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Indonesian Journal of Green Chemistry
E-ISSN: 3064-4720
DOI: https://doi.org/10.69930/ijgc.v2i2.557
Research Article

ScienceTech Group

Vol. 2 (2), 2025
Page: 84-89

Determination of Water-Soluble and Fat-Soluble Vitamins in Margarines Marketed in Nigeria

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Abstract. *This study involved the determination of water-soluble and fat-soluble vitamins in margarines marketed in Nigeria. The vitamin content was quantified using UV-visible spectrophotometer along with standard procedures. The results obtained showed vitamins A, D, E, B (1,2,3,6 and 12), and C were detected in most of the samples analyzed, and the most predominant vitamin in all the margarine samples was vitamin E, which ranged from 2.03-3.45 mg/100g, with sample M3 having the least content, and sample M7 having the highest content, the second most predominant vitamin was vitamin C, ranging from 0.75-1.42 mg/100g, with sample M6 having the least content and sample M1 having the highest content. The other vitamins were in the following ranges: vitamin A (0.00-0.46 mg/100g), D (0.02-0.13 mg/100g), B_1 (0.006-0.032 mg/100g), B_2 (0.012-0.023 mg/100g), B_3 (0.39-0.66 mg/100g), B_6 (0.18-0.33 mg/100g), and B_{12} (0.00-0.14 mg/100g). The most common vitamin in all the margarine samples was vitamin E, ranging from 2.03 to 3.45 mg/100g. Sample M7 had the highest content and also provided the most overall vitamins, followed by sample M1, which had the highest levels of vitamins C, B_1, and B_{12}. These findings suggest that Nigerian margarines contain significant amounts of vitamins, especially vitamins E and C, and may provide a small boost to dietary vitamin intake.*

Keywords: *Margarine, water-soluble vitamin, fat-soluble vitamin, spectrophotometer, Nigeria*

1. Introduction

Vitamins are vital organic compounds that provide no calorie but are important for cellular and metabolic reactions (Ismail *et al*., 2013). Vitamins are required for cell formation, growth and development, deficiency in them causes health issues. Many plants and microorganisms synthesize vitamins, except humans and some other animals; hence they need to be supplied through diet to the human body (Ihenetu *et al*., 2019). Most vitamins are present in required amounts in fresh and natural foods available in both plants and animal source, they are required in tiny amounts because of their inactivation in the body as they undergo catalytic reaction in the cell and act as coenzyme or part of coenzyme and enzyme systems (Ihenetu *et al*., 2019).

Vitamins can be classified according to their solubility as water-soluble vitamins e.g. vitamin C, B_1, B_2, B_3, B_5, B_6, B_8, B_9 and B_{12} and fat-soluble vitamins e.g. vitamin A, K, D and E. Fat soluble vitamins can be stored in body cells, but excess of water-soluble vitamins are excreted and cannot be absorbed (Ismail *et al*., 2013). Vitamins are not only vital for normal growth and regulation, but also overcomes different chronic diseases and helps to keep the body healthy. In Nigeria, margarines are often fortified with essential vitamins during production to improve their nutritional value, particularly fat-soluble vitamins A, D, and E. This fortification helps provide dietary support, especially for people with limited access to a

Received: 27 September 2025 / Accepted: 30 October 2025 / Available online: 31 October 2025

variety of food sources. Hence, the aim of this study was to determine the water-soluble and fat-soluble vitamins present in margarines marketed in Nigeria.

2. Methods

2.1. Sample collection and preparation

Ten (10) different brands each of margarines were randomly purchased at various retail outlets and markets in Anambra state, Nigeria. The choice of the brands was based on the highest consumption among those available in Nigerian food market. Random sampling technique was used in selecting the various samples. The margarine brands; Mr. 2 margarine, Simas margarine, Super Delicieux margarine, Hanno Cooking margarine, Napa Cooking margarine, Blue band Margarine, Golden penny margarine, Mamador margarine, Moi margarine, and Devon kings margarine were coded as M1, M2, M3, M4, M5, M6, M7, M8, M9, and M10 respectively. The margarine samples (1g) were melted in an oven at 60^{o}C to allow phase separation, so as to obtain the fat phase and this phase (decanted oil) was removed by centrifugation and dried (freed of moisture) using anhydrous sodium sulfate and finally stored frozen prior to analysis.

2.2. Determination of vitamins

2.2.1. Determination of vitamin A (Retinol), (Bayfield and Cole, 1980).

Principle: The assay is based on the spectrophotometric estimation of the colour produced by vitamin A acetate or palmitate with trichloroacetic acid (TCA).

Procedure: All procedures were carried out in the dark to avoid the interference of light. 1g of sample was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60°C in the dark. The tubes were cooled and 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml of (40°C-60°C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60℃. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5g were pipetted out into a series of test tubes. The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as mg/kg.

2.2.2. Determination of vitamin E (Tocopherol).

Vitamin E was estimated in the samples by the Emmerie-Engel reaction as reported by Rosenberg (1992).

Principle: The Emmerie-Engel reaction is based on the reduction of ferric to ferrous ions by Vitamin Es, which, with 2,2'-dipyridyl, forms a red colour. Vitamin Es and carotenes are first sampled with xylene and read at 460nm to measure carotenes. A correction is made for these after adding ferric chloride and read at 520nm.

Extraction of vitamin E: The sample (2.5g) was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation.

Procedure: Into 3 stoppered centrifuge tubes, 1.5ml of sample, 1.5ml of the standard and 1.5ml of water were pipetted out separately. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. The mixture (1.5ml) was pipetted out into a cuvette and the extinction (absorbance) was read at 460nm.

2.2.3. Determination of vitamin C (Ascorbic acid), (Roe and Keuther, 1943).

Principle: Absorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2,4-dinitrophenylhydrazine (DNPH) to form osazones. These osazanes produce an orange-coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Extraction of vitamin C: Ascorbate was extracted from 1g of the sample using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifuging at 2000rpm for 10minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. The charcoal particles were removed by centrifugation and aliquots were used for the estimation.

Procedure: Standard ascorbate ranging between 0.2 – 1.0ml and 0.5ml and 1.0ml of the supernatant were taken. The volume was made up to 2.0ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37^0c for 3 hours resulting in the formation of osazane crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540nm in a spectrophotometer.

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the sample was calculated and expressed in terms of mg/kg of sample.

2.2.4. Determination of vitamin B_1 (Thiamine) and B_2 (Riboflavin), (Kirk and Sowyer, 1991).

1g of sample was weighed into a conical flask and dissolved with 100ml of deionized water. This was shaken thoroughly, heated for 5 minutes and allowed to cool and then filtered. The filtrate was poured into cuvette and their respective wavelength for the vitamins set to read the absorbance using spectrophotometer, with vitamin B_1 = 261n m and vitamin B_2 = 242 nm

$$Concentration\ (mg\%) = \frac{A \times D.F \times volume\ of\ cuvette\ (pathlength)}{E}$$ (Nwolisah *et al.*, 2024)

Where; A = absorbance

E = extinction coefficient = 25 for B_1 and B_2

DF = dilution factor

The term "volume of cuvette" refers to the pathlength of the cuvette used in spectrophotometric analysis, which is usually 1 cm. The extinction coefficient (E = 25) was applied equally for both vitamins B1 and B2, according to the method described by Nwolisah et al. (2024). This approach recognizes that although these vitamins have different structures, a simplified method was used for comparative estimation under the same conditions.

3. Results and Discussion

Table 1. Vitamin contents of margarine samples

Vitamins	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
A (mg/100g)	0.29	0.34	0.21	0.18	0.46	0.11	0.07	0.12	0.03	ND
D (mg/100g)	0.05	0.10	0.04	0.03	0.06	0.08	0.09	0.13	0.02	0.04
E (mg/100g)	2.93	2.40	2.03	3.06	2.69	2.65	3.45	2.53	2.41	2.54
C (mg/100g)	1.42	1.18	1.01	1.41	0.84	0.75	0.97	0.91	1.27	1.17
B_1(mg/100g)	0.031	0.032	0.024	0.023	0.029	0.028	0.020	0.019	0.011	0.006
B_2(mg/100g)	0.014	0.014	0.012	0.012	0.017	0.016	0.019	0.018	0.021	0.023
B_3(mg/100g)	0.54	0.61	0.52	0.57	0.46	0.44	0.66	0.61	0.39	0.48
B_6(mg/100g)	0.30	0.30	0.33	0.31	0.27	0.26	0.30	0.29	0.27	0.18
B_{12}(mg/100g)	0.14	0.13	0.08	0.06	ND	ND	0.04	0.02	ND	ND

Note: ND = Not detected, Limit of Detection (LOD) for each vitamin ranged between 0.0001–0.0003 mg/100g depending on the vitamin and method used.

From Table 1 it was observed that the most predominant vitamin in all the margarine samples was vitamin E, which ranged from 2.03-3.45 mg/100g, with sample M3 having the least content, and sample M7 had the highest overall vitamin content, followed by M1. The high concentration of fat-soluble vitamin E shows it's benefit, as it functions as a potent antioxidant, protecting cells from damage by free radicals, it's role in anti-inflammatory processes, it's inhibition of platelet aggregation and it immune enhancing activity (Lukaski, 2004), the second most predominant vitamin was vitamin C, ranging from 0.75-1.42 mg/100g, with sample M6 having the least content and sample M1 having the highest content, the ascorbic acid's (vitamin C) antioxidant property is crucial for maintaining normal connective tissues, promoting wound healing, and facilitating the absorption of dietary iron from the intestine (Aburawi, 2021; Button, 2004).

To provide context for these findings, the Recommended Dietary Allowance (RDA) for vitamin E is 15 mg per day for adults. A 20g serving of margarine with 3.45 mg per 100g of vitamin E (as in sample M7) offers about 0.69 mg, or roughly 4.6% of the RDA. Likewise, the RDA for vitamin C is between 75 and 90 mg per day. The highest concentration found (1.42 mg per 100g in sample M1) contributes less than 1% per serving. The values of vitamin E obtained here were higher than the range of 0.15-1.47 mg/100g reported by Sami *et al*. (2014) for Okra pods but closely aligned with the 3.265 (mg/l) reported by Uzoekwe *et al*. 2021 for *Solanum erianthum*. Comparable studies on fortified margarine products have reported similar vitamin E levels. This suggests that Nigerian brands follow international fortification practices. The values obtained here for Vitamin C were lower than the range of 11.60-27.14 mg/100g reported by Sami *et al*. (2014). Vitamin B_2 recorded the lowest values (least predominant) when compared to the other vitamins in the margarine samples studied, it was found to be maximum (0.023 mg/100g) in sample M10 and minimum (0.012 mg/100g) in samples M3 and M4, the values were lower than the range of 0.043-0.136 mg/100g reported previously by Mina *et al*. 2016 for fresh vegetables but closely aligned with the range of 0.016-

0.100 mg/100g reported by Ismail *et al*. 2013 for fruits and vegetables. Sample M7 had the highest positive vitamin contributions among the ten margarine samples studied, it had the highest content of vitamin E (3.45 mg/100g) and vitamin B_3 (0.66 mg/100g), this was followed by sample M1 which had the highest content for vitamin C (1.42 mg/100g) and vitamin B_{12} (0.14 mg/100g). The values of vitamin B_3 obtained here were higher than the range of 0.010-0.100 mg/100g reported by Ismail *et al*. 2013 for fruits and vegetables. Sample M10 had the least positive vitamin contributions among the ten margarine samples analyzed, it had the least content for vitamin A where it was not detected in it, vitamin B_1 (0.006 mg/100g), vitamin B_6 (0.18 mg/100g), and vitamin B_{12} where no detectable quantities of the vitamin were observed in it along with sample M5, M6 and M9. Sample M5 had the highest vitamin A content (0.46 mg/100g), which is an essential vitamin for bone maintenance, the vitamin D content of the margarine samples ranged from 0.02-0.13 mg/100g, with sample M9 having the least content and sample M8 having the highest content.

Conclusions

The findings from this work revealed that sample M7 had the highest total vitamin contribution among the ten margarine samples studied, this was followed by sample M1 which had the highest vitamin C, B_1, and B_{12} contents. Generally, the margarine samples contained significant amounts of vitamins. Vitamin E was the most abundant, followed by vitamin C.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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