the storage in the dark and refrigerated conditions. However, the difference between average riboflavin content of pasta products stored under daylight and the other two conditions was statistically significant ($P<0.05$).

Riboflavin level was determined in the range of 4.00-4.09 mg/kg in the samples stored in the dark and refrigerated conditions and these values are compatible with the value indicated on the label (4 mg/kg). However, under daylight conditions, riboflavin levels decreased below the value indicated on the label (4 mg/kg) even after the first month of storage and continued to decrease further with extended storage time.

Watanabe and Ciacco (1990) indicated that 78% of riboflavin was retained during 3 months of storage in dark conditions. In another study, it was stated that little or no riboflavin loss was observed during storage of pasta for 1 year under moderate temperature and in dark conditions (Kamman *et al.*, 1981). Bui and Small (2009) indicated that higher loss occurred in the yellow alkaline noodles compared to white salted noodles for short-time storage. Storage at refrigeration temperature enhanced the stability of riboflavin.

Furuya and Warthesen (1984a) indicated that approximately 70% riboflavin reduction was observed in pasta products which have transparent packaging material stored under daylight condition in retail markets. Light intensities did not affect the riboflavin degradation. On the other hand, pasta products packaged in paper board cartons with or without transparent windows had approximately 100% riboflavin retention after 1 week.

It has been shown that riboflavin is resistant to heating, acid solutions and oxidation whereas is highly sensitive to light (especially at high temperature and pH). Lumichrome has been described as a photodegradation product of riboflavin in pasta products. Woodcock *et al.* (1982) reported that riboflavin degradation in macaroni occurred in two phases; a rapid initial phase followed by a slower phase. In that study (storage conditions: 27.87 lm/m^2, 55 °C, and 0.44 a_w), more than 50% of the riboflavin content of macaroni was lost within one day. The degradation rate of riboflavin to lumichrome was affected by light intensity. When protected from light, the amount of riboflavin remained fairly constant. Furuya and Warthesen (1984b) stated that 50% of riboflavin content of enriched elbow macaroni photodegraded after only 3 hours of storage (1,615 lm/m^2, ambient temperature and 44% relative humidity). After

Table 4. Riboflavin content of pasta products during 8 months of storage under different conditions.[1]

Storage time (month)	Ambient conditions		Refrigerator (mg/kg)	Label value (mg/kg)
	Dark (mg/kg)	Daylight (mg/kg)		
0	4.09 a	4.09 a	4.09 a	4.00
1	4.06 a	2.07 b	4.01 a	4.00
2	4.02 a	1.23 c	4.00 a	4.00
3	4.06 a	0.95 d	4.01 a	4.00
4	4.02 a	0.93 d	4.05 a	4.00
5	4.01 a	0.93 d	4.05 a	4.00
6	4.07 a	0.87 d	4.05 a	4.00
7	4.00 a	0.87 d	4.00 a	4.00
8	4.06 a	0.89 d	4.04 a	4.00

[1] Average values of three repetitions were given in the table. Values followed by the same letter in the same column are not statistically significant.

that, riboflavin gradually decreased and during 3 months more than 80% of the riboflavin was lost.

The packaging material of pasta products is a key factor in preventing the degradation of the radiosensitive riboflavin. Three types of packaging materials are used for the pasta products, mainly transparent, cardboard and cardboard with transparent window. Such packaging materials provide different levels of light protection On the other hand, the amount of macaroni exposed to light on the surface, and the penetration of light into the interior was the other factor affecting riboflavin loss (Code of Federal Regulations, 1979), (Woodcock *et al.*, 1982). Therefore; the packaging material plays a crucial role in keeping the level of riboflavin in the enriched pasta products in the range specified by the legislation.

Niacin analyses

Niacin contents of vitamin enriched pasta products stored under different conditions and storage periods were determined and the results are presented in Table 5.

One-way ANOVA results indicated that there were statistically significant differences between the niacin content of enriched pasta products and niacin content decreased gradually over 8 months of storage under all storage conditions. The 5.4 to 6.4% reduction was observed in niacin content of pasta products stored under different condition. According to t-test results; there were no significant differences between average niacin content of the samples in different storage conditions (in the dark, under daylight and refrigerated conditions; Table 5). Therefore; niacin content of pasta products decreased within 8 month of storage time but not affected from different storage conditions.

The niacin level was determined in the range of 82.57-88.24 mg/kg in the study. These analysis results were a little higher than the niacin content indicated on the label (61 mg/kg). This might be due to the tendency of the pasta manufacturers to add more niacin to enriched products to compensate the possible niacin loses during processing and prevent the niacin content to decrease below the level indicated on the label.

In the literature, there are a limited number of studies on niacin loss in pasta products during storage. Watanabe and Ciacco (1990) indicated that 94% of niacin content in spaghetti was retained during 3 months of storage in dark conditions. It was also indicated that; there were no significant differences on niacin content of various food products during processing (Eitenmiller and Landen, 1995; Lešková *et al.*, 2006). However, in these studies, the niacin content was reduced due to the processing of food rather than the storage.

Vitamin B results in cooking water

The amounts of vitamins in dry pasta and the amounts leached out to the cooking water from the enriched pasta, stored 8 months under different conditions, are given in Table 6.

It was observed that the loss of niacin to cooking water was in the range of 86.6-90.2% while the losses for thiamine and riboflavin were in the ranges of 14.4-14.6 and 15.3-19.1%, respectively (Table 6) Niacin seems to be more easily separated from the pasta structure during the cooking process and a larger proportion is leached out to the cooking water. Therefore, it can be concluded that pasta products should not be cooked in excess water and the cooking water should not be discarded to avoid vitamin losses.

Table 5. Niacin content of pasta products during 8 months of storage under different conditions.

Storage time (month)	Ambient conditions		Refrigerator (mg/kg)	Label value (mg/kg)
	Dark (mg/kg)	Daylight (mg/kg)		
0	88.24 a	88.24 a	88.24 a	61.00
1	87.11 bc	87.53 b	87.97 a	61.00
2	87.16 bc	86.88 bc	87.36 b	61.00
3	87.41 b	87.48 b	87.01 b	61.00
4	86.77 cd	86.07 d	86.20 c	61.00
5	86.70 cd	86.74 bcd	85.65 d	61.00
6	86.29 d	86.14 cd	85.93 cd	61.00
7	83.27 e	83.85 e	84.81e	61.00
8	82.57 f	82.67 f	83.48 f	61.00

[1] Average values of three repetitions were given in the table. Values followed by the same letter in the same column are not statistically significant.

Table 6. The amount of vitamins leached out to the cooking water.

B Vitamin	Storage conditions	Vitamin content of dry pasta (mg/kg)	Vitamin content of cooking water (mg/kg)	Vitamin loss (%)
Thiamine	Dark	11.31	1.63	14.4
	Daylight	11.31	1.64	14.5
	Refrigerator	11.37	1.66	14.6
Riboflavin	Dark	4.06	0.62	15.3
	Daylight	0.89	0.17	19.1
	Refrigerator	4.04	0.70	17.3
Niacin	Dark	82.57	74.45	90.2
	Daylight	82.67	72.87	88.2
	Refrigerator	83.48	72.27	86.6

In the literature, 25% riboflavin loss was observed in a noodle (Bui and Small, 2009), 56%-58% thiamine loss, 45%-51% riboflavin loss and 33%-44% niacin losses were observed in a spaghetti sample (Watanabe and Ciacco, 1990) by the cooking process. It was also stated that the cooking process applied to the noodle caused significant vitamin loses and longer cooking times caused greater effect. In noodle-type products, enrichment is effective for compensating the vitamin losses due to cooking (Bui and Small, 2008, 2009). Furthermore, Silveira *et al.* (2017) indicated that the cooking time and the type of fortification is effective in the retention percentages of thiamine.

4. Conclusion

In this study, the effects of different storage conditions on vitamin B contents of an enriched pasta product were investigated. Thiamine content did not change significantly at different storage periods (until 8 months) and conditions (ambient conditions under daylight, ambient conditions in the dark, and in a refrigerator). Riboflavin content halved starting from the first month and decreased gradually under daylight conditions, however did not change significantly during storage in the dark and refrigerated conditions. Niacin content did not change due to storage under different conditions but gradually decreased during storage. However, this depletion was not as high as the one observed in riboflavin stored under daylight conditions. Although, pasta products are not normally stored in refrigerated conditions, it was included in the present study to investigate potential differences between ambient and refrigerated conditions. There were no considerable differences between B vitamin losses during storage under ambient in the dark and refrigerated conditions. Therefore, it can be concluded that there is no need to store pasta under refrigerated conditions in terms of B vitamin losses.

In the study, the amount of vitamins leached out to the cooking water was also analysed. It was determined that the highest vitamin loss to cooking water was observed in niacin. The level of riboflavin and thiamine losses to cooking water was 6-7 times less than that of niacin. Among the B vitamins investigated in the present study, niacin seems to be more easily separated from the structure of the pasta product by the cooking process and a larger proportion of niacin is leached out to the cooking water. Hence, in order to avoid vitamin losses pasta products should be cooked in sufficient amount of water and the cooking water should not be discarded.

Validation studies indicated that the equipment, method and system performance are suitable for the purposes of the study. In the present study, the methods modified by Bilgi Boyaci (2008) and Bilgi Boyaci *et al.* (2012) for vitamin analysis in bread were used. In our study, these methods were tested for pasta. The validation studies showed that the methods modified for bread gave acceptable results for pasta.

It can be concluded that riboflavin was the most sensitive vitamin to daylight. Taking into consideration of riboflavin depletion due to light, the vitamin enriched pasta products must be protected from light. For this purpose, an opaque or light (UV and fluorescence) impermeable material can be suggested for pasta packaging. Furthermore, a short expression related to protecting vitamin enriched pasta products from direct light can be placed on the package.

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