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Minerals Vitamins and Anti-Nutritional Properties of Biscuits Produced from Defatted and Undefatted Debittered Orange Seed Flours

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Abstract

Purpose: The aim of the research was to identify the amounts of minerals, vitamins, and ant nutrients present in biscuits made from the combination of orange seed flour and wheat flour.

Materials and Methods: Firstly, the orange seeds were submerged in water, after which they were boiled, ground, and half part of the ground flour was defatted for biscuits production, the orange seed flours were used to alternate wheat flour.

Findings: The biscuit samples have 2.52 – 16.95 mg/100g vitamin B9, 0.00 – 1.33 mg/100g vitamin B12, 0.00 – 650.5 IU vitamin A, 2.77 – 26.34 mg/100g vitamin D, 0.00 – 35.3 mg/100g vitamin E, 0.06 – 0.11 mg/100g vitamin B1, 0.034 – 4.09 mg/100g vitamin B2, 0.38 – 3.20 mg/100g vitamin B3. The minerals content of the biscuits are potassium (124.44 – 241.48 mg/100g), sodium (23.14 – 79.9 mg/100g), copper (0.011 – 0.05 mg/100g), zinc (0.2 – 1.55 mg/100g), iron (0.20 – 1.55 mg/100g) and calcium (4.00 – 875.41 mg/100g). The antinutrients content of the biscuits are 23.82 – 85.97 mg/100g oxalate, 0.00 – 46.05 Hu/mg haemaglutinin, 0.00 – 9.99 mg/100g tanins and 79.44 – 149.29 mg/100g phytate. The biscuits containing undefatted and defatted orange seed flours had higher values of vitamins and minerals.

Implications to Theory, Practice and Policy: The production of orange seed flour would serve as a waste disposal strategy in addition to being a source of flour for the processing of biscuits.

Keywords: *Vitamin, Minerals, Antinutrients, Orange-Seed Flour, Biscuits*

1.0 INTRODUCTION

According to forecasts, the world's orange production will reach 47.5 million tons in 2019–2020, with China, Brazil, Mexico, and the European Union as the pinnacle exporters (USDA, 2020). Peels and seeds from the processing of fruit contribute significantly to waste in the environment. Usually, proper disposing of these items is difficult, which is made worse by poor regulatory restrictions (Emojorho and Okonkwo, 2022). As a waste product from the production of orange juice, sweet orange seeds can pollute the environment if they are not properly disposed (Emojorho and Akubor, 2016). This produces an unpleasant environment with a diminished aesthetic outlook and releases odors that serve as a breeding place for insects (Oikeh et al., 2013). Fresh ideas on how to use these wastes as byproducts to make high-value food supplements or additives have drawn interest because they are high-value goods with nutritional potential (Emojorho and Okonkwo, 2022). A promising agricultural byproduct that is mineral-rich is orange seeds flour (Emojorho and Akubor, 2016). Micronutrients and macronutrients are the two main types of nutrients. The body needs macronutrients in large quantities, while micronutrients are desirable in much smaller amounts. Micronutrients (vitamins and minerals) are necessary for the body's proper operation, as the body cannot synthesis micronutrients, minerals and vitamins are required in tiny amounts and must thus be provided in the food (Akram et al, 2020).

The confectionary known as a biscuit has very low moisture content, Nwosu, (2013) defines a biscuit as a little, thin, crispy cake prepared from dough. In addition to being a staple of the human diet and typically consumed with tea, biscuits also serve as a weaning snack for young children. Simple components of biscuits include soft wheat flour, sugar, oil, and eggs (Nwosu, 2013; Adegbanke et al., 2020). These components are regarded as having low nutritional and biological qualities since the soft wheat flour used to make biscuits lacks a number of essential elements, such as several vitamins and minerals (Adegbanke, 2020). Orange seeds are rich in minerals and vitamins; they can be added to wheat when making biscuits to improve its nutritive quality.

There has been a lot of study done on recovering, reusing, and upgrading citrus trash like peels and seeds into valuable goods (Reddy and Yang, 2005; Kang et al., 2006; Li et al., 2006). Conversely, due to their nutritional and technological features, these wastes are attractive commodities in the food industry (Atta and El-shenawi, 2013). According to Saidani et al. (2004), citrus seeds are a good supply of K, Ca, Na, Fe and Mg and contained 26 to 42 % of oil. The purpose of this research was to examine the minerals, vitamins and antinutrients composition of biscuits produced from orange seed flours and wheat flour blends with improved nutrients quality.

2.0 MATERIALS AND METHODS

Sweet orange fruits (Citrus sinensis) Bende CIT/NH 10 were bought from Obollo and Ibagwa markets in Enugu State, Nigeria. Wheat flour, baking fat, sugar, powdered milk, eggs, baking powder, and table salt were purchased at the Ogige main market in Nsukka Township, Enugu State, Nigeria, for the biscuit production.

Preparation of Debittered-Defatted and Undefatted Orange Seed Flour

Orange fruits were, sorted, washed, peeled and the seeds were manually removed from the sweet orange fruits and sun-dried after using clean sharp knife to cut into halves. The dried orange seeds were washed for 12 hours (1:10 seed: water) with tap water. In the tap water, (20 kg) of the orange seeds were soaked for 12 hours. The soaked seeds were then boiled for 120 minutes. The samples were manually dehulled, removal of chaff (winnowed), and oven dried at 60 °C for 12 hours before being ground using a Corolla hand grinding machine. Then with the help of ethanol, half portion of ground seed flour was defatted using a Soxhlet apparatus before dried. Which was later sieved through a 60 mesh sieve. The defatted and undefatted orange seed flours were used to combined with wheat flour in the process of making biscuits.. Figure 1 shows the process flow for making undefatted and debittered-defatted orange seed flour.

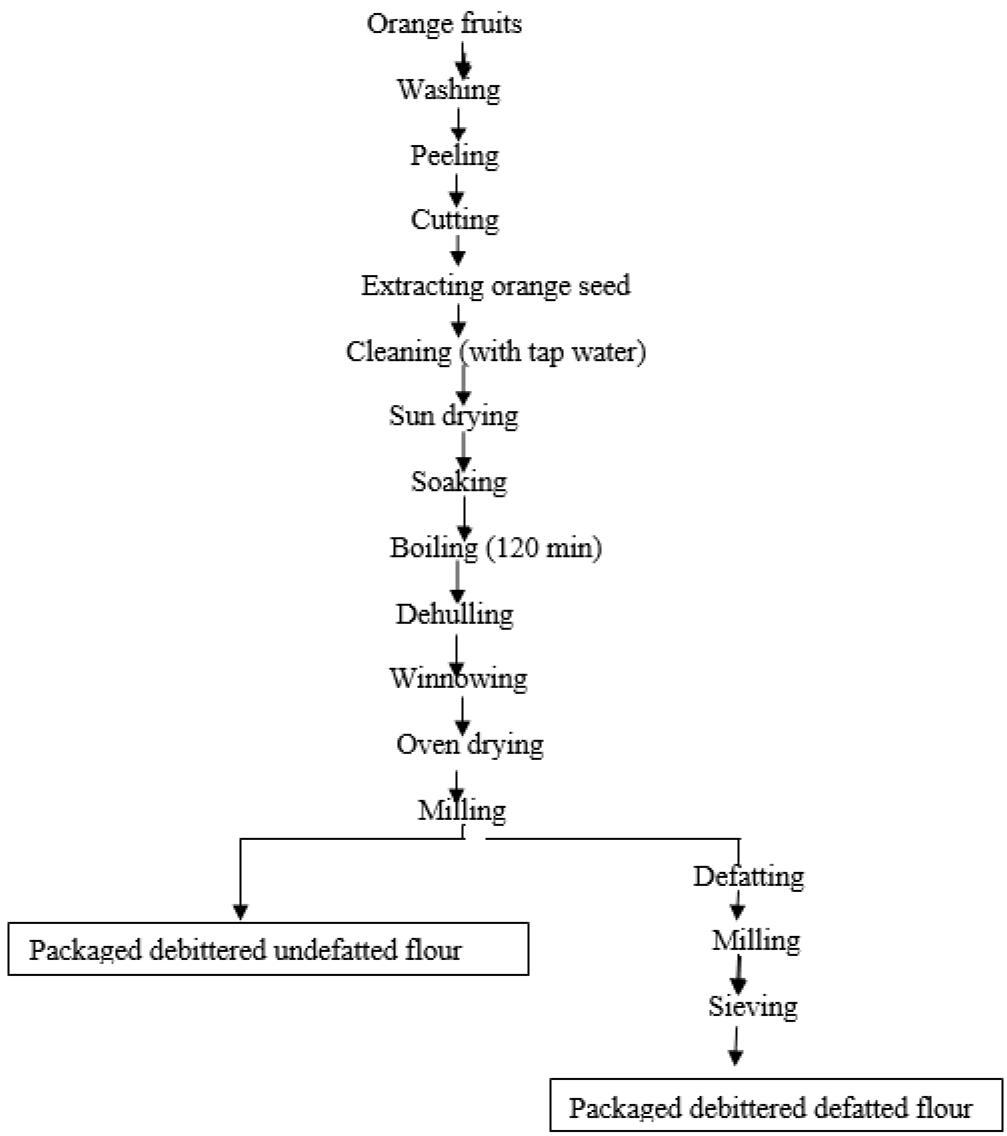

Figure 1: Flow Chart for Processing of Defatted/Undefatted Orange Seed Flour Defatted Orange Seed Flour

Preparation of Biscuits

Ten (10), 20, 30, and 40 % wheat flour were substituted with the debittered (undefatted and defatted) orange seed flours in a food blender that was run at full speed (120 rpm) for 10 minutes. Biscuits were made in accordance with Okaka (1997) procedures. Fat and sugar were combined to form cream in a mixer, and then the other dry ingredients were added. Thereafter, the eggs were added. The addition of water was then made (5 ml). The doughs were mixed for 30 minutes in a dough mixer. The dough was cut into shapes with a manual biscuit cutter. The cut-out dough was positioned on greased trays and then baked for 20 minutes at 200 °C. After cooling for 15 minutes, the hot baked biscuits were packaged in high-density polyethylene (HDPE) bags. The 100 % wheat flour biscuit served as control.

Analysis of Antinutrients

Phytate content was determined according to the technique described by Pearson (1979). The titration technique of AOAC (2010) was used to determine the oxalic acid in the sample. Tannin was identified using the Folin-Denis spectrophotometric technique as described by Pearson (1976). Hemagglutinating activity in the extracts was predictable by a serial dilution procedure according to the methods of Trugo and von Baer (1998).

Determination of Mineral Composition:

Using the method outlined by AOAC (2010) the samples' mineral contents (Fe, Mg, Mn, Se, Zn and Cu) were determined. Kirk and Sawyers (1991) technique was used to examine calcium content, phosphorus was determined using the process APHA (1995). The sodium and potassium content were determined using James (1996) atomic absorption spectrophotometry method.

Determination of Vitamins

Kirk and Sawyer's (1991) method was used to examine vitamin B1 and vitamin B2 levels. The technique of AOVC (1966) was carried to examine Vitamin B3, B6, B9, B12, vitamin E, cobalamine and vitamin K. The AOAC (2010) technique was used to examine vitamin C and vitamin D. Determination of vitamin A was carried out by the method devised by Jakutowicz et al. (1997).

Statistical Analysis

A complete randomized split-plot design was used to conduct the experiment. Analysis of variance was used to examine the data that was gathered (ANOVA). For the purpose of identifying significantly different means, (LSD) least significant difference test was applied ($p < 0.050$) and was measured significant.

3.0 FINDINGS

The Ant-Nutrients Composition of Biscuits Produced from Blends of Wheat and Orange Seed Flours

The anti-nutrients composition of biscuits made from blends of orange seed flours and wheat is revealed in Table 1.

Oxalate Content of Orange Seed Flour Biscuits

Table 1 shows the oxalate content of the biscuits ranged from 23.82 mg/100g to 85.97 mg/100g. The defatted orange seed flour biscuits had lower oxalate contents (33.16mg/100g – 85.97mg/100g) compared with undefatted orange seed flours biscuits (48.50 mg/100g – 85.97 mg/100g) even as the highest value was detected in the biscuits containing 100 % debittered-undefatted orange seed flour (85.97 mg/100g). Significantly differences ($p< 0.05$) existed in the oxalate contents among the biscuits.

The biscuits containing 100 % wheat flour biscuit had the lowest oxalate content of 23.82 %. The oxalate contents rise significantly ($p<0.05$) with increase in the level of orange seed flour in the biscuits. Since orange seed flour had higher oxalate content than wheat flour, the cause was likely an additional effect. Significantly difference in the interactions between biscuits samples ($p < 0.05$) existed. The oxalate content of the orange seed biscuits behaved differently with increase in the level of orange seed flours. The significant ($p<0.05$) interaction showed that the oxalate content of the undefatted and defatted orange seed biscuits samples increased gradually with increase in the level of orange seed flours but not at the same degree. Diets with high oxalates can make it more likely for kidneys to absorb calcium (Onimawo and Akubor, 2012).

Haemaglutinin Content of Orange Seed Flour Biscuits

The haemaglutinin content of the biscuits ranged from 0.00 Hu/mg to 46.05 Hu/mg (Table 1). Haemaglutinin was not detected in biscuits produce from wheat and defatted orange seed flour biscuits. The haemaglutinin content of undefatted orange seed flours biscuits ranged from 11.50 Hu/mg to 46.05 Hu/mg, however, the highest value was found in the biscuits made with 100 % debittered-undefatted orange seed flour (46.05 Hu/mg). In the haemaglutinin contents, significantly differences ($p < 0.05$) existed among the biscuits. The haemaglutinin contents rises significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. Significantly different ($p<0.05$) interactions existed between the biscuit samples. The significant interaction showed that the haemaglutinin content of the undefatted and defatted orange seed biscuits samples increased gradually with increase in the level of orange seed flours but not at the same degree.

Tannins Content of Orange Seed Flour Biscuits

The tannin content of the biscuits ranged from 0.00 mg/100g to 4.23 mg/100g as shown in Table 1. Tannin was not detected in biscuits produce from wheat and defatted orange seed flour biscuits. The tannins content of undefatted orange seed flours biscuits ranged from 4.23 mg/100g to 9.99 mg/100g, while the highest value was observed in the biscuits containing 100 % undefatted orange seed flour (9.99 mg/100g). The higher tannins contents recorded in the supplemented biscuits with orange seed flours than the control compared favorably with the higher tannins contents in debittered orange seed flours than wheat flour reported by Emojorho and Akubor, (2016). The increase in tannins content could be due to additional effect. The significant ($p<0.05$) interactions shown that the tannins content of the undefatted and defatted orange seed biscuits samples rises with the level of orange seed flours but not at the same degree.

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Table 1: Anti-Nutrient Composition of Biscuits from Blends of Wheat Flour and Orange Seed Flour

FLOUR	BLEND	OXALATE (mg/100g)	HAEMAGLUTININ (Hu/mg)	TANINS (mg/100g)	PHYTATE (mg/100g)
DEFATTED	W100	23.82^{f}±0.16	ND	ND	79.44^{f}±0.57
	W:DOSF(90:10)	33.16^{e}±2.2	ND	ND	82.97^{e}±0.24
	W:DOSF(80:20)	37.07^{d}±1.8	ND	ND	89.82^{d}±0.02
	W:DOSF(70:30)	53.84^{c}±2.45	ND	ND	97.09^{c}±2.06
	W:DOSF(60:40)	66.85^{b}±0.76	ND	ND	118.85^{b}±0.42
	DOSF 100	85.87^{a}±0.87	ND	ND	124.75^{a}±0.18
UNDEFATTED	W100	23.82^{f}±0.16	ND	ND	79.44^{f}±0.57
	W:UOSF(90:10)	48.5^{e}±1.8	11.50^{e}±0.71	4.23^{d}±0.4	89.44^{e}±0.33
	W:UOSF(80:20)	61.22^{d}±0.32	27.00^{d}±1.41	5.78^{c}±0.23	94.62^{d}±0.54
	W:UOSF(70:30)	77.25^{c}±0.83	31.5^{c}±0.71	5.97^{c}±0.21	104.57^{c}±4.19
	W:UOSF(60:40)	78.56^{b}±0.49	43.00^{b}±1.41	7.16^{b}±0.21	120.28^{b}±1.97
	UOSF 100	85.97^{a}±3.8	46.05^{a}±0	9.99^{a}±0.02	149.26^{a}±7.43

Values are means ± standard deviation of 3 replications. Values in the same column carrying different superscript are significantly different (*P < 0.05*): Keys: DOSF = defatted orange seed flour UOSF = Undefatted orange seed boiled and W= Wheat flour, W100 = 100 % wheat flour biscuits (Control)

Phytate Content of Orange Seed Flour Biscuits

The phytate content of the biscuits ranged from 79.44 mg/100g to 149.26 mg/100g (Table 1). The defatted orange seed flour biscuits had lower phytate contents (82.97 mg/100g – 124.75 mg/100g) when compared to undefatted orange seed flours biscuits (89.44 mg/100g – 149.26 mg/100g). The 100 % undefatted orange seed flour biscuit had the highest value (149.26 mg/100g). Significantly differences ($p< 0.05$) existed in the phytate contents among the biscuits. The phytate contents increased significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. The differences in the phytate content interactions among the biscuit samples were significant ($p<0.05$). Phytate can impact digestibility by chelating with calcium, which bind with substances, or interacting with proteolytic enzymes (Onimawo and Akubor, 2012)

Mineral Composition of Biscuit

Table 2 shows the biscuits mineral composition prepared from wheat flour blends and debittered orange seed flour. The biscuits potassium contents ranged from 159.49 mg/100g to 270.27 mg/100g (Table 2). The potassium contents were higher in the supplemented biscuits. The 100 % wheat flour biscuit had the lowest potassium content of 159.49 mg/100g. The defatted orange seed flour biscuits had lesser potassium contents (177.79 mg/100g – 226.23 mg/100g) than undefatted orange seed flours biscuits (177.98 mg/100g – 270.27 mg/100g), while the biscuits with 100% debittered-undefatted orange seed flour were found to have the highest value of 270.27 mg/100g. The significant ($P < 0.05$) interactions showed the potassium content of the samples of undefatted and defatted orange seed biscuits increased with the level of orange seed flours, although not to the same extent. The values of potassium content of biscuits supplemented with orange seed flours were greater than 5.20 to 7.12 mg/100g for biscuits supplemented with Bambara groundnut flour reported by Adegbanke et al. (2020) and also 40.63 to 154.07 mg/100g reported by Bello et al. (2017) for biscuits produced from mushroom-wheat composite flour.

Table 2 shows the biscuits sodium contents, which ranged from 17.38 mg/100g to 35.92 mg/100g. The 100 % wheat biscuits had the least sodium content of 17.38 mg/100g, while the highest value was obtained in the 100 % undefatted orange seed flour biscuit (35.92 mg/100g). Defatted orange seed flour biscuits had lower sodium contents (22.28 mg/100g – 34.33 mg/100g) than undefatted orange seed flours biscuits (25.59 mg/100g – 35.92 mg/100g). The higher values of sodium content in biscuits supplemented with orange seed flours than 100 % wheat biscuits was in agreement with Emojorho and Akubor (2016), findings of higher sodium content of orange seed flour than wheat flour, this could be due to additional effect of orange seed flours in the biscuits processing. Significantly differences ($p < 0.05$) existed in the sodium contents among the biscuits. The values of sodium content of biscuits supplemented with orange seed flours were higher than 4.32 to 4.77 mg/100g for biscuits supplemented with Bambara groundnut flour reported by Adegbanke et al. (2020) and also 36.15 to 65.92 mg/100g stated by Bello et al. (2017) for biscuits produced from mushroom-wheat composite flour.

The copper contents of the biscuits as shown in Table 2 ranged from 0.024 mg/100g to 0.043 mg/100g. The copper contents were higher in the supplemented biscuits. The values increased progressively as the level of orange seed flour increased in the biscuits. The defatted orange seed flour biscuits had lower copper contents (0.027 mg/100g – 0.039 mg/100g) than undefatted orange seed flours biscuits (0.029 mg/100g – 0.043 mg/100g) whereas the highest assessment was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (0.043 mg/100g). Significantly differences ($p < 0.05$) existed in the copper contents among the biscuits. The copper contents increased significantly ($p<0.05$) with increase in orange seed flours. The values of copper content of biscuits supplemented with orange seed flours were higher than 0.03 to 0.05mg/100g for biscuits supplemented with Bambara groundnut flour reported by Adegbanke et al. (2020) and also 0.04 to 0.13 mg/100g reported by Bello et al. (2017) for biscuits produced from mushroom-wheat composite flour.

As shown in Table 2, the biscuits magnesium contents ranged from 127.76 mg/100g to 244.21 mg/100g. The 100 % wheat biscuits had the lowest magnesium content of 127.76 mg/100g though the highest-value was obtained for the 100 % undefatted orange seed biscuit (244.21 mg/100g). The biscuits produced from defatted orange seed flour had lower magnesium contents (134.36 mg/100g – 215.45 mg/100g) than undefatted orange seed flours (147.25 mg/100g – 244.21 mg/100g). The magnesium contents increased significantly ($p<0.05$) with increase in the level of orange seed flour in the biscuits, due to additional effect. The interactions of the magnesium content between the biscuit samples varied significantly ($p<0.05$). In humans, prolonged vomiting, malabsorption, along with very bad diarrhea, could be caused by magnesium deficiency (Okaka et al., 2011). The values of magnesium content of biscuits supplemented with orange seed flours were higher than 7.02 to 14.00 mg/100g for biscuits supplemented with mushroom flour reported by Bello et al. (2017), however, Bello et al. (2017) also stated increase in magnesium content of biscuits produced from mushroom wheat composite flour.

The selenium content of the biscuits ranged from 9.63 mg/100g to 36.87 mg/100g (Table 2). The selenium contents were higher in the supplemented biscuits. The values of selenium increased progressively in the biscuits with increase in orange seed flour percentage. The defatted orange seed flour biscuits had lower selenium contents (11.72 mg/100g – 19.79 mg/100g) than undefatted orange seed flours biscuits (15.30 mg/100g – 36.87 mg/100g) though the maximum value was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (36.87 mg/100g). Significantly differences ($p < 0.05$) existed in the selenium content among the biscuits. Selenium plays a crucial role in how tocopherols are metabolized and is helpful in the management of protein and calorie malnutrition (Okaka et al., 2011). Mineral called selenium has anti-cancer properties against breast cancer, colon cancer, and possibly extra malignancies (Hoffmann and Berry 2008). Naturally occurring antioxidant (Gaman et al. 2014)

The zinc contents of the biscuits ranged from 0.20 mg/100g to 1.02 mg/100g (Table 2). The 100 % wheat biscuits had the lowest zinc content of 0.20 mg/100g while the highest value was obtained for the 100 % undefatted orange seed biscuit (1.02 mg/100g). The defatted orange seed flour biscuits had lower zinc contents (0.23 mg/100g – 0.43 mg/100g) than undefatted orange seed flours biscuits (0.3 mg/100g – 1.02 mg/100g). The zinc contents rise significantly (p<0.05) with rise in the level of orange seed flour in the biscuits. The zinc content interactions between biscuits samples were significantly (P < 0.05) different. Cell growth, proliferation, sexual development, and fertility are all made possible by this component of metalloenzymes. It improves immunity, appetite, and taste. Iron and copper reduce its absorption (Akram, 2020). The values of zinc content of biscuits supplemented with orange seed flours were higher than 2.04 to 2.73 mg/100g for biscuits supplemented with Bambara groundnut flour described by Adegbanke et al. (2020). Similar increases in zinc content of biscuits were noted by Bello et al. (2017) along with increases in mushroom flour.

Table 2: Minerals Composition of Biscuits from Blends of Wheat Flours and Orange Seed Flours

Flour	Blend	K mg/100g	Na mg/100g	Cu mg/100g	Mg mg/100g	Se mg/100g	Zn mg/100g	Fe mg/100g	Mn mg/100g	P mg/100g	Ca mg/100g
Defatted	W100	159.49f±0.97	17.38f±1.19	0.024e±0	127.76f±0.98	9.63f±0.68	0.2f±0	1.66a±0.02	1.96f±0.02	40.65f±0.09	419.42f±0.05
	W:DOSF(90:10)	177.79e±1.21	22.28e±0.30	0.027d±0	134.36e±0.34	11.72e±0.4	0.23e±0.01	0.96b±0.05	2.3e±0.03	43.42e±0.35	444.95e±0.12
	W:DOSF(80:20)	184.22d±0.23	24.89d±0.06	0.029d±0	144.39d±0.79	12.18d±0.1	0.28d±0	0.85bc±0.06	2.77d±0.08	49.52d±0.52	512.00d±0.07
	W:DOSF(70:30)	198.71c±1	28.46c±0.06	0.030c±0	148.93c±13.77	12.73c±0.46	0.31c±0.01	0.76bc±0.03	3.43c±0.03	54.78c±0.07	556.95c±0.04
	W:DOSF(60:40)	211.82b±0.25	32.06b±1.22	0.032b±0	168.11b±0.24	13.48b±0.04	0.33b±0.01	0.46c±0.04	3.86b±0.08	60.65b±0.32	580.37b±0.03
	DOSF 100	226.23a±3.05	34.33a±0.18	0.039a±0	215.45a±0.22	19.79a±0.06	0.43a±0.01	0.24d±0.02	5.91a±0.03	77.79a±0.95	611.76a±0.10
Undefatted	W100	159.49f±0.97	17.38f±1.19	0.024e±0	127.76f±0.98	9.63f±0.68	0.2f±0	1.66a±0.02	1.96f±0.02	40.65f±0.09	419.42f±0.08
	W:UOSF(90:10)	177.98e±1.47	25.59e±0.19	0.029d±0	147.52e±0.65	15.3e±0.42	0.3e±0	1.52b±0.01	2.76e±0.03	45.82e±0.55	474.99e±0.13
	W:UOSF(80:20)	196.38d±0.78	27.59d±0.19	0.029d±0	164.78d±0.28	19.72d±0.57	0.35d±0.01	1.35bc±0.05	2.98d±0.06	52.27d±0.9	523.53d±0.02
	W:UOSF(70:30)	223.3c±3.25	31.33c±0.48	0.031c±0	181.57c±1	22.68c±0.93	0.38c±0.01	1.28bc±0.01	3.4c±0.01	61.37c±1.29	597.09c±0.06
	W:UOSF(60:40)	241.81b±1.15	33.71b±0.23	0.036b±0	197.51b±1.83	33.99b±0.18	0.64b±0.01	1.47c±0.52	4.06b±0.07	78.81b±0.9	612.60b±0.09
	UOSF 100	270.27a±0.22	35.92a±0.10	0.043a±0	244.21a±2.39	36.87a±0.11	1.02a±0	0.85d±0.00	6.12a±0.7	91.57a±0.47	686.33a±0.05

Values are means ± standard deviation of 3 replications. Values in the same column carrying different superscript are significantly different (*P < 0.05*): Keys: DOSF = defatted orange seed flour UOSF = Undefatted orange seed boiled and W= Wheat flour, W100 = 100 % wheat flour biscuits (Control)

The significant (p<0.05) interactions showed that the zinc content increased but not to the same extent in the samples of undefatted and defatted orange seed biscuits. An insufficient intake of food, hypogeusia (impaired perception of taste) and delayed development are symptoms of a moderate zinc deficiency (Okaka et al., 2011). Table 2 shows the biscuits iron contents, which ranged from 0.24 mg/100g to 1.66 mg/100g. The iron contents were lower in the supplemented biscuits. The iron content values decreased gradually as the level of orange seed flour rises in the biscuits. The 100 % wheat flour biscuit had the maximum iron content of 1.66 mg/100g. The defatted orange seed flour biscuits had lower iron contents (0.24 mg/100g – 0.96 mg/100g) than undefatted orange seed flours biscuits (0.85 mg/100g – 1.52 mg/100g). The undefatted and defatted orange seed biscuit samples' iron content decreased with an increase in the amount of orange seed flours, but not to the same extent, according to the significant

($p<0.05$) interaction. Hypochromic microcytic anemia is brought on by an iron deficiency. It participates in the exchange of oxygen among blood and tissues as a component of myoglobin and hemoglobin (Akram, 2020).

Table (Table 2) shows the manganese contents of the biscuits ranged from 1.96 mg/100g to 6.12 mg/100g. The 100 % wheat flour biscuit had the lowest manganese content of 1.96 mg/100g while the highest value (6.12 mg/100g) was observed in the biscuits containing 100 % undefatted-debittered orange seed flour. The defatted orange seed flour biscuits had lower manganese contents (2.30 mg/100g – 5.91 mg/100g) compared with undefatted orange seed flours biscuits (1.96 mg/100g – 6.12 mg/100g). In the development of embryonic bones in other species, manganese deficiency results in congenital defects (Soetan et al., 2010). Slip tendon disease is brought on by a deficit (Okaka et al., 2011).

The biscuits phosphorus contents ranged from 40.65 mg/100g to 91.57 mg/100g (Table 2). The values of the phosphorus content increased progressively in the biscuits with increase orange seed flours. The wheat flour biscuit with 100 % had the lowest phosphorus content of 40.65 mg/100g. The defatted orange seed flour biscuits had lower phosphorus contents (43.42 mg/100g – 77.79 mg/100g) than the undefatted orange seed flours biscuits (45.85 mg/100g – 91.57 mg/100g). The highest value was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (91.57 mg/100g). The phosphorus contents increased significantly ($p<0.05$) due to an additional effect, as orange seed flour had a higher phosphorus content than wheat flour. Significantly differences ($p< 0.05$) existed in the phosphorus content between biscuits samples. The significant ($p<0.05$) interactions showed that the phosphorus content of the undefatted and defatted orange seed biscuits samples rises with increase in the level of orange seed flours but not at the same degree. Lack of phosphorus can cause rickets in children and osteomalacia in adults (Okaka et al., 2011). Bello et al. (2017) also reported similar increase in phosphorus content of biscuits with increase in mushroom flour with wheat flour.

The biscuits calcium content ranged from 419.42 mg/100g to 686.33 mg/100g (Table 2). The calcium contents were higher in the supplemented biscuits than 100 % wheat biscuits. The 100 % wheat flour biscuit had the lowest calcium content of 419.42 mg/100g. Defatted orange seed flour biscuits had lower calcium contents (444.95 mg/100g – 611.76 mg/100g) than undefatted orange seed flours biscuits (474.99 mg/100g – 686.33 mg/100g) even as the highest value was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (686.33 mg/100g). The higher calcium contents recorded in the supplemented biscuits with undefatted and defatted orange seed flour than the 100 % wheat flour biscuits was in agreement with the higher zinc contents in debittered orange seed flour than wheat flour reported by Emojorho and Akubor, (2016). The calcium contents increased significantly ($p<0.05$) as the amount of orange seed flour in the biscuits rises, because of the addition effect. The significant ($p<0.05$) interactions showed that, the calcium content of the undefated and defatted orange seed biscuits samples rises with increase in the level of orange seed flours but not at the same degree. Cell structure is kept intact by calcium (Orlov et al., 2005). The values of calcium content of biscuits supplemented with orange seed flours were higher than 2.08 mg/100g for biscuits from wheat and bambara groundnut flour reported by Adegbanke et al. (2020) and also 12.10 to 47.00 mg/100g stated by Bello et al. (2017) for biscuits produced from mushroom wheat composite flour.

Vitamin Composition of Biscuit

Table 3 shows the vitamin content of biscuits made with wheat flour and debittered orange seed flour blends. The vitamin A contents of the biscuits ranged from 15.5 IU to 88.26 IU (Table 3). Progressively, the Vitamin A contents increased as the level of orange seed flour increased in the biscuits. Wheat flour biscuit with 100 % had the least vitamin A content of 15.5 IU, while the biscuits with 100 % undefatted-debittered orange seed flour were found to have the highest value (88.26 IU). The defatted orange seed flour biscuits had lower vitamin A contents (34.3 IU – 81.23 IU) than undefatted orange seed flours biscuits (39.52 IU – 88.26 IU. The significant ($p<0.05$) interaction showed also that the Vitamin A content of the defatted orange seed biscuits samples and the undefatted orange seed biscuits samples increased with increase in the level of orange seed flours but at different rate. Lack of vitamin A slows growth and can cause night blindness or poor vision in low light (Onimawo and Akubor, 2011). The rod cells in the retinal, vitamin A and the protein opsin combine to form rhodopsin. Vitamin A also supports bone growth and the immune functions (Akram, 2020).

The biscuits vitamin D content ranged from 7.04 mg per 100g to 10.20 mg per 100g (Table 3). The biscuit made of 100 % wheat flour had the least level of vitamin D (7.04 mg/100g). The undefatted orange seed flour biscuits (8.42 mg/100g – 10.20 mg/100g) had higher of vitamin D content than the defatted orange seed flour biscuits (7.66 mg–9.66 mg/100g). The dietary illnesses rickets and osteomalacia (softening of the bones), are linked to vitamin D deficiency (Onimawo and Akubor, 2011). The most well-known benefit of vitamin D is its ability to regulate blood phosphorus and calcium levels. Additionally, vitamin D promotes normal bone mineralization and boosts phosphate absorption through the tubules. Additionally, it has antioxidant qualities (Akram, 2020).

Table 3: Vitamin Composition of Biscuits from Blends of Wheat Flour and Orange Seed Flours

FLOUR	BLEND	A (IU)	D (mg/100g)	E (mg/100g)	K (mg/100g)	B1 (mg/100g)	B2 (mg/100g)	B3 (mg/100g)	B6 (mg/100g)	B9 (mg/100g)	B12 (mg/100g)
DOSF	W100	15.5f±0.71	7.04f±0.04	4.56f±0.36	0.23e±0.02	0.07a±0	0.95a±0.04	0.56b±0.01	2.3a±0.02	1.49e±0.11	ND
	W:DOSF(90:10)	34.3e±0.21	7.66e±0.21	4.73e±0.03	0.34d±0.02	0.04b±0	0.9a±0.02	0.65b±0.05	2.42a±0.01	1.95d±0.02	ND
	W:DOSF(80:20)	58.24d±0.01	8.06d±0.07	5.33d±0.01	0.4d±0.01	0.04bc±0	0.62b±0.01	0.68b±0.01	2.93a±0.02	1.99d±0.01	ND
	W:DOSF(70:30)	62.86c±0.39	8.66c±0.17	6.51c±0.29	0.47c±0.02	0.04c±0	0.55c±0.05	0.7ab±0.01	3.55a±0.05	2.41c±0.01	ND
	W:DOSF(60:40)	71.11b±1.36	8.9b±0.01	7.31b±0.28	0.52b±0.01	0.03d±0	0.33d±0.01	0.89a±0.01	3.96a±0.04	2.69b±0.01	ND
	DOSF 100	81.23a±1.1	9.66a±0.17	9.35a±0.07	0.66a±0.01	0.03e±0	0.17e±0.03	0.95a±0.01	4.82a±0.11	2.87a±0.03	ND
UOSF	W100	15.5f±0.71	7.04f±0.04	4.56f±0.36	0.23e±0.02	0.07a±0	0.95a±0.04	0.56b±0.01	2.3a±0.02	1.49e±0.11	ND
	W:UOSF(90:10)	39.52e±1.31	8.42e±0.01	5.52e±0.13	0.36d±0.02	0.05b±0	0.91a±0.02	0.79b±0.01	2.7a±0.02	1.96d±0.03	ND
	W:UOSF(80:20)	60.61d±0.54	8.88d±0.04	6.05d±0.1	0.4d±0.03	0.05bc±0	0.66b±0.01	0.88b±0.02	3.07a±0.05	2.06d±0.05	ND
	W:UOSF(70:30)	71.27c±1.2	9.38c±0.08	7.07c±0.08	0.51c±0.03	0.05c±0	0.58c±0.01	0.97ab±0.01	3.91a±0.01	2.74c±0.06	ND
	W:UOSF(60:40)	78.77b±0.49	9.77b±0.07	8.89b±0.04	0.61b±0.03	0.04d±0	0.5d±0.03	1.56a±0.03	4.28a±0.03	2.87b±0.04	ND
	UOSF 100	88.26a±0.57	10.2a±0.02	11.7a±0.14	0.72a±0.03	0.03e±0	0.3e±0.03	1.63a±0.55	6.19a±0.05	3.02a±0.01	ND

Values are means ± standard deviation of 3 replications. Values in the same column carrying different superscript are significantly different (*P < 0.05*): Keys: DOSF = defatted orange seed flour UOSF = Undefatted orange seed boiled and W= Wheat flour, W100 = 100 % wheat flour biscuits (Control)

The biscuits contained 4.56 mg to 11.70 mg of vitamin E per 100g (Table 3). The 100 % wheat flour biscuit had the lowest vitamin E content of 4.56 mg/100g. The defatted orange seed flour biscuits had lower vitamin E contents (4.73 mg/100g – 9.35 mg/100g) than undefatted orange seed flours biscuits (5.52 mg/100g – 11.70 mg/100g) even as the highest value was detected in the biscuits containing 100 %

debittered-undefatted orange seed flour (11.70 mg/100g). The vitamin E contents rises significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. The significant interaction ($p<0.05$) showed that the vitamin E content of the defatted orange seed biscuits samples rises with the level of defatted orange seed flours and the vitamin E content of undefatted orange seed biscuits samples as well increased with rise in the level of undefatted orange seed flours but at different level. Vitamin E main role is as an antioxidant, protecting other nutrients and preventing lipid peroxidation (Yamauchi, 1997). A natural antioxidant, vitamin E also has anti-sterility properties. It plays a role in immunity and the recovery of wounds (Akram, 2020). Table 3 shows the biscuits vitamin K contents which ranged from 0.23 mg/100g to 0.72 mg/100g. Progressively, the values rises as the level of orange seed flour increased in the biscuits. The 100 % wheat flour biscuit had the least vitamin k content of 0.23 mg/100g. The defatted orange seed flour biscuits had lower vitamin k contents (0.23 mg/100g – 0.66 mg/100g) than undefatted orange seed flours biscuits (0.36 mg/100g – 0.72 mg/100g) while the highest value was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (0.72 mg/100g). Undefatted and defatted orange seed biscuit samples had significantly different vitamin K interactions ($P < 0.05$). The significant ($p<0.05$) interaction showed that the Vitamin K content of the defatted orange seed biscuits samples rises with the level of defatted orange seed flours and the Vitamin K content of undefatted orange seed biscuits samples also rises with rise in the level of undefatted orange seed flours but at different level. The blood clotting process involves vitamin K. (Onimawo and Akubor, 2011). A lack of vitamin K can cause pervasive bleeding, the onset of hemorrhagic disease in newborns, and a protracted time for blood to clot in adults (Akram, 2020).

Table 3 shows the biscuits vitamin B1 contents which ranged from 0.03 mg/100g to 0.07 mg/100g. The vitamin B1 contents were lower in the supplemented biscuits. Progressively, as the level of orange seed flour increased in the biscuits the values of vitamin B1 contents decreased. The biscuit made entirely of wheat flour contained the highest vitamin B1 content (0.07 mg/100g). The defatted orange seed flour biscuits had lower vitamin B1 contents (0.03 mg/100g – 0.04 mg/100g) than undefatted orange seed flours biscuits (0.03 mg/100g – 0.05 mg/100g) while the lowest value was noticed in the biscuits containing 100 % debittered-defatted orange seed flour (0.03 mg/100g). The vitamin B1 contents reduced significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. Vitamin B1 interactions different significantly ($p<0.05$) between samples of orange seed biscuits. Hence the orange seed biscuits were behaving differently with increase in the level of orange seed flours. Thiamine deficiency results in beri-beri, weight gain, loss of skin sensation, oedema in kids (water logged), gastro intestinal problems, and cardiac failure (heart failure) (Onimawo and Akubor, 2011). The heart, nervous system, and muscles all depend on thiamine, which also serves as a co-catalyst in the digestion of sugar (Akram, 2020).

The vitamin B2 contents of the biscuits ranged from 0.17 mg/100g to 0.95 mg/100g (Table 3). The vitamin B2 contents were lower in the supplemented biscuits. The values decreased progressively as the level of orange seed flour increased in the biscuits. The biscuit made entirely (100 %) of wheat flour had the maximum level of vitamin B2 (0.95 mg/100g). The defatted orange seed flour biscuits had lower vitamin B2 contents (0.17 mg/100g – 0.95 mg/100g) than undefatted orange seed flours biscuits (0.30 mg/100g – 0.91 mg/100g) while the lowest value was noticed in the biscuits containing 100 % debittered-defatted orange seed flour (0.17 mg/100g). Vitamin B2 contents reduced significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. The Vitamin B2 interactions between the samples of orange seed biscuits that were defatted and those that were not were significantly different ($p<0.05$). Man suffers from glossitis, a condition marked by swollen tongues, lips, and scaly mouth corners, which are brought on by riboflavin deficiency (Onimawo and Akubor, 2011). It contributes to the respiration of tissues. Ariboflavinosis is a condition caused by its deficiency of vitamin B2, the symptoms of ariboflavinosis include cheilosis (textured desquamation of the skin around the mouth), glossitis (sparkly red and painful tongue), soreness in the lips, eye disturbances, photophobia (light sensitivity), oily skin on the nose, and scrotal dermatitis (Henriques et al., 2010).

The vitamin B3 contents of the biscuits ranged from 0.56 mg/100g to 1.63 mg/100g (Table 3). The 100 % wheat flour biscuit had the lowest vitamin B3 content of 0.56 mg/100g. Defatted orange seed flour biscuits had lower vitamin B3 contents (0.65 mg/100g – 0.95 mg/100g) than undefatted orange seed flours biscuits (0.79 mg/100g – 1.63mg/100g) whereas the greater value was noticed in the biscuits containing 100 % debittered-undefatted orange seed flour (1.63 mg/100g). The vitamin B3 contents increased significantly ($p<0.05$) with increased level of orange seed flours. The interactions of vitamin B3 content among the biscuit samples varied significantly ($p<0.05$). Pellagra is caused by a niacin deficiency and is known as the "three-D disease" due to its primary symptoms of diarrhea, dermatitis, and delirium (Lule et al., 2016). Niacin can be used to treat diabetes mellitus as well as lower cholesterol (Elam et al., 2000).

The vitamin B6 contents of the biscuits ranged from 2.30 mg/100g to 6.19 mg/100g. Table 3 shows that, the 100 % wheat flour biscuit had the least vitamin B6 content of 6.19 mg/100g. Defatted orange seed flour biscuits had lower vitamin B6 contents (2.42 mg/100g – 4.82 mg/100g) compared with undefatted orange seed flours biscuits (2.70 mg/100g – 6.19 mg/100g) whereas the greater value was noticed in the biscuits containing 100 % undefatted-debittered orange seed flour (6.19 mg/100g). Vitamin B6 contents rises significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. Perhaps as a result of an additional effect given that orange seed flour had higher vitamin B6 content than wheat flour. B6 deficiency brought on by illnesses like "severe diarrhoea, malabsorption, congenital metabolic dysfunction, hyperthyroidism, renal and hepatic disease, congestive heart failure, alcoholism, drug-induced illnesses, and during pregnancy and lactation (McCormick, 2014).

Table 3 shows the biscuits vitamin B9 contents which ranged from 1.46 mg/100g to 3.02 mg/100g. As the values increased progressively, the level of orange seed flour increased in the biscuits. The defatted orange seed flour biscuits had lower vitamin B9 contents (1.95 mg/100g – 2.87 mg/100g) than undefatted orange seed flours biscuits (1.95 mg/100g – 3.02 mg/100g) whereas the highest value was noticed in the biscuits containing 100 % undefatted-debittered orange seed flour (3.02 mg/100g). Vitamin B9 contents rises significantly ($p<0.05$) with rise in the level of orange seed flour in the biscuits. The significant ($p<0.05$) interaction showed that, vitamin B9 content of the samples of undefatted and defatted orange seed biscuits increased with the level of orange seed flours although not at the same rate. Folate is necessary for the creation of good red blood cells during periods of fast expansion, such as pregnancy and fetal development (Greenberg et al., 2011). It works in conjunction with vitamin B12 to produce blood, preventing anemia (Onimawo and Akubor, 2011).

Vitamin B12 could not be detected in any of the products including biscuits containing 100 % wheat flour. It is either that vitamin B12 quantity was too small to be detected by the method used or that the vitamin is completely absent. It is a crucial component of the molecule that creates nucleic acids (DNA and RNA). Pregnant women must take folate supplements as a preventive measure because folate shortage can cause neural tube defects (Akram, 2020).

4.0 CONCLUSION AND RECOMMENDATIONS

Undefatted and defatted orange seed flour increased the mineral and vitamin contents of the biscuit when compared to wheat flour biscuit. Wheat flour could be supplemented with debittered-defatted and undefatted orange seed flour for biscuit production as this will improve the nutritional value and also reduce over dependency on wheat flour. There was increase in anti-nutritional factors with increase in orange seed flours in the biscuit, but lower in biscuits supplemented with defatted orange seed flours than undefatted orange seed flours.

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Authors' Contributions

Ernest Eguono Emojorho was responsible for the conceptualization, project administration, writing of original draft, reviewing and provision of resources for the research, while Maryann Nkemakonam Anene also was involved with writing and editing of the manuscript, supervised the research process, assisted with necessary software and Udeh Charles Chiedu assisted greatly the research methodology and also provision of relevant materials, writing and reviewing of manuscript.

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