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EDITORIAL TEAM

Journal of Food Research; Vol. 9, No. 6; 2020
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CONTENTS

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Impacts of Protease Treatment on the Contents of Tocopherols and B Vitamins in Peanuts

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Abstract

This study investigated the changes of tocopherols and B vitamins in raw peanuts as a result of protease treatment which was used to reduce peanut allergens. Raw peanut kernels were treated with Alcalase, bromelain, Neutrase and papain separately at different concentrations, vacuum dried, and then ground into paste. The paste was defatted using hexane containing 0.02% BHT to obtained crude oil and defatted peanut flour which were used for tocopherol and B-vitamin analysis, respectively. The protease treatment significantly reduced the contents of all tocopherols and B-vitamins in the peanuts in enzyme concentration-dependent manner ($P<0.0001$). The highest losses of α-, γ-, and δ-tocopherols were 60.87 %, 40.60 % and 36.89 %, respectively, while the maximum losses of vitamins B1, B2, B3 and B6 were 63.29 %, 44.83 %, 40.56 % and 49.59 %, respectively. Among tocopherols, α-tocopherol was the most affected while δ-tocopherol was the least affected. Among B-vitamins, B1 was the most affected and B3 the least affected. This study demonstrated that although the protease treatment approach (including enzyme treatment and drying) for peanut allergen reduction resulted in different degrees of losses in tocopherols and B vitamins in raw peanuts, the enzyme treated peanuts is still a good source of tocopherols and vitamin B3 comparing to most cooked legumes and vegetable.

Keywords: peanuts, protease treatment, tocopherols, B vitamins, vitamin loss, HPLC analysis

1. Introduction

Peanuts are excellent source of fat, proteins, vitamins and minerals. The proximate composition of raw peanut kernels includes 47 % fat, 25 % protein, 5 % moisture, 17 % carbohydrate, and 2 % ash (Yadav, Edukondalu, Patel, & Rao, 2018). Peanuts are rich in vitamin E and some B vitamins. The vitamin E is fat-soluble and the most abundant in peanuts. It functions as an antioxidant for cell membrane protection (Chen et al., 2011; Settaluril et al., 2012) and can be stored in the liver and fatty tissues (Whitney & Rolfes, 2010). Vitamin E is not a single compound but a group of eight compounds which act as antioxidants maintaining the stability of cell membranes against oxidative stress. These compounds are α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols, collectively known as tocochromanols (Ahsan et al., 2015). The main tocopherols in peanuts were reported to be α-, γ- and δ- tocopherol with δ- tocopherol having the least concentration (Medina-Juárez et al., 2009). The concentrations of α-, γ-, and δ-tocopherol in peanut oil are 8.86–30.4, 0 - 0.38, 3.50–19.2, 0.85–3.10 mg/100g oil, respectively (Shahidi & Costa De Camargo, 2016). The concentration ranges of these tocopherols in peanuts are 11.9-25.3, 10.4-34.2 and 0.58-2.5 mg/100g (Ahmed & Young, 1982). The B vitamins include B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B8 (biotin), B9 (folic acid), and B12 (cyanocobalamine) are water soluble. They function as coenzymes during biochemical processes in the body. Peanut is a rich source of thiamine (B1), niacin (B3) and choline (B4). Vitamin B4 (choline) is a vitamin-like nutrient which can be synthesized by the body provided the precursors folic acid and vitamin B12 are sufficient (Cai et al., 2019). However, there is limited information on the determination of B vitamins in peanut because the analysis of B vitamins (extraction and purification methods) in oilseed is very tedious. Another major challenge is the tendency of the vitamin degradation due to exposure to air and light (Trang, 2013). The contents of vitamin B1, B2, B3 and B6 in Virginia peanuts were reported by the United States Department of Agriculture (USDA) database as 0.653, 0.131, 12.375, and 0.346 mg/100g (USDA, 2018). Peanut oil also contains a substantial amount of phytosterols which have anticancer properties and impair the absorption of cholesterol

from the digestive tract (Awad et al., 2000; Abumweis et al., 2008; Hashemian et al., 2017). According to the USDA nutrient composition database, 100 g peanuts consumed may provide up to 75 % RDA of niacin, 60 % RDA of folate, 53 % RDA of thiamine, 10 % RDA of riboflavin, 35 % RDA of pantothenic acid, 27 % RDA of pyridoxine, 55.5 % RDA of vitamin E (USDA, 2018).

Although peanut contains about 25% protein, the majority of the protein is allergenic which affect 2-2.5% of populations in the developed countries. In the United States 2.5 % children and 1.8 % of adults are allergic to peanut (Jiang et al., 2018; Gupta et al., 2019). Peanut allergy prevalence in Europe is about 2.2 % (Nwaru et al., 2014). Peanut allergy is one of the most fatal food allergies, which accounts for about 59 % death caused by food allergy (Bock et al., 2007). Scientists have proposed different approaches to mitigate peanut allergy. Proteolytic hydrolysis using non-specific proteases such as Alcalase, bromelain, Neutrase and papain has been reported to tremendously reduce the contents of major allergenic proteins such as Ara h 1, Ara h 2 and Ara h 6 in the peanuts (Yu et al., 2015; Yu & Mikiashvili, 2020). However, the effect of the proteolytic hydrolysis on the nutrient composition is unknown. The purpose of this study was to evaluate the influence of protease treatment on the tocopherol contents in peanut kernels.

2. Materials and Methods

2.1 Materials

Raw Virginia peanuts were purchased from Good Earth Peanut Company (Skippers, VA, USA). The enzymes used in the study, including Alcalase, bromelain, Neutrase, and papain were purchased from Fisher Scientific (Suwanee, GA, USA). These are non-selective proteases. The standards of B Vitamins (including B1, B2, B3, B6) and tocopherols (including the α-, γ-, and δ-tocopherols) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). The chemical reagents and solvents including hexane, methanol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), hydrochloric acid (HCl), sodium dioctylsulfosuccinate, formic acid, potassium hydroxide (KOH) were all purchased from the Fisher Scientific (Hampton, NH, USA).

2.2 Enzymatic Treatment

Raw peanuts (50 g each) were treated with individual proteases (Alcalase, bromelain, Neutrase, and papain) separately in phosphate buffer (20 mM) at different concentrations and incubated at the optimal temperature and pH of each enzyme for 3 hours as described previously (Yu & Mikiashvili, 2020). The concentrations of Alcalase, bromelain, Neutrase and papain used in different treatments were shown in Table 1. The optimal pHs and temperatures of Alcalase, papain, Neutrase, and bromelain are 7.5, 6.0, 7.0, and 7.0 and 50, 48, 50, and 45 °C, respectively. Due to the solubility limitation, the maximum concentrations of bromelain and papain could be used in the treatment were 0.2% and 0.5% (w/w), respectively. The treated peanut kernels were dried in a vacuum oven at 75 ℃ for 16-18 hours to remove the moisture, ground into paste and stored at 4 °C. The untreated raw peanut sample was used as control, and untreated vacuum dried peanut sample was used to examine the influence of vacuum drying on the contents of tocopherols and B vitamins in the peanuts.

Table 1. Concentrations of proteases used in the study

	Enzyme Concentration (%)			
Treatment	Alcalase*	Bromelain†	Neutrase*	Papain†
T1	1.0	0.02	0.2	0.1
T2	2.0	0.04	0.4	0.2
T3	3.0	0.08	0.8	0.3
T4	3.5	0.16	1.2	0.4
T5	4.0	0.20	1.6	0.5

*Alcalase and Neutrase were liquid forms and their concentrations (enzyme to peanut ratios) were volume to weight (v/w).

†Bromelain and papain were solid forms and their concentrations (enzyme to peanut ratios) were weight to weight (w/w).

2.3 Extraction of Peanut Oil

Tocopherols are fat-soluble and they concentrate in the oil. Therefore, peanut oil was extracted using hexane containing 0.02 % butylated hydroxytoluene (BHT) for tocopherol quantification. The peanut paste (20.00 g) was weighed into a glass flask and then 30 ml of the extraction solvent was added and magnetic stirred for 1 hour.

The sample was centrifuged at 3000g for 15 minutes using a 5810 R centrifuge (Eppendorf Corporation, Hamburg, Germany) and the supernatant was poured into a pre-weighed evaporating flasks. The precipitate was re-extracted by another 30 ml of solvent. The combined supernatant was concentrated at 40 ℃ under reduced pressure using a R-300 Rotavapor (Büchi, Flawil, Switzerland) to remove the hexane. The resulted oil was quantified, collected into an amber glass vial and stored at 4 ℃ for tocopherol analysis. The oil yield was calculated based on the amount of oil extracted and the weight of peanut paste for oil extraction. Both oil extraction and concentration were conducted under dim light.

2.4 Extraction and Purification of B Vitamins

The precipitates from oil extraction were dried in a 285A Isotemp vacuum oven (Fisher Scientific (Suwanee, GA, USA) at 40 ℃ for 3 hours to obtain the defatted peanut flour, which were stored at 4 ℃ for the B vitamins extraction. The method described by Woollard & Indyk (2002) was used to extract B vitamins from peanut flour. The defatted peanut flour (1.00 g) was suspended in 5 ml warm deionized water (37 ℃), and then 10 ml of 0.3 M TCA was added. The sample was shaken in a GmbH SW22 water bath (Julabo, Seelbach, Germany) for 15 minutes at 37 ℃, and then centrifuged at 3000g for 10 minutes. The supernatant was purified using Sep-Pak Vac solid-phase extraction (SPE) columns (3cc, C_{18}) where acidified water (pH 4.2) and methanol were used as mobile phases for elution. The elute (purified sample) was concentrated using a rotary evaporator at 40 ℃ to remove the methanol. The concentrated sample was transferred into an amber glass vial and stored at 4 ℃ for analysis within one week. For each peanut flour sample, the extraction was conducted in triplicate. The extraction, purification and concentration were conducted under dim light.

2.5 Determination of Tocopherols in Peanut Oils

For each peanut oil extract, 100mg sample was diluted with a mixture of hexane: methanol (20:80, v/v) to 1 ml. The sample was vortex mixed and filtered through a 0.22 μm glass fiber syringe filter into an amber HPLC vial and then directly injected into an HPLC system (Waters Corporation., Milford, MA) equipped with a UV-Vis Dual Wavelength Detector and a Florescence Detector. The tocopherols in the sample were separated on an Accucore XL C18 column (3 mm×150 mm, 4 μm), eluted with 96 % methanol in water (v/v) at 0.8 ml/min and the peak areas were detected by the UV-Vis detector at 230 nm. The tocopherols were identified by comparing the retention times of samples with the corresponding standards. The concentrations of tocopherols in the diluted oil samples were calculated using their calibration curves, and then converted to mg per 100 g peanuts according to dilution factor and oil yield. The standard curve of each tocopherol was obtained using a set of properly diluted standard solutions with concentrations of 1, 5, 10, 15, and 20 μg/ml under the same chromatography condition for the peanut oil samples. The total α-tocopherol in oil sample was the sum of α-tocopherol and α-tocopherol acetate. For each peanut oil extract, the analysis was conducted in triplicate.

2.6 Determination of B Vitamins

The purified B vitamin extracts were quantitatively diluted using 0.3 M TCA solution. An aliquot of the sample was filtered through a 0.22 μm syringe filter and analyzed by a Waters HPLC system. The B vitamins were separated by a Kinetex C_{18} column (150 × 4.6 mm, 5 μm) and eluted with mobile phase isocratically at 1 ml/min. The mobile phase for thiamine (B1) elution was prepared by dissolving 1.00 g of sodium dioctylsulfosuccinate in 550 ml methanol containing 10 ml of concentrated formic acid. The solution was adjusted pH to 4.4 with 50 % KOH solution, and then diluted to 1 L using DI water. The mobile phase for riboflavin (B2), nicotinic acid, nicotinamide, and pyridoxine (B6) elution was prepared by dissolving 1.00 g of sodium dioctylsulfosuccinate in 250 ml methanol containing 10 ml of concentrated formic acid, the solution was adjusted pH to 2.8, and then diluted to 1 L with DI water. The B1 was detected by the UV-Vis detector at 254 nm, while the B2, nicotinic acid, nicotinamide, and B6 were detected by the Fluorescence detector at the excitation wavelength of 290 nm and the emission wavelength of 390 nm. The standard curve of each B vitamin was obtained using a set of properly diluted standard solutions of the B vitamin. The concentration ranges of B1, B2 and B6 were 1-25 μg/ml, 0.025-0.025 μg/ml, and 1-100 μg/ml, respectively. Vitamin B3 includes nicotinic acid and nicotinamide, and their concentrations for standard curve development were 1-12 μg/ml and 1-100 μg/ml, respectively. The concentration of each B vitamin in the extract was calculated by using the peak area and the corresponding calibration equation, and then converted to mg per 100 g peanuts according to the dilution factor, extract volume and sample weight.

2.7 Recovery Test

For the tocopherols, a known amount of a specific tocopherol standard was added to 100 mg of peanut oil with known concentration of the tocopherol. The mixture was then diluted to 1 ml with hexane: methanol (20:80, v/v) and the tocopherol concentration was determined as described in subsection 2.5. For the B vitamins, 1.000 g of

the defatted peanut flour was suspended in 5 ml warm distilled water at a temperature of 37 ℃ in an Elementary flask and a known amount of a specific B vitamin standard was added to the suspension under constant stirring, and then stored at 4 ℃ overnight with stopper on. The sample was warmed to 37 ℃. The vitamin was extracted and purified as described in subsection 2.4, and then analyzed by HPLC, as described in subsection 2.6. The recovery rate for each vitamin was calculated by the equation below and used to adjust the content the specific vitamin in the peanut oil sample. For each vitamin, the recovery experiment was conducted in triplicate.

$$\text{Recovery } (\%) = (Ct - Cp) * 100 / (Ca + Cp) \quad (1)$$

Where Ct - the total vitamin content determined, Cp – the vitamin content in peanut oil or flour, Ca – the amount of vitamin added.

2.8 Data Analysis

The results were expressed as mean ± standard deviation and statistically analyzed using ANOVA using SAS version 9.4 (SAS Institute, Cary, NC, USA). The means were compared using Duncan Multiple Range tests with a significance level of <0.05.

3. Results

3.1 Oil Yield of Extraction Method

The oil yield was used as an indicator to evaluate whether the oil was completely extracted from peanuts because the tocopherol content of peanut was determined using extracted oil. Table 2 shows that oil yields of untreated and treated peanuts were in the range of 48-52% which indicate that the oil in peanut samples was completely extracted by the method and procedure used in this study because the crude fat content of raw peanuts (all types) is reported to be 49.24±0.30% (USDA, 2015).

Table 2. Oil yield of protease treated and untreated peanut samples

	Oil Yield (%)				
Treatment	Untreated	Alcalase	Bromelain	Neutrase	Papain
UNT	50.81	-	-	-	-
UNT-VD	48.00	-	-	-	-
T1	-	48.60	51.35	48.75	49.34
T2	-	49.65	49.82	49.39	50.09
T3	-	50.01	49.35	50.47	51.46
T4	-	51.30	51.24	48.02	52.00
T5	-	50.96	51.42	49.67	50.15

Note: UNT: untreated raw peanuts; UNT-VD: untreated vacuum dried raw peanut; T1, T2, T3, T4 and T5-represent 5 different protease concentrations.

3.2 Recovery of Tocopherols and B Vitamins during Extraction, Purification, and Analysis

The recovery tests were conducted to validate the vitamin extraction, purification, and HPLC methods used in the study to ensure the accurate quantification. The mean recoveries for α-, α_A-, γ-, and δ-tocopherols added to the peanut oil sample was 106%, 206.61%, 111%, and 116%, respectively, while the mean recoveries for thiamine (B1), riboflavin (B2), niacin (B3) and pyridoxine (B6) added to the peanut flour samples were 105%, 108%, 105%, and 96%, respectively. This indicates that the contents of vitamins with exception of pyridoxine in the peanuts were overestimated and adjustments were needed. The concentrations of each vitamin in the peanuts reported in this study were adjusted with the recovery rate of the specific vitamin.

3.3 Effect of Alcalase Treatment on the Contents of Tocopherols and B Vitamins in Raw Peanuts

Figure 1 shows that α-T and B3 were the richest tocopherol and B vitamin in peanuts and Alcalase treatment resulted in significant reductions of all tocopherols and B vitamins in enzyme concentration-dependent manner ($P<0.0001$). Figure 1A shows that α-T and ɣ -T were significantly reduced compared to the untreated at lower Alcalase concentrations (T1 - T3); increasing Alcalase concentration from T3 to T5 resulted in further reduction in α-T and δ-T ($P<0.05$) but not ɣ-T contents. Figure 1B shows that the contents of all tested B vitamins in peanuts were significantly reduced at low Alcalase concentration T1 ($P<0.0001$), but the effects of Alcalase concentration on B1, B2, B3 and B6 varied. The B6 content decreased almost linearly with increasing Alcalase concentration ($R^2 = 0.939$, $P<0.05$), while the changes of B1, B2 and B3 with Alcalase concentration were nonlinear. The B1 contents were not significantly different at enzyme concentrations T1, T2 and T3, but were

significantly lower at T4 and T5 ($P<0.05$). The B2 content was not affected until Alcalase concentration T3 ($P<0.05$).

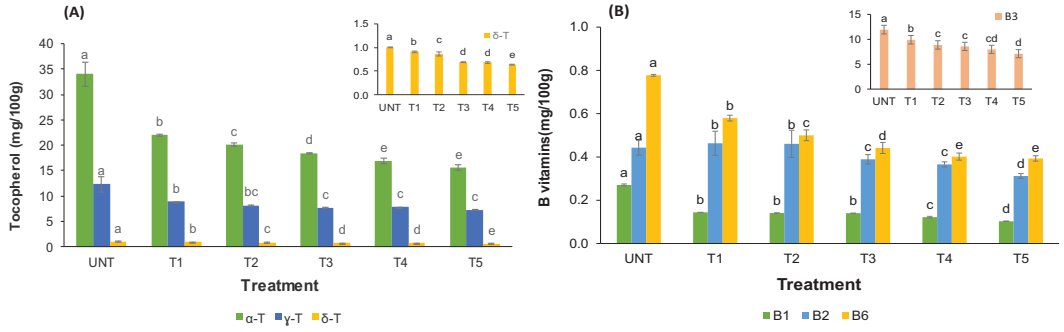

Figure 1. Effects of Alcalase treatment on concentrations of Tocopherols (A) and B-vitamins (B) in raw peanuts. Different letters on the top of data bars for the same vitamin indicate significant differences ($P<0.05$, n=3). UNT: untreated sample, T1-T5: Alcalase concentrations (T1-1%, T2-2%, T3-3%, T4-3.5% and T5-4%)

3.4 Effect of Bromelain Treatment on the Contents of Tocopherols and B Vitamins in Raw Peanuts

Figure 2A shows that the treatment of raw peanuts with bromelain resulted in a significant decrease in the α-T content at all enzyme concentrations, and the reduction of α-T increased with increasing bromelain concentration ($P<0.0001$). Although bromelain treatment also decreased ɣ-T and δ-T contents in the peanuts, particularly at high enzyme concentration (T5) ($P<0.05$), the influence was much smaller than that on α-tocopherol within the range of bromelain concentration used in this study. Figure 2B shows that the contents of all tested B vitamins were significantly reduced at low bromelain concentration (T1) compared with those in untreated samples ($P<0.0001$). However, increasing bromelain concentration from T1 to T3 did not significantly increase B vitamin loss with exception of B2. Slight but statistically significant reductions of B1, B2, B6 and B3 contents were observed as enzyme concentration increased to T4 and T5 ($P<0.05$). Data in Figure 2 indicate that the effects of bromelain treatment on tocopherol and B vitamins are limited with exceptions of α-tocopherol and vitamin B1.

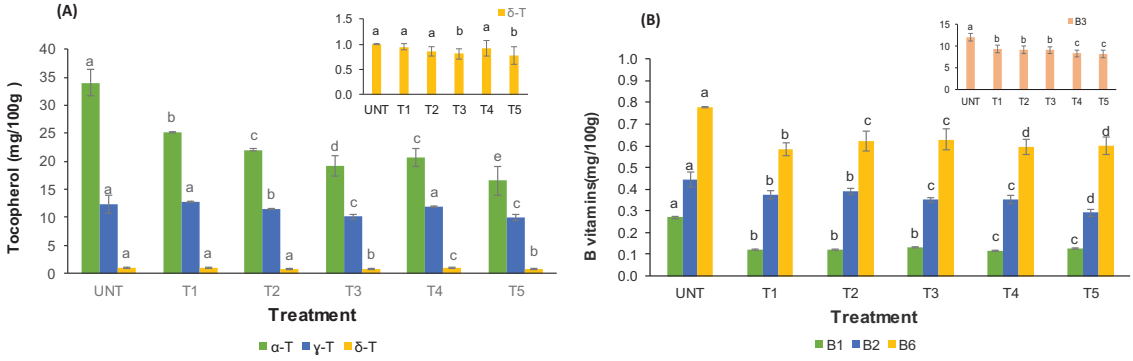

Figure 2. Effects of bromelain treatment on concentrations of Tocopherols (A) and B-vitamins (B) in raw peanuts. Different letters on the top of data bars for the same vitamin indicate significant difference ($P<0.05$, n=3). UNT: untreated sample, T1-T5: bromelain concentrations (T1-0.02%, T2-0.04%, T3-0.08%, T4-0.16% and T5-0.20%)

3.5 Effect of Neutrase Treatment on the Contents of Tocopherols and B Vitamins in Raw Peanuts

Figure 3A shows that at low concentration T1 Neutrase treatment resulted in significant reduction of α-T and γ-T contents in the peanuts ($P<0.0001$); there was no significant difference in α-T among samples treated at Neutrase concentrations T1, T2, and T3; but further reduction of α-T was observed when enzyme concentration increased to T4 and T5 ($P<0.05$). Similar changes of γ-T with Neutrase level were observed. The δ-T contents of the peanuts treated at lower Neutrase concentrations (T1-T3) were significantly higher than that of untreated

(P<0.05), but it decreased as Neutrase concentration increasing. The δ-T content at T4 and T5 was slightly but significantly lower than that of untreated (P<0.05). Figure 3B depicts that Neutrase treatment of peanuts resulted in significant reduction in B1, B2, B3, and B6 contents (P<0.0001). Similar to the bromelain treatment, significant loss of B vitamins with exception of B2 was observed at low Neutrase concentration (T1) (P<0.05). There was no further reduction in B1 and B3 when the enzyme concentration increased from T1 to T3, but further reductions of B1 and B3 were observed at T4 and T5 (P<0.05). The significant reduction of B2 content was observed at T2 and higher Neutrase concentration. B6 content was significantly reduced at T1 but remained constant until T5.

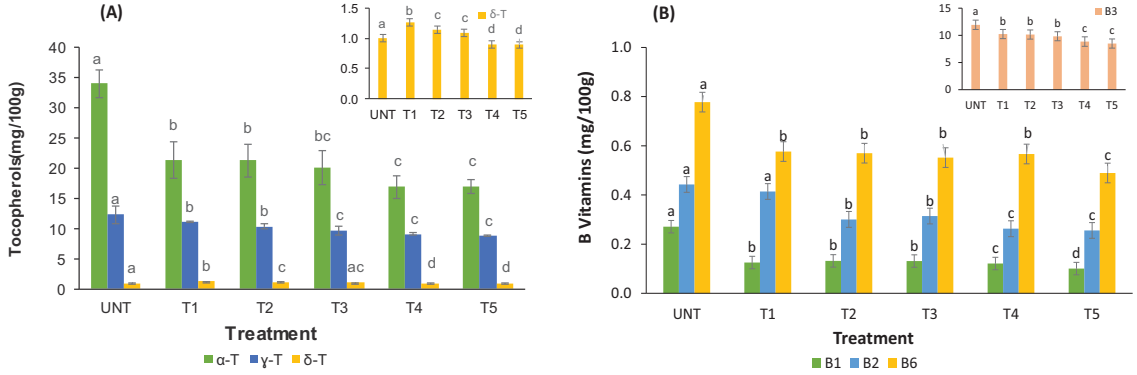

Figure 3. Effects of Neutrase treatment on concentrations of Tocopherols (A) and B-vitamins (B) in raw peanuts. Different letters on the top of data bars for the same vitamin indicate significant differences (P<0.05, n=3). UNT: untreated sample, T1-T5: Neutrase concentrations (T1-02%, T2-0.4%, T3-0.8%, T4-1.2%, T5-1.6%)

3.6 Effect of Papain Treatment on the Contents of Tocopherols and B Vitamins in Raw Peanuts

Figure 4A exhibits that the papain treatment of peanuts had significant impacts on the contents of all tocopherols compared with untreated (P<0.0001). The α-T content decreased with increasing papain concentration but was not significantly different at enzyme concentrations T2 and T3. The content of γ-T was not reduced until papain concentration T2, and then remained unchanged until T5 (P<0.05). Similar to Neutrase treated peanuts, the δ-T content increased significantly (P<0.05) at T1, then gradually decreased with increasing papain concentration, and was lower than that of untreated peanuts at T5 (P<0.05). Figure 4B shows that papain treatment significantly reduced the contents of all B vitamins in peanuts at low papain concentration T1 (P<0.0001). The B1 content decreased significantly with increasing papain concentration. However, B2 and B3 were not significantly reduced until papain concentration T4. Although B6 was significantly reduced at T1, its level remained constant until T5. Therefore, the effects of papain concentration on B2, B3, and B6 were not as strong as those on B1.

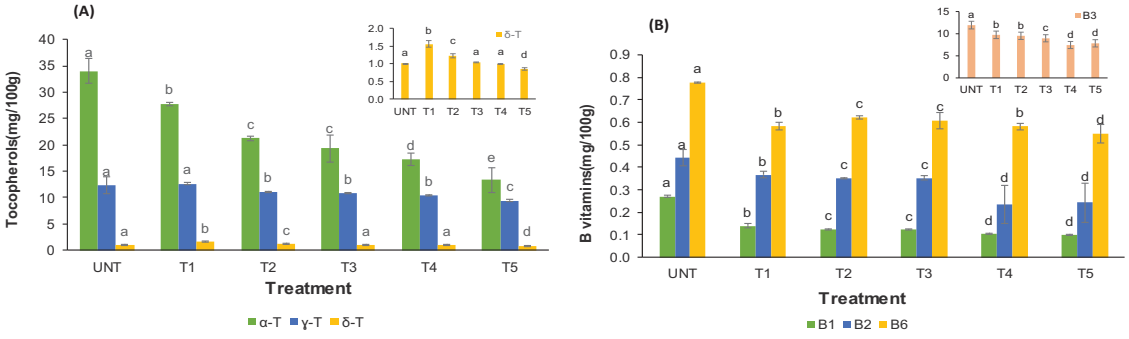

Figure 4. Effects of papain treatment on concentrations of Tocopherols (A) and B-vitamins (B) in raw peanuts. Different letters on the top of data bars for the same vitamin indicate significant differences (P<0.05, n=3). UNT: untreated sample (control), T1-T5: papain concentrations (T1-0.1%, T2-0.2%, T3-0.3%, T4-0.4% and T5-0.5%)

3.7 Vitamin B3 Content of Raw Peanuts Treated with different Proteases

Vitamin B3 (niacin) consists of nicotinic acid and nicotinamide; therefore, the impacts of different protease

treatments on nicotinic acid and nicotinamide are given separately from other B vitamins. Table 3 shows that the nicotinamide, nicotinic acid, and total niacin content of untreated peanuts were 1.99, 9.94, and 11.94 mg/100g of peanuts, respectively. All enzyme treatments resulted in significant reductions of nicotinamide and nicotinic acid, thus the content of vitamin B3 ($P<0.0001$). Regardless of the type of protease, the B3 reduction increased with enzyme concentration ($P<0.05$). The treatment with Alcalase had the strongest impact on the B3 level, followed by papain, bromelain, and Neutrase.

Table 3. Nicotinamide, nicotinic acid, and total vitamin B3 contents of raw peanut treated with different proteases

Enzyme	Treatment	Enzyme conc. (%)	Nicotinamid mg/100g Peanuts	Nicotinic acid mg/100g Peanuts	Total B3 vitamins mg/100g Peanuts
Alcalase	UNT*	0	1.99 ± 0.05^{a}	9.94 ± 0.79^{a}	11.94 ± 0.78^{a}
	T1	1.0	1.66 ± 0.08^{b}	8.25 ± 0.13^{b}	9.92 ± 0.13^{b}
	T2	2.0	1.52 ± 0.02^{c}	7.34 ± 0.27^{bc}	8.87 ± 0.27^{c}
	T3	3.0	1.52 ± 0.02^{c}	7.04 ± 0.08^{c}	8.57 ± 0.09^{c}
	T4	3.5	1.12 ± 0.01^{d}	6.86 ± 0.55^{c}	7.98 ± 0.55^{cd}
	T5	4.0	0.94 ± 0.01^{e}	6.15 ± 0.49^{c}	7.07 ± 0.48^{d}
Bromelain	T1	0.02	1.43 ± 0.013^{b}	7.89 ± 0.34^{bc}	9.32 ± 0.33^{b}
	T2	0.04	1.40 ± 0.011^{b}	7.75 ± 0.46^{c}	9.16 ± 0.46^{b}
	T3	0.08	1.48 ± 0.032^{c}	7.54 ± 0.11^{c}	9.02 ± 0.13^{b}
	T4	0.16	1.30 ± 0.008^{d}	6.95 ± 0.27^{c}	8.25 ± 0.27^{c}
	T5	0.20	1.19 ± 0.005^{e}	6.94 ± 0.26^{c}	8.13 ± 0.26^{c}
Neutrase	T1	0.2	1.62 ± 0.007^{b}	8.62 ± 0.47^{b}	10.24 ± 0.47^{b}
	T2	0.4	1.62 ± 0.045^{b}	8.52 ± 0.47^{b}	10.14 ± 0.46^{b}
	T3	0.8	1.52 ± 0.023^{c}	8.30 ± 0.45^{bc}	9.83 ± 0.48^{b}
	T4	1.2	1.15 ± 0.043^{d}	7.69 ± 0.32^{bc}	8.85 ± 0.30^{c}
	T5	1.6	1.21 ± 0.005^{e}	7.28 ± 0.33^{c}	8.49 ± 0.33^{c}
Papain	T1	0.1	1.53 ± 0.005^{b}	8.21 ± 0.48^{bc}	9.75 ± 0.48^{b}
	T2	0.2	1.54 ± 0.070^{b}	7.99 ± 0.47^{bc}	9.54 ± 0.43^{b}
	T3	0.3	1.48 ± 0.045^{b}	7.47 ± 0.24^{c}	8.95 ± 0.21^{c}
	T4	0.4	1.17 ± 0.013^{c}	6.26 ± 0.33^{d}	7.44 ± 0.33^{d}
	T5	0.5	1.26 ± 0.018^{d}	6.56 ± 0.21^{dc}	7.82 ± 0.21^{d}

UNT*: untreated peanut. For a specific protease, data with different superscripts in the same column are significantly different at $P<0.05$.

3.8 Percentage Loss of Vitamins in Raw Peanuts Due to Protease Treatments

Table 4 shows that the loss of extractable vitamins in peanuts caused by protease treatments varied with the type and concentration of protease, as well as type of vitamin. Overall, higher protease concentration resulted in a larger loss of all vitamins. Among all tocopherols, α-T was the most affected, and δ-T was the least affected. Neutrase and papain treatments increased δ-T at lower enzyme concentrations (T1 to T3) but decreased δ-T at higher enzyme concentrations (T4 and T5). The highest losses of α-, γ-, and δ-tocopherols were 60.87%, 40.60% and 36.89%, respectively. Among B vitamins, B1 (Thiamine), was the most affected by Neutrase followed by Papain and Alcalase, whereas B2 was the most affected by papain but the least affected by Alcalase. Niacin (B3) was the most affected by Alcalase but least affected by Neutrase. B6 was the most affected by Alcalase but least affected by bromelain. The maximum losses of vitamins B1, B2, B3 and B6 were 63.29 %, 44.83 %, 40.56 % and 49.59 %, respectively. As part of protease treatment approach, vacuum drying reduced α-T by 23.9%, but increased ɣ-T and δ-T by 21.46% and 110.86%. The reductions of vitamin B1, B2, B3 and B6 due to drying were 21.04%, -2.82%, 9.90% and 10.10%, respectively. Thereby, the changes of tocopherols and B-vitamins include the changes caused by enzyme treatment and drying thereafter.

Table 4. Percentage losses of tocopherols and B vitamins after treatment with four different proteases

Protease	Treatment	Enzyme Conc. (%)	Loss of tocopherols and B vitamins (%)						
			α-T	γ-T	δ-T	B1	B2	B3	B6
	UNT-VD*	0.00	23.92	**-21.46**	**-110.86**	21.04	**-2.82**	11.84	10.10
Alcalase	T1	1.00	35.39	28.06	9.40	46.70	**-5.26**	16.95	25.69
	T2	2.00	40.99	33.95	13.80	47.79	**-4.63**	25.74	35.91
	T3	3.00	46.01	37.98	31.36	48.16	11.56	28.25	43.41
	T4	3.50	50.17	36.78	31.87	54.91	16.91	33.17	48.52
	T5	4.00	54.17	40.60	36.89	61.86	29.13	40.56	49.59
Bromelain	T1	0.02	25.90	**-3.63**	5.52	54.17	14.75	21.91	24.89
	T2	0.04	35.28	7.26	14.53	55.18	11.17	23.26	20.29
	T3	0.08	43.62	18.23	19.72	51.51	20.33	24.42	19.42
	T4	0.16	39.48	3.26	8.60	56.46	20.19	30.88	23.62
	T5	0.20	51.51	19.18	23.67	52.89	33.77	31.88	23.11
Neutrase	T1	0.20	37.43	9.81	**-26.03**	55.86	10.49	14.27	29.66
	T2	0.40	37.36	15.56	**-14.09**	52.33	33.38	15.07	28.72
	T3	0.80	41.07	21.41	**-8.54**	50.43	27.26	17.68	27.92
	T4	1.20	50.10	25.90	9.63	58.59	44.83	25.88	32.88
	T5	1.60	50.15	28.25	11.13	63.29	42.63	28.84	38.06
Papain	T1	0.10	18.50	**-2.51**	**-55.40**	49.89	18.70	18.37	27.34
	T2	0.20	37.59	9.91	**-21.71**	53.96	19.68	20.12	19.90
	T3	0.30	43.09	11.72	**-3.21**	51.62	15.26	25.00	17.38
	T4	0.40	49.41	15.33	0.22	57.58	42.19	37.69	19.14
	T5	0.50	60.87	23.48	15.47	62.71	44.42	34.51	28.90

*UNT-VD: Untreated, vacuum dried peanuts.

Bold data: The negative values indicate increased detectable vitamin due to drying or protease treatment at low enzyme concentrations compared with untreated raw peanut sample.

4. Discussion

4.1 The Effects of Protease Treatments on the Contents of Vitamins in Raw Peanuts

In this study, three main tocopherols identified in raw peanuts were α-, γ-, and δ- tocopherols. Their contents in the raw untreated peanuts (UNT) detected by HPLC were 34.02, 12.32 and 1.0 mg/100g, respectively, in the order of α-T> γ-T > δ-T, suggesting that the α-T is the dominant tocopherol in peanut. The contents of these tocopherols in untreated raw peanuts are in the range reported by Ahmed & Young (1982). The concentrations of vitamins B1, B2, B3, and B6 in the untreated raw peanuts (UNT) were 0.27, 0.44, 11.88, and 0.78 mg/100g, respectively, within the ranges reported by Ahmed & Young (1982) but were lower than those reported in another study (King, Blumberg, Ingwersen, Jenab, & Tucker, 2008). This might be due to difference in peanut cultivar, growing location, harvest time, processing etc. (Pattee, Isleib, Giesbrecht, & McFeeters, 2000).

The influence of protease treatment on the content each vitamin varied with the type of enzyme, enzyme concentration, and individual vitamin stability (Figure 1-4). High protease concentrations resulted in larger loss of tocopherols and B vitamins although some vitamins were less affected by protease concentration (Table 4). The highest tocopherol loss occurred at Alcalase concentration 3.5-4% where highest allergen reduction and lowest *in vitro* allergenicity were achieved (Yu & Mikiashvili, 2020). The extent of tocopherol reduction in the peanuts due to proteolytic hydrolysis was in the order of α-T > γ-T > δ-T. The higher loss of α-T is because α-tocopherol is the most reactive and less stable due to its lower bond dissociation energies (Silva et al., 2010; Keen & Hassan, 2016; Shahidi & Costa de Camargo, 2016; Decker & Elias, 2016). Some studies about the effects of enzyme treatment on tocopherol content of oil seeds presented contradict results. For example, one study found that enzymatic aqueous (Alcalase 2.4 L) extraction of sunflower seeds resulted in similar oil yield but lower total tocopherol content in the oil than common cold press (Ribeiro et al., 2016); while another study found that the enzyme-assisted oil extraction with a mixture of α-amylase, Alcalase and Celluclast increased tocopherol content in the tiger nut oil (Ezeh et al., 2016). Due the differences in food matrix and enzyme treatment condition, it is difficult to compare the results of our study with the results of other studies. Because protease treatment of peanuts was conducted in the water-based enzyme solution and B vitamins are water-soluble, significant losses in B vitamins due to dissolution/leaching were expected. For example, the loss

of B vitamins in vegetables by steaming is much lower than by cooking in water (Hwang et al., 2012; Veda et al., 2006).

The losses of vitamins during enzyme treatment is most likely caused by dissolution and protease treatment could enhance vitamin dissolution because the breakdown of protein molecules could create paths which allow vitamins leaching out from the inside of peanut kernels. In addition, the selectivity of protease used to treat peanuts might contribute to the differences in vitamin loss. Less selective protease has more cleave sites and will lead to more complete hydrolysis of proteins, thus more vitamin leaching. Among the four proteases used in this study, Alcalase is the least selective and can hydrolyze most of the peptide bonds (Yu & Mikiashvili, 2020). This could explain the higher loss of B-vitamins in Alcalase treated peanuts (Table 4). Furthermore, the difference in treatment pH may be one of the contributing factors of B vitamin loss because the stability of B vitamins is affected by the pH of the food system. For instance, vitamin B1 is unstable in alkaline medium, B6 is more stable in acidic medium than in alkaline medium, while vitamin B2 and B3 are stable in both media (Gadient, 1986; Saidi & Warthesen, 1983). The optimal pHs of Alcalase, bromelain, Neutrase, and papain are 7.5, 6.0, 7.0, and 7.0, respectively, and peanuts were treated at the optimal pH for each enzyme. This may partially explain the higher loss of vitamin B1 caused by Alcalase treatment and lower loss of B2, B3 and B6 caused by bromelain and papain treatment.

4.2 Effect of Post-enzyme Treatment Drying on Vitamin Content in Peanuts

In food industry, heating oil seeds prior to oil extraction is usually used to increase oil yield, but it can also induce changes to various components of the seed (Kraljić et al., 2018). Some studies found that heat treatment such as blanching and roasting caused loss of α-tocopherol (Camargo et al., 2016; Shi et al., 2018), while other studies found 14 and 11 % increase of γ-, and δ- tocopherol levels in peanuts after roasting at 140 °C for 20 min (Eitenmiller et al., 2011). Air-drying or microwave heating increased α-, β- and γ-tocopherol, but decreased δ-tocopherol contents of grapeseed (Oomah et al., 1998). Roasting corn germ for 60 min at 125-175°C or microwave irradiation at 40-80W for 4-8 min also significantly increased the oil yield and α-, β- and γ-tocopherol contents but did not change δ- tocopherol content in the corn oil (Zheng et al., 2018). It was reported that roasting decreased the content of thiamine in tree nuts such as almond, walnut, and hazelnut, but minimally affected riboflavin content (Stuetz et al., 2017). A recent study also found that riboflavin was heat-stable while the other vitamins were heat-labile (Tylicki et al., 2018). This study shows significant loss of α-tocopherol and B vitamins, as well as the increase of γ- and δ- tocopherols caused by vacuum drying. These examples suggest that the influence of heat treatment on tocopherols and B vitamins of oil seed varies with the food matrix, temperature and treatment time. Thus, the increase γ- and δ- tocopherols in enzyme-treated peanuts might be caused by drying at low temperature (75°C) which might increase the extractability of these tocopherols. Because drying was part of the treatment in this study, and the total loss of tocopherols and B-vitamins should be the sum of the loss during enzyme treatment and the loss/gain caused by vacuum drying.

4.3 The Effect of Protease Treatment on Vitamin Intake from Peanuts

The recommended daily allowance (RDA) of vitamin E for both male and female adults aged 14 years and above 15 mg (NIH, 2020). The levels of α-tocopherol and total vitamin E in the peanuts treated with Alcalase, bromelain, Neutrase, and papain at their highest concentrations (T5) used in this study were 15.64, 16.54, 17.00, and 13.35 mg/100g peanuts, and 23.54, 27.22, 26.69, and 23.59 mg/100g peanuts, respectively. Consuming one serving (28 g) of enzyme-treated peanut per day can provide more than 1/3 of daily recommended vitamin E intake. Therefore, even though the treatments of peanuts reduced the contents of tocopherols, the treated peanuts still a good source of vitamin E.

Similarly, the RDA of vitamin B1, B2, B3 and B6 for adults are 1.1-1.2, 1.0-1.3, 14-16, 1.2-1.3 mg (NIH, 2020). At the protease concentrations where highest allergen reduction was achieved, the remaining vitamin B1, B2, B3 and B6 contents in peanuts were 0.12, 0.37, 10.02 and 0.40 mg/100g, respectively. This shows that consumption of 3 servings (approximately 100 g) of peanuts treated with enzymes may provide 10-12 % of RDA of thiamine, 21-33 % RDA of riboflavin, 71-76 % RDA of niacin and 31-48 % of pyridoxine. These values, except thiamine, are consistent with the RDA equivalent per 100 g of untreated peanuts reported in the USDA nutrient composition database (Arya et al., 2016). Human beings usually obtain their B vitamins from grain, meat and eggs, while vegans may need consume more nuts or take B vitamin supplement to meet the dietary requirement of B vitamin intake.

5. Conclusion

In conclusion, this study demonstrates that peanut allergen reduction approach using non-specific proteases (including enzyme treatment and drying) resulted in significant losses of tocopherols and B vitamins although

the treatment greatly reduced the allergen content and immunoreactivity of peanuts as shown in our previous studies (Yu & Mikiashvili, 2020). The degree of vitamin loss was influenced by the type of enzyme, enzyme concentration, and individual vitamin stability. Overall, papain and Alcalase treatments had larger impacts on tocopherols than bromelain and Neutrase. The α-tocopherol was the most affected among all tocopherols due to its instability, while δ-tocopherol was the least affected. Among all B vitamins investigated, vitamin B1 was the most affected and B3 was the least affected. Higher enzyme concentration resulted in a more significant loss of each vitamin. However, the enzyme treated peanuts is still a good source of tocopherols and vitamin B3 comparing to most cooked legumes (Margier et al., 2018) and vegetables (Knecht et al., 2015). Consuming one serving (28 g) of enzyme-treated peanut per day can provide more than 1/3 of daily recommended vitamin E intake and 20-25% of vitamin B3 intake. Vitamin enrichment may not be required if treated peanuts are consumed with other food products rich in B vitamins, but the enrichment may be necessary if the enzyme treated peanuts are used to combat malnutrition.

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Conflicts of Interest

The authors declare no conflict of interest.

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Changes in the Physicochemical Properties of Kashkaval of Pindos Cheese Produced with Different Salting Methods during Ripening

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Abstract

Kashkaval of Pindos cheeses were produced either with sheep (100%) or mixture of sheep (90%) - goat (10%) milk. Sheep milk cheeses were manufactured either by dry salting or by immersion in 18% w/w salt (NaCl) brine. Cheeses made with the mixture of sheep- goat milk were directly immersed in brine of 15% or 18% w/w NaCl concentration. These are common practices of cheese salting used by the traditional cheese-makers. The physicochemical characteristics of all cheeses were monitored during a ripening period of 3-months. The results have shown that the physicochemical characteristics of both cheeses were not affected by the different salting methods. Therefore, Kashkaval of Pindos cheese can be salted either with dry salt or with immersion in brine, without altering their main composition and organoleptic characteristics. Furthermore, some historical data about Kashkaval of Pindos cheese are included.

Keywords: traditional, cheese, Kashkaval of Pindos, salting

1. Introduction

The endurance of rural human population in less favoured areas assures the safeguard and survival of biodiversity and leads to the manufacture of traditional products. A traditional product is a result of several factors such as raw material, transformation process and sensory characteristics (Scintu & Piredda, 2007). In this respect, it is of major importance to support the maintenance of the production of local cheeses. Greece has a wide variety of artisanal cheeses, each one with its own distinctive texture, flavour and aroma; characteristics that clearly reflect their terroir of production. Kashkaval of Pindos is a traditional paste filata cheese produced seasonally in the mountains of Pindos as a farmhouse product. Nowadays, there is an increasing demand by consumers for this local cheese. However, in order to produce a cheese with consistent physicochemical characteristics in respect to its traditional character, it is necessary to study the various technological parameters that are commonly used by different cheesemakers, on farm level. It is known that in traditional production of this type of cheese, salt can be added by either dry salting or direct immersion of cheese in brine (Pejic, 1956; Scott, 1981; Kindstedt et al., 2004).

The traditional cheese-making technology of Kashkaval cheese has been recorded since the beginning of the previous century (Dimitriadis, 1900; Liambeis, 1900; Polychroniadis, 1912) and the biochemical characteristics of the artisanal cheese have been studied before (Pappa et al., 2019; Samelis et al., 2019). The aim of the present work was a) to study the effect of the salting methods that are commonly used in its manufacture by the different cheese-makers and b) to provide some historical data regarding the Kashkaval of Pindos cheese. Therefore, in the present study, the physicochemical characteristics of Kashkaval of Pindos cheese manufactured with sheep milk and salted with two different methods: dry salting or immersion in brine (18% w/w), at different ripening dates were studied. Moreover, the physico-chemical parameters of Kashkaval of Pindos cheese manufactured with mixture of sheep (90%)-goat (10%) milk and salted by direct immersion in brine with concentration 15% or 18% w/w were assessed during ripening.

2. Materials and Methods

2.1 Cheesemaking

Kashkaval of Pindos cheeses were produced at the pilot plant of Dairy Research Department, Institute of Technology of Agricultural Products, using 100% sheep (Sh-K) or mixture of 90% sheep-10% goat (mix-K) milk following the procedure as described elsewhere (Pappa et al., 2020) and presented in Table 1.

Table 1. Protocol of Kashkaval of Pindos manufacture

Step	Description
1	Milk 100% sheep milk (Sh-K) or mixture of 90% sheep and 10% goat milk (mix-K) was filtered
2	Pasteurization Milk was heated at 63^{o}C for 30 min and then cooled at 37^{o}C
3	Starter culture addition Addition of freeze-dried and direct to vat set starter culture to milk (*Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus,* RSF-736, Hansen)
4	Coagulation Addition of $CaCl_2$ (20g/100 kg) and rennet (10.000 strength, NATUREN Extra NB, Hansen) to milk (milk temperature: 35^{o}C, coagulation time: 40 min, rennet quantity: according to instructions)
5	Cutting and cooking of the curd size of curd pieces: 6-8mm; rate of temperature scalding-up: 1^{o}C/ 3min until final temperature 42^{o}C and keeping at 42^{o}C, under gentle stirring, for 15 min
6	Curd forming and pressing Curd is placed at pierced stainless steel moulds containing a cheese cloth. Pressing with a weight of 4kg and after 30min curd was turned upside down in the mould and the weight was put again. This was repeated three times and then the weight was removed
7	Acidification of the curd The curd was left at 17^{o}C to ripen until the pH reached ~5.2 (next day)
8	Texturing of the curd
9	The acidified curd was cut into long thin slices which were immersed in stainless steel containers with hot water (~80^{o}C) and manipulated with a wooden stick until a homogenous compact texture is obtained (pasta filata)
10	Moulding of the curd The texturized curd was transferred to a cheese-table; it was kneaded by hand, moulded and transferred to 17^{o}C. The following day (3rd day from manufacture) the moulds were removed.
11	Salting of the cheese Half Sh-K cheeses were salted by immersing them in brine (18%) for 4 days at 17^{o}C, while the other half cheeses were dry salted (15g/10kg cheese). Cheeses dry salting and turning lasted for ten days (four salting took place). Half mix-K cheeses were immersed in brine 18%, while the other half cheeses in brine 15% for 4 days at 17^{o}C.
12	Ripening of the cheese The cheeses were transferred to 12^{o}C for maturation until they were 90-days-old

2.2 Analysis of Cheese

The milk for the cheesemaking was analyzed for physicochemical parameters, i.e. fat, protein, lactose, total solid by Milko-Scan, model 6000 (Foss Electric, Hillerød, Denmark). Milk pH was measured directly with a pH meter (Micro pH 2002; Crison, Barcelona, Spain). Microbiological evaluation was carried out by assessing the total viable counts (TVC) using the Bactoscan FC (Foss Electric, Hillerød, Denmark).

Cheese samples were examined for pH electrometrically (Micro pH 2002; Crison, Barcelona, Spain) and were analyzed for their fat according to the Gerber-Van Gulik method (Schneider, 1954), salt according to the modified Volhard method (Kosikowski, 1982) and moisture content by drying to constant weight at 102±1°C (International Dairy Federation, 1982). The fat-in-dry-matter (FDM) content was calculated by the formula: FDM% = fat% x 100/100- moisture%.

Cheeses were, also, assessed organoleptically by five trained panel members who were permanent staff of the

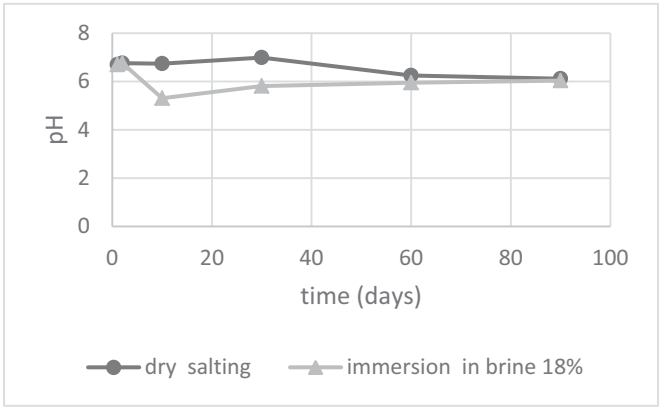
Dairy Research Department; all of them well experienced and familiar with pasta filata cheeses.

3. Results and Discussion

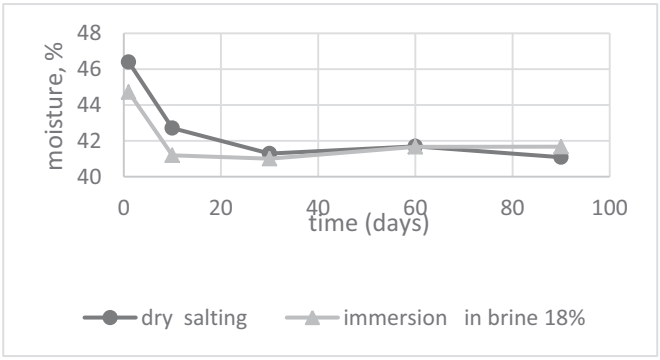
3.1 Effect of the Different Salting Methods on the Physicochemical Characteristics of Kashkaval of Pindos Cheese

The composition of the milk used for the manufacture of Kashkaval of Pindos cheese was fat 5.50%, protein 4.76%, lactose 4.99%, total solids 16.09% and its pH was 6.74 for the sheep milk (Sh-K) and 6.06%, 4.72%, 4.86%, 16.46% and 6.74 respectively for the mixture of 90% sheep and 10% goat milk (mix-K). The total viable counts of sheep milk were 4.79 log cfu/mL and of mixture of sheep-goat 5.88 log cfu/mL. The above results show that the milk used for the manufacture of cheeses was of good quality.

The physicochemical characteristics of Sh-K cheeses are presented in Figure 1 (a-d) and of mix-K cheeses in Figure 2 (a-d). The pH, moisture, fat and salt content of Kashkaval of Pindos cheese made with 100%sheep milk exhibited the same trend regardless the way of salting (i,e. dry salting or immersion in brine, at all sampling days (Figure 1 a-d). Also, cheeses manufactured using a mixture of 90%sheep -10%goat milk and salted with immersion in brine 15% or 18% showed similar pH, moisture, fat and salt content, at all sampling days (Figure 2 a-d). The results have shown that in general, at 30 days of ripening the physicochemical characteristics of Kashkaval of Pindos cheeses, regardless the type of salting method used, reached equilibrium. The physicochemical characteristics found in this study were in accordance with the data found by others (Kindsted et al., 2004; Alichanidis, & Polychroniadou, 2008; Pappa et al., 2019; Pappa et al., 2020) for pasta filata and Kashkaval cheeses.



(a)



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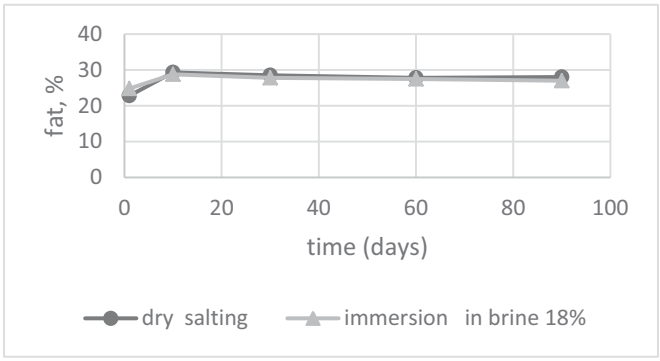

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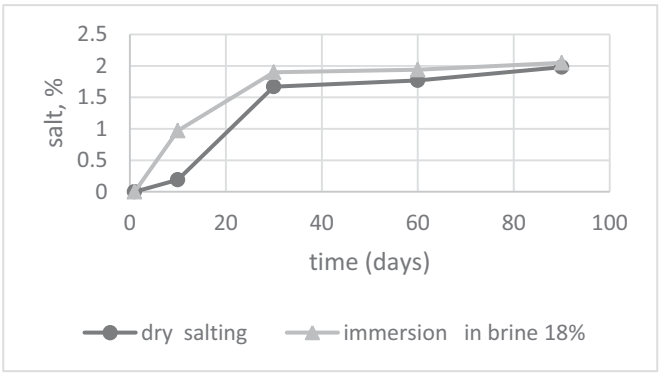
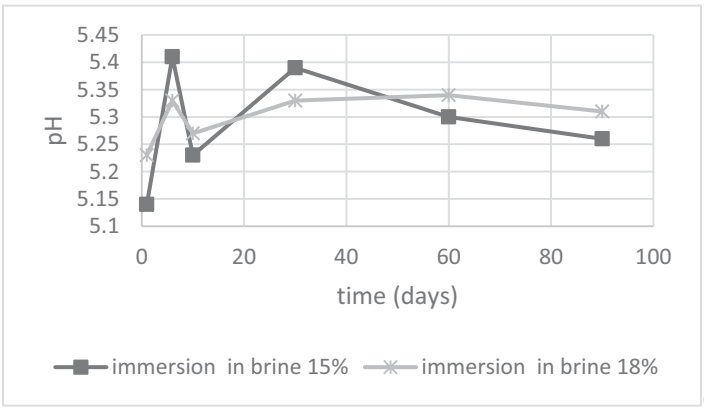
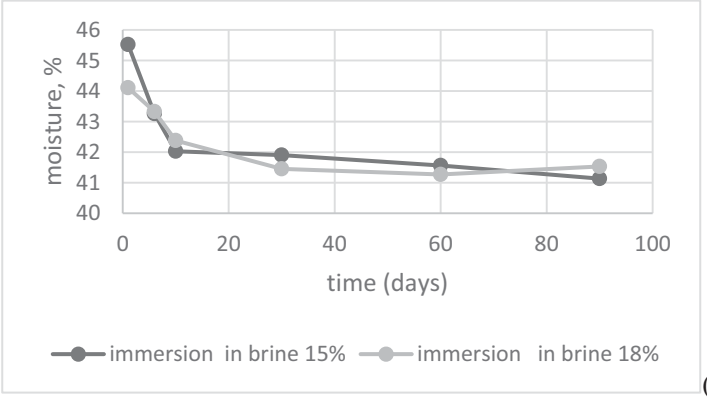


(d)

Figure 1. Effect of type of salting on the pH (a), moisture (b), fat (c) and salt content (d) of Kashkaval of Pindos cheese made with 100%sheep milk



(a)



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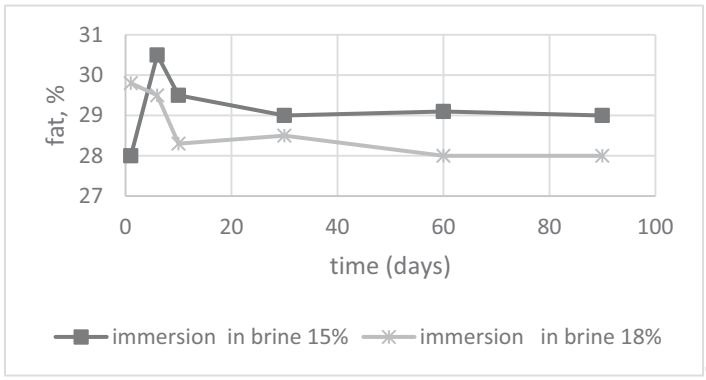

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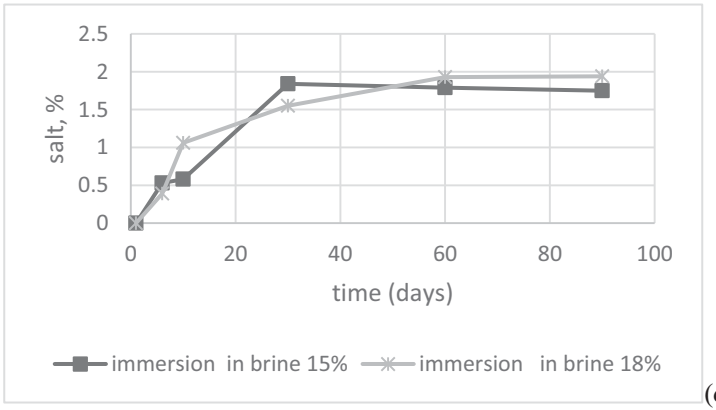

(d)

Figure 2. Effect of brine concentration on the pH (a), moisture (b), fat (c) and salt content (d) of Kashkaval of Pindos cheese made with mixture of 90%sheep -10%goat milk

Initially, there was a decrease in the pH values of the cheese due to the accumulation of lactic acid produced by the action of microorganisms to the residual lactose. Then, from day 1 to the end of salting there was an increase in the pH values and that could be attributed to the buffering effect of milk salts transferred in the cheese (Perna et al., 2014). Also, plasticization of the curd had a major influence on the composition, structure and ripening of Kashkaval cheese, as the cheese mass is washed to some extent, resulted in an increase of the pH values (Gobbetti et al., 2002). An increase in the pH values were also observed in Kashkaval cheese (Omar & El-Zayat, 1986).

The moisture content decreased until the end of salting time, probably due to the syneresis of cheeses (Pappa et al., 2006). The dry matter content (i.e. DM%= 100-moisture%) of all produced cheeses over 60 days-old ranged from 58.31-58.92% (data not shown).

Fat content of cheeses increased from day 1 until the end of salting. This trend is due to loss of moisture during the early stages of ripening. The FDM content of over 60-day cheeses regardless the type of milk or the salting method ranged from 47.14-49.79%. All Kashkaval of Pindos cheeses of this study fulfilled the requirements of regulation No 1225/90 of European Economic Community, i.e. fat-in-dry matter >45% and dry matter>58%.

The NaCl percentage of the Sh-K cheeses salted with the different methods, i.e. dry salting or immersion in 18% brine was similar (Figure 1d). Also, the salt content of mix-K cheeses salted with immersion in brine 18% or 15% did not differ (Figure 2d), especially at the end of ripening (90 days). Therefore, the different salting methods did not affect the salt content of the Sh-K and mix-K cheeses.

According to the Greek Codex Alimentarius (2014) Kashkaval of Pindos cheese can be sold in the market after 90 days of ripening. At that age, the organoleptic evaluation has shown that all cheeses in this study were very much appreciated by the five trained panel members. Their appearance was fairly yellow, with a smooth and thin rind and with occasionally technological slits. The texture was firm and elastic and the flavour was mild, with piquant, fruity and buttery notes. Panellists did not observe any difference regarding the salty taste of the cheeses produced with the different salting methods. No quality defects were found such as red or dirty white rind, gas holes etc as described by Caric (1993) for Kashkaval cheese and no goaty flavor was reported in the mix-K

cheeses.

3.2 Historical Data of Kashkaval of Pindos Cheese

Greece is a mountainous country and Pindos is its biggest mountain range with many picturesque villages such as Metsovo, Samarina, Syrako etc around it, with a long history in sheep and goat breeding and cheese making. The main agricultural activity of the nomadic and non-nomadic populations that lived for many years in the mountains of Pindos was sheep and goat breeding so as to provide milk, meat and wool for their families. In order to preserve milk for a longer time, they used to manufacture cheeses. Kashkaval of Pindos has been one of the most traditional and popular cheese during the centuries of the prevail of the Ottoman Empire in Greece. As this cheese belongs to pasta-filata group, its manufacture consists of two stages, the acidification of the curd after its production and the texturizing of the acidified curd with heating, kneading and stretching by soaking in hot water or in brine. Its manufacture was very popular especially during the hot summer. The coagulation and drainage of the curd was made in the mountains of Pindos to prolong the shelf-life of milk and then the drained cheese curds were gathered together and transported to the lowlands for further processing. Moreover, raw milk or milk with an increased acidity could be used for its production as during the texturizing of the acidified curd in hot water or brine (≈ 80^{o}C) a partial elimination or control of the pathogenic microflora could be achieved.

At that time, living conditions in Greece were very difficult, including unbearable taxes, robberies, killings etc. Residents of Pindos's villages were extremely familiar with the mountain trails and since the borders among the countries were not clearly demarcated, they managed to develop trade all over the Balkan Peninsula by travelling a lot with their horses, carrying various products such as wheat, oil, wool and cheese and selling them in markets. Furthermore, during the 18th century, population from the regions of Epirus and West Macedonia emigrated due to persecution or economic reasons to places such as Serbia, Austria-Hungary, Eastern Rumelia and others, exchanging with the locals, habits, culture, recipes, products etc. Based on written historical documents during the 19th century, merchants from villages in the mountains of Pindos (such as Metsovo) were commercialized in Venice, Instanbul, Alexandria, Odessa, Vienna, Boudapest, selling products such as barrels, wool textiles and Kashkaval cheese (Pouqueville, 1820; Leake, 1835; Berard, 1911; Wace & Thomson, 1914).

Mijacenic and Bulajic (2004) stated that Kashkaval cheese in the beginning was produced exclusively from sheep's milk during the grazing period, and nomads from Greece implemented the tradition of Kashkaval cheese on Stara Planina 100 years ago. Historical data showed that in the year 1903, 160 wagons of Kashkaval cheese were exported to Vienna and Budapest.

While in ancient times Kashkaval production was limited to the Greek and Roman empires as well as their colonies, nowadays it is produced in many parts of the world i.e. Crimea, South Ukraine, the Caucasus and Turkey, Greece, Italy, Bulgaria, Romania, Yugoslavia etc (Kindsted et al., 2004). In the Mediterranean region it is called simply Kashkaval with small differences in spelling. However, variations of that name can be found (Alichanidis & Polychroniadou, 2008). Kashkaval has also been given different commercial names according to the production district such as Pirdop in Bulgaria, Epir in Greece, Sarplaninski and Pirotski Kaskaval in Yugoslavia (Pejic, 1956). The Italian version is Caciocavallo and in Egypt the name Romy is commonly used.

4. Conclusions

As there is an increasing demand of Kashkaval of Pindos cheese, it is important to study the effect of the different salting methods (dry salt or immersion in brine 18% or 15%, w/w) which are commonly used by traditional cheese-makers. The results have shown that physicochemical characteristics of both cheeses were not affected by the different salting methods. Therefore, Kashkaval of Pindos cheese can be salted either with dry salt or with immersion in brine (15% or 18% concentration). However more work must be done in order to find if there is an effect of these two different methods on its level of proteolysis, lipolysis and its microbiological data.

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Nutrient and Anti-Nutrient Composition of Extruded Cereal Flours Fortified with Grain Amaranth, Baobab and Orange-fleshed Sweet Potato Powder

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Abstract

A majority of households in Sub-Saharan Africa utilize cereal-based flours in the preparation of most of their staples. However, the micronutrient contents of these cereal-based flours are low with higher levels of anti-nutrients. Food to food fortification is being used as an alternative to improve the micronutrient content of the cereal-based flours. This study sought to develop an extruded composite flour using sorghum and maize as the cereal base and baobab, grain amaranth and orange-fleshed sweet potatoes as the fortificants. A completely randomized design in factorial arrangement with ingredient ratio and extrusion as factors and seven levels was used to develop different formulations of the composites. Fortification of sorghum-maize cereal flour blends with amaranth, baobab and orange-fleshed sweet potato powder resulted in a significant ($p<0.05$) increase in the protein, beta-carotene, iron and zinc contents, on average, 8.99 ± 1.03 g/100g, 895.90 ± 346.85 mg/100g, 11.81 ± 9.73 mg/100g and 1.74 ± 0.18 mg/100g dry weight basis respectively. High grain amaranth levels in the formulations significantly ($p<0.05$) increased the phytate content whereas tannin content was significantly lower in low sorghum formulations. Conversely, extrusion of the composite flours significantly ($p<0.05$) reduced protein and beta-carotene contents by 4.7% and 40.9% respectively. Extrusion and its interaction with ingredient ratio significantly ($p<0.05$) affected the proximate, mineral and anti-nutrient composition of the composite flours. Both the fortificants and extrusion play a role in the reduction of anti-nutrients and therefore future studies should focus on other treatments that can be used together with extrusion to reduce anti-nutrients.

Keywords: Anti-nutrients, composite flours, extrusion, fortificant, micronutrient

1. Introduction

Food to food fortification is one of the nutrition interventions and strategies employed to alleviate micronutrient deficiencies in resource-poor countries (Chadare et al., 2019; De Groote et al., 2020). Micronutrient deficiency in Sub-Saharan Africa (SSA) is as high as 49% among households (Fraval et al., 2019). Harika et al., (2017) estimated that children below the age of five years had a 35-63%, 32-63% and 15-35% zinc, iron and vitamin A deficiencies respectively. 24% of Kenyan children below the age of five are stunted due to poverty and poor nutrition (Ndemwa et al., 2017). Milk, eggs, fish and meat are good sources of protein but their high costs affect their availability in most developing countries households hence the need to improve the nutrient composition of readily available cereals (Manary & Callaghan-Gillespie, 2020).

Considering that the diets of most of the communities in sub-Saharan Africa (SSA) are cereal-based (Ekpa et al., 2019; Van Ittersum et al., 2016), nutrients such as protein, iron, zinc, beta carotene and folate have been incorporated in them for delivery to the population. Fortification of wheat and maize flour has been necessitated by insufficient levels and limited bioavailability of these micronutrients (Aslam et al., 2018). The ever-evolving nature and diversified processing seeking to address various gaps in the nutritional and physicochemical quality preferences by various populations also necessitates fortification of these cereal flours (Asaam et al., 2018; Mitchell et al., 2019).

According to Brown et al., (2010), the recommended zinc fortification levels for cereal flours across the globe were 1.4-3.3 mg $100g^{-1}$. The blending of flours rather than the use of mineral supplements has been proposed as one of the cost-effective and sustainable techniques of food fortification in SSA. Other than improving micronutrient levels in the flours, blending also induces other nutritional and health benefits such as improved protein and fibre contents. Adeyeye (Adeyeye, 2016) reported that whereas wheat had a fibre content of 1.42 ± 0.05%, sorghum recorded a higher content of 2.32 ± 0.14% which makes it a good fortificant of fibre. Since sorghum starch has been shown to have poor digestibility (Kulamarva, Sosle, & Raghavan, 2009), compositing it with other flours promotes its acceptability and sensory attributes. According to Stefoska-Needham et al., (2015) and Vila-Real et al., (2017), incorporation of other foods into cereal flours is necessary due to the low level of mineral and protein contents.

Foods rich in micronutrients such as orange-fleshed sweet potato (OFSP), baobab pulp and grain amaranth have been composited with cereal flours in efforts to improve the micronutrient contents. The beta-carotene rich property of OFSP has enabled its utilization for mitigation of vitamin A deficiencies (VAD) through its incorporation into other foods (Owade et al., 2018). Although Abong' et al., (2020) indicated that the leaves of OFSP have higher beta carotene content than the roots, the roots are still the most consumed edible part of the OFSP. Incorporation of OFSP flour in maize increased the crude fibre and carotenoids by 2.19-2.69% and 160.26-205.22 µg/g respectively (Ukom et al., 2019).

Baobab, a deciduous tree that originated in Africa is mainly found in scrubland and savannah vegetation (Abdulkarim et al., 2014). The baobab fruit is rich in vitamin C, fibre, potassium, calcium, iron and magnesium (Muthai et al., 2017). Studies on flour blends using baobab pulp powder have shown improved rheological and mineral content of cereal flours (Mounjouenpou et al., 2018). According to Tanimola et al., (2016), grain amaranth is rich in micronutrients such as zinc (6.27 mg/100g), iron (11.00 mg/100g) and calcium (33.29mg/100g), of which their deficiency is of great public health importance. Easy availability of OFSP, baobab and grain amaranth in SSA makes their utilization in composite flour formulation viable. However, evaluation of the three ingredients as possible fortificants of cereal flours has not been extensively done. The current study evaluates a novel product developed through fortification of cereal flours for improved nutritional composition.

2. Materials and Methods

2.1 Raw Material Acquisition

All the raw materials were purchased from different parts of Kenya. White maize was purchased from Eldoret market. Pale-red sorghum (E71) from Busia was purchased through McKnight Sorghum research team and pale cream grain amaranth was purchased from Bungoma. Baobab powder was purchased from Mombasa and stored at the University of Eldoret Food Processing Centre at 25 ℃. Sweet potato puree, was purchased form Organi Ltd in Homabay and stored in a deep freezer at -20 ℃. Each of the raw materials was purchased in packs of 10 kg

2.2 Sample Preparation

The grains were washed and dried in a forced draft oven at 60 ℃ for 24 h to a moisture content of 12%. The frozen orange-fleshed sweet potato puree was thawed in warm water before drying it in the oven at 60 ℃ for 6 h. They were then milled in a hammer mill fitted with 800 µm sieve to obtain whole-milled flours and stored in clean buckets.

2.3 Formulation of the Composite Flours

A completely randomized design in factorial arrangement with extrusion and formulation as main factors was used in the production of the flours. The formulations were based on findings of the nutritional profile of each of the ingredients to meet the Recommended Dietary Allowance of children below the age of five as per World Health Organization recommendations. Nutrisurvey linear programming software embedded with WHO RDA for children was used in the formulation of the flours. The formulations targeted 25 % of beta-carotene, iron and zinc contents and 15% of protein. Seven different formulations with varying ratios of maize, sorghum, grain amaranth, baobab and orange-fleshed potatoes were arrived at using Nutrisurvey as presented in (Table 1). The raw materials were mixed and half of each mixture stored separately for use in comparison to the extruded mixture. The moisture content of the other half was raised to 35% by adding water and mixing thoroughly and extruded at 160°Cin a single screw extruder (TechnoChem, Indiana, USA) with a screw rotation of 800rpm.The extruded products were dried at 50°C for 4 h, milled and vacuum-packed.

Table 1. Composite flour formulations

Formulation	Ingredient proportion (%)					Description
	Maize	Sorghum	Amaranth	OFSP	Baobab	
A	30	35	20	10	5	Varying cereals and fortificants
B	42.5	22.5	5	15	15	More maize than sorghum with constant fortificants
C	22.5	42.5	5	15	15	More sorghum than maize with constant fortificants
D	32.5	32.5	5	15	15	Equal maize and sorghum, constant fortificants
E	65	0	5	15	15	Maize plus constant fortificants
F	0	65	5	15	15	Sorghum plus constant fortificants
G	20	45	5	15	15	Variant of formulation C

2.4 Analytical Methods

2.4.1 Proximate Composition Determination

Proximate composition was determined according to AOAC 2012 (AOAC, 2012) methods. Ash content was analyzed as per method number AOAC 942.05:2012, moisture content was according to method number AOAC 976.08:2012, the nitrogen content was done by Kjeldahl method number AOAC 988.05:2012 and converted to protein by multiplying with a factor of 6.25. The crude fibre was determined by gravimetric method according to method number AOAC 958.06:2012 while fat content was determined by Soxhlet method number AOAC 942.05:2012.

Carbohydrates were determined by the difference:

Carbohydrates= (100%-[%protein+%fat+%moisture+%ash+%Fiber])

Energy content was determined by the WHO/FAO (WHO/FAO, 2003) factor; Energy= 4 kcal/g (protein)+ 9 kcal/g (fat)+ 4 kcal/g (carbohydrates).

2.4.2 Determination of Beta-carotene Content

Beta-carotene was done according to a method described by Biswas et al (Biswas et al., 2011). A 2 g sample was extracted using a pestle and motor with small portions of acetone until the residual turned colourless. All the extract was then combined in a 100 mL volumetric flask. Approximately 25 mL of the extract was transferred to a 50 mL round-bottomed flask and evaporated to dryness in a rotary evaporator at 60 ˚C. Petroleum spirit, approximately 1 mL, was added to dissolve the β-carotene. The β-carotene was then eluted through a packed column and the absorbance of the eluent read at 450 nm. The β-carotene content was then calculated from the β-carotene standard curve.

2.4.3 Determination of Iron and Zinc Contents

Iron and zinc were determined by a modification of the method described by Puwastien et al (Puwastien et al., 2011) using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Approximately 0.5 g of the samples, blanks, positive control and negative controls were weighed into Teflon tubes and 6 mL of concentrated HNO_3 and 3 mL of H_2O_2 added. The vessels were left to vent in a fume hood for 30 min and then capped. They were then placed on the rotor and inserted into the microwave oven (Anton Paar MULTI WAVE PRO 50 HZ 10HF100) with the microwave power set at a maximum of 1500W and digested according to manufacturer's instructions until a clear digest was obtained. They were then transferred to centrifuge tubes and the volume adjusted to 50 mL. The samples and quality control standards were then placed on the autosampler (Agilent Technologies 5110) for analysis and the results read. The Zn and Fe content of the sample expressed in mg/Kg of the product was calculated using the formula:

$$\textbf{Zn/Fe content} = (ci - cb) * v * d.f/w$$

Where;

ci = Zn or Fe content of test solution expressed in mg/l read from the calibration curve.

cb = Content of blank solution in mg/l read from calibration curve

d. f = Dilution factor

w= Sample weight

2.4.4 Determination of Phytate Content

Phytic acid content was determined using the method by Latta et al (Latta & Eskin, 1980). Approximately 1g of the sample was defatted by addition of 10 mL of petroleum ether and left to stand for two hours. The supernatant was discarded and the samples allowed to dry; 10 mL of 10% hydrochloric acid was added and the suspension centrifuged (Dr Ngerber, K. Schneider & Co, Zurich) at 482.97g for 10 min and the supernatant transferred into 100 mL volumetric flask. This was repeated 4 times. Approximately 2 mL of the sample was transferred to a 50 mL volumetric flask and 2 mL of Wade reagent (0.03% iron chloride+ 0.3% Sulfosalicylic acid) added and topped up to 10 mL with water. Absorbance was read using a single-beam spectrophotometer (Spectronic 1001, Milton Roy Company, USA) at 500 nm wavelength. The phytate content was calculated using the phytic acid standard curve and the results expressed in g/100g dry weight.

2.4.5 Determination of Tannin Content

Tannin content was analyzed according to AOAC (2012) method number 952.0:2012. Briefly,0.5g of the sample was weighed and 50 mL of water added and vortexed for 5 min and allowed to settle. The supernatant was then decanted into a clean conical flask. Approximately 2 mL of Folin Denis reagent, prepared according to Ferreira et al., (2004) was added to 75 mL of distilled water followed by the addition of 2 mL of the sample and 5 mL of concentrated sodium carbonate. The volume was then adjusted to 100 mL by addition of distilled water and allowed to stand for 40 min. Absorbance was then read at 725nm using a single beam spectrophotometer (Spectronic 1001, Milton Roy Company, USA) at 500 nm wavelength and the results expressed in g/100g dry weight.

2.5 Statistical Analysis

Statistical analysis of the data was done in the *R* Project for *Statistical* Computing, R-3.6.3 (R Core Team, 2019). The nutrient and anti-nutrient contents were converted to dry weight basis (dwb) and descriptive statistics including the mean and the standard deviation obtained. Normality of the data was tested using the Wilk's Shapiro test. Exploratory analysis of the data was done using the Pearson correlation. Inferential statistics were done by ANOVA, whereby means that were statistically different were separated using the Tukey's HSD test. Significant differences were tested at $p<0.05$.

3. Results

3.1 Proximate Composition of the Raw Materials

Proximate composition of the raw materials was statistically ($p<0.05$) different as shown in Table 2. Grain amaranth had a fat, protein and fibre content of 9.03 ± 0.23, 15.26 ± 0.34 and 9.28 ± 0.44 g/100g dwb, respectively, which was significantly ($p<0.05$) higher than the combination of these nutrients reported in other raw flour samples whereas the baobab powder had the highest ash content (9.25 ± 0.09 g/100g dwb).

The micronutrient content of the raw flour significantly ($p<0.05$) differed from each other (Table 3). Iron, zinc and beta-carotene contents were the micronutrients of interest in this study and they were highest in sorghum, grain amaranth and orange-fleshed sweet potatoes respectively.

3.2 Anti-nutrient Content of Raw Flours

Tannins were highest in sorghum flour (335.08 ± 16.53 mg/100g) whereas the phytates were highest in baobab powder (191.95 ± 0.41 mg/100g), $p<0.05$ (Figure 1).

Table 2. Proximate composition of raw flours used in formulating composite flours (per 100g dwb)

Raw flour	Moisture (g)	Protein (g)	Fat (g)	Fibre* (g)	Ash* (g)	Carbohydrates* (g)	Energy value (Kcal)
Maize	13.76±0.60^{b}	4.89±0.07^{c}	4.56±0.12^{c}	2.33±0.03^{b}	1.35±0.02^{e}	86.87±0.00^{a}	408.06±0.78^{b}
Sorghum	11.06±0.15^{c}	7.19±0.05^{b}	6.32±0.91^{b}	3.03±0.07^{b}	1.68±0.00^{d}	81.79±0.92^{b}	412.77±4.30^{a}
Amaranth	12.30±0.69^{bc}	15.26±0.34^{a}	9.03±0.23^{a}	9.28±0.44^{a}	2.95±0.05^{c}	63.48±0.17^{d}	396.25±2.74^{c}
Baobab	17.42±0.62^{a}	0.42±0.01^{d}	0.70±0.06^{d}	10.48±0.41^{a}	9.25±0.09^{a}	79.15±0.43^{c}	324.58±2.31^{e}
OFSP	14.06±0.02^{b}	6.09±0.02^{c}	0.54±0.03^{d}	2.89±0.03^{b}	5.03±0.01^{b}	85.46±0.09^{a}	371.00±0.01^{d}
%CV	16.7	75.7	82.6	65.0	72.7	11.1	11.2
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The values are mean of duplicate samples ± SD. Values with different letters in the superscript along a column are statistically different at p<0.001. Moisture values are in g/100g wet weight basis (wwb).

Table 3. Micronutrient composition of raw flours used in formulating blended flours (per 100g dwb)

Raw flour	Iron (mg)	Zinc (mg)	Beta-carotene (mg)
Maize	2.09±0.02^{e}	0.20±0.02^{b}	Nd
Sorghum	18.57±0.03^{a}	0.14±0.00^{c}	Nd
Amaranth	12.30±0.14^{c}	0.62±0.01^{a}	Nd
Baobab	16.07±0.27^{b}	0.12±0.00^{c}	Nd
OFSP	6.50±0.02^{d}	0.16±0.00^{c}	3268.45±6.64
%CV	58.1	81.8	N/A
p-value	<0.001	<0.001	

The values are mean of duplicate samples ± SD. Values with different letters in the superscript are statistically different at p<0.05. Nd- not detected.

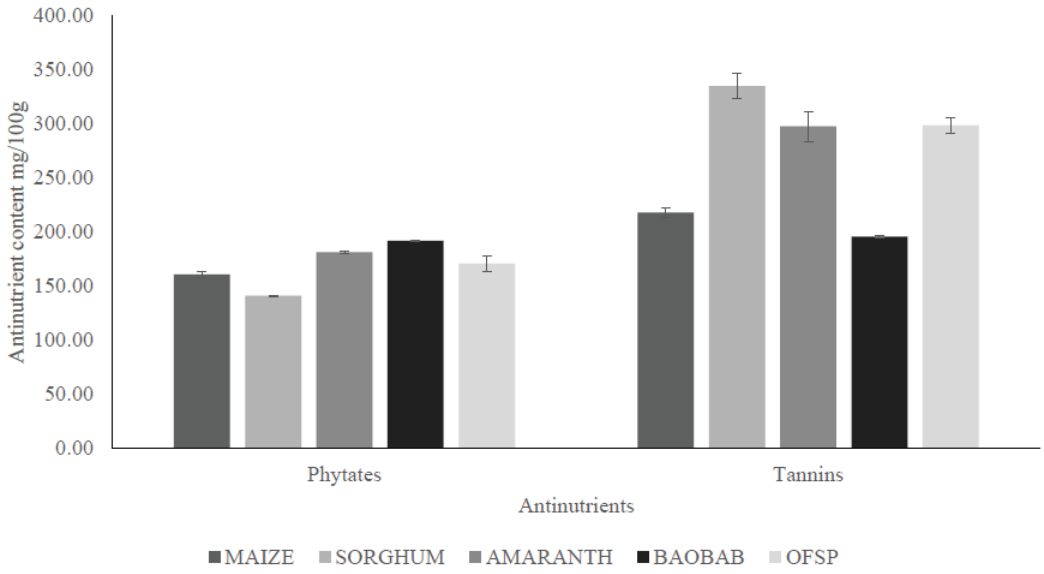

Figure 1. Antinutrient content (mg/100g dry weight) of raw flours used in formulating blended flours

3.3 Proximate Composition of Composite Flours

Proximate composition of flours was significantly affected by the ratio of the ingredients, extrusion and the interaction of the two factors. Addition of the fortificants significantly (p<0.05) affected the protein, ash and carbohydrate contents (Table 4)**.** Sample A in which the highest content of amaranth grain (20%) was added had significantly (p<0.001) high contents of protein and ash. Increasing maize levels resulted in significantly (p<0.05) higher carbohydrate contents compared to sorghum.

Extrusion of the composite flours significantly improved the fat and fibre contents and the energy values while reducing the ash, moisture, protein, fibre and carbohydrate contents as shown in Figure 2.

Table 4. Effect of fortification of cereal flours with baobab, orange-fleshed sweet potato and amaranth grain powders on their proximate composition (per 100g dwb)

Blended flours	Moisture (g)	Protein (g)	Fat (g)	Fibre (g)	Ash (g)	Carbohydrates (g)	Energy values (Kcal)
A	10.59±3.39^{a}	10.36±0.89^{a}	4.33±0.99^{a}	0.87±0.04^{a}	0.72±0.02^{a}	84.08±0.30^{ab}	416.70±5.13^{a}
B	8.82±4.96^{a}	8.85±0.33^{b}	4.16±1.56^{a}	0.98±0.16^{a}	0.61±0.05^{b}	85.41±1.77^{ab}	414.42±8.29^{a}
C	9.23±4.38^{a}	9.24±0.04^{ab}	3.87±1.24^{a}	0.95±0.08^{a}	0.63±0.04^{b}	85.32±1.14^{ab}	413.04±6.63^{a}
D	11.68±1.85^{a}	8.35±0.78^{b}	3.91±1.29^{a}	0.91±0.19^{a}	0.63±0.03^{b}	86.36±0.24^{ab}	414.05±8.02^{a}
E	11.22±3.11^{a}	7.24±0.33^{c}	3.74±1.15^{a}	0.97±0.24^{a}	0.56±0.01^{c}	87.62±0.52^{a}	413.10±7.36^{a}
F	10.55±2.34^{a}	9.41±0.41^{ab}	3.77±1.45^{a}	0.91±0.10^{a}	0.62±0.02^{b}	85.29±0.96^{ab}	412.71±7.58^{a}
G	7.84±5.57^{a}	9.45±0.27^{ab}	3.88±1.69^{a}	1.07±0.01^{a}	0.54±0.01^{c}	85.05±1.95^{ab}	412.95±8.49^{a}
%CV	36.6	11.5	30.7	14.4	9.6	1.7	1.7
p-value	0.790	<0.001	0.996	0.576	<0.001	0.012	0.989

Values are means of triplicates for a sample. All values are in g per 100g whereas those of carbohydrates and energy values are to be multiplied with a factor of 10 and 100, respectively. All the values are in dry weight basis (dwb) except for moisture (wwb). The error bars represent the standard deviation.

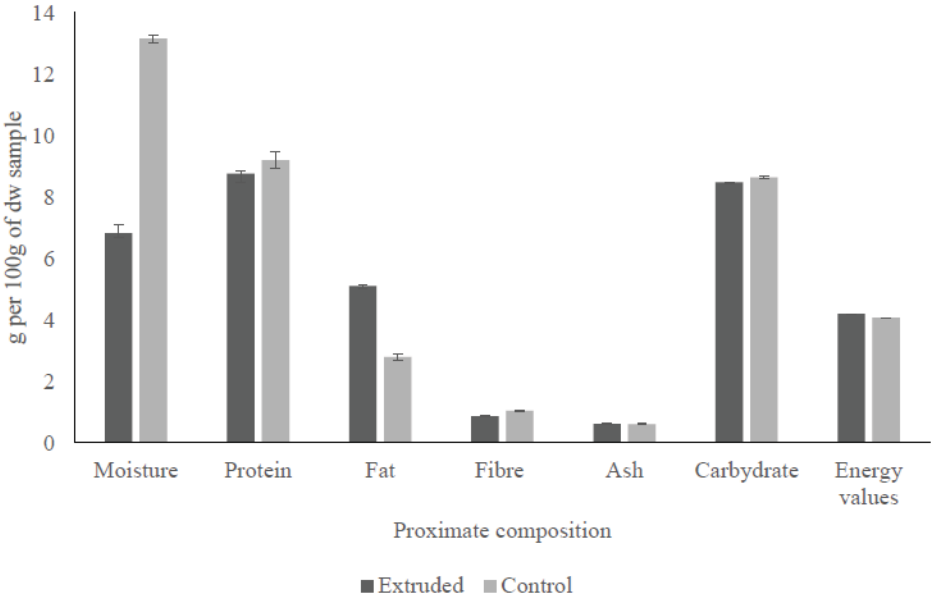

Figure 2. Effect of extrusion on the proximate composition of fortified cereal flours

All values are in g per 100g except and moisture which is on a wwb. Carbohydrate and energy values are to be multiplied by a factor of 10 and 100, respectively. The error bars represent the standard deviations.

Extrusion and ingredient ratio interaction significantly ($p<0.05$) affected the proximate composition of the composite flours as shown in Table 5. Extrusion significantly ($p<0.001$) increased the energy contents of high sorghum formulations. Increasing grain amaranth in the formulations resulted in significantly ($p<0.001$) higher protein and ash contents in both the extruded and non-extruded treatments. Extrusion induced higher fat contents while decreasing the moisture contents in all the formulations as compared to the non-extruded samples ($p<0.001$). The formulations that underwent extrusion all had significantly ($p<0.05$) higher carbohydrate contents notwithstanding the ratio of ingredients incorporated. An increasing proportion of maize in the formulation resulted in higher levels of fibre both in the extruded and non-extruded formulations ($p<0.05$).

Table 5. Effect of interaction between formulation and extrusion on the proximate composition of the composite flours (per 100g)

Formulation		Moisture content(g)	Protein (g)	Fat (g)	Fibre (g)	Ash(g)	Carbohydrate (g)	Energy values (Kcal)
A	Extruded	7.65±0.01^{d}	9.63±0.01^{e}	5.18±0.02^{e}	0.90±0.03^{d}	0.70±0.02^{fg}	83.94±0.43^{b}	420.90±1.90^{d}
	Control	13.53±0.01^{l}	11.09±0.51^{f}	3.47±0.00^{c}	0.84±0.02^{bc}	0.73±0.02^{g}	84.22±0.04^{c}	412.50±1.09^{b}
B	Extruded	4.53±0.03^{b}	9.12±0.11^{d}	5.51±0.03^{f}	0.84±0.00^{bc}	0.65±0.03^{e}	83.88±0.11^{b}	421.60±0.30^{d}
	Control	13.12±0.04^{j}	8.57±0.06^{c}	2.80±0.01^{b}	1.12±0.01^{f}	0.57±0.01^{abc}	86.94±0.09^{g}	407.20±0.00^{a}
C	Extruded	5.44±0.03^{c}	9.24±0.07^{d}	4.94±0.01^{d}	0.89±0.07^{c}	0.59±0.01^{bcd}	84.33±0.12^{cd}	418.80±0.20^{c}
	Control	13.03±0.02^{i}	9.24±0.01^{d}	2.79±0.03^{b}	1.01±0.01^{e}	0.66±0.01^{ef}	86.30±0.06^{ef}	407.30±0.10^{a}
D	Extruded	10.08±0.01^{g}	7.68±0.13^{b}	5.02±0.20^{d}	0.75±0.05^{a}	0.63±0.02^{de}	86.24±0.33^{e}	420.90±2.60^{c}
	Control	13.28±0.03^{k}	9.03±0.04^{d}	2.80±0.00^{b}	1.08±0.01^{f}	0.62±0.04^{d}	86.47±0.09^{f}	407.20±0.20^{a}
E	Extruded	8.52±0.03^{e}	6.96±0.06^{a}	4.73±0.02^{c}	0.77±0.01^{a}	0.57±0.02^{abc}	87.25±0.49^{h}	419.40±1.60^{c}
	Control	13.91±0.01^{m}	7.53±0.00^{b}	2.74±0.02^{b}	1.18±0.01^{g}	0.55±0.01^{ab}	88.00±0.03^{h}	406.80±0.00^{a}
F	Extruded	8.53±0.00^{e}	9.06±0.10^{d}	5.02±0.01^{d}	0.83±0.01^{b}	0.63±0.00^{de}	84.46±0.12^{d}	419.30±0.00^{cd}
	Control	12.58±0.01^{g}	9.76±0.01^{e}	2.51±0.01^{a}	1.00±0.02^{e}	0.60±0.01^{cd}	86.13±0.05^{e}	406.1±0.10^{a}
G	Extruded	3.02±0.03^{a}	9.68±0.02^{e}	5.35±0.01^{f}	1.07±0.00^{f}	0.54±0.00^{ab}	83.36±0.03^{a}	420.30±0.00^{d}
	Control	12.67±0.02^{h}	9.22±0.00^{d}	2.42±0.02^{a}	1.06±0.01^{ef}	0.54±0.01^{a}	86.75±0.05^{g}	405.60±0.10^{a}

Values are means of triplicates for a sample. Values with different letters in the superscript along a column are statistically different at $p<0.05$. All the values are in dry weight basis (dwb) except moisture which is on wwb. Formulation A had varying fortificants, formulation B had more maize and equal fortificants, formulation C had more sorghum and equal fortificants, formulation D had equal maize and sorghum with constant fortificants, formulation E had maize and fortificants, formulation F had sorghum and the fortificants and formulation G was a variant of formulation C

3.4 Micronutrient and Antinutrient Content of Blended Flours

Beta carotene, phytates and tannin contents in the formulations were significantly ($p<0.01$) affected by the ratio of the ingredients, extrusion and the interaction of the two factors. However, the zinc content of the flours was only significantly ($p<0.001$) affected by the ratio of the ingredients and the interaction between the ratio of the ingredients and extrusion. Neither the incorporation of the fortificants nor the process of extrusion significantly ($p>0.05$) affected the iron content of the formulations.

The beta-carotene content of the fortified cereal flours was significantly predicted by the moisture content at $p<0.05$ with a variance of 53.1%. The regression equation of the predictor model was as shown in equation 1.

Equation 1

$$y = 3 + 0.01x \quad (1)$$

Whereby y is the beta carotene content and x is the moisture content. $R^2=0.53$

Whereas extrusion significantly ($p<0.001$) reduced the beta-carotene content of the cereal flours, it had no significant ($p>0.05$) effect on the iron and zinc contents. Treatment with the highest proportion of amaranth (20%) had the highest level (1.97 ± 0.12 mg/100g dwb) of zinc content at $p<0.001$. The trends did not change with the inclusion of the second factor (extrusion) as the incorporation of the amaranth grain significantly increased the zinc content (1.88-2.06 mg/100g dwb). Incorporating OFSP and amaranth grain powder significantly increased the beta carotene content of the cereal flour (Table 6). Extrusion resulted in higher degradation of beta-carotene in cereal flour with OFSP as compared to that with amaranth grain, $p<0.05$.

There was significantly ($p<0.001$) higher phytate and tannin contents in non-extruded cereal flours than the extruded (Table 7). A higher proportion of sorghum significantly ($p<0.05$) increased the tannin and phytate contents with the samples with 65% sorghum having the phytate and tannin contents of 11.47 ± 2.17 and 1329.9 ± 265.2 mg/100g dwb, respectively. The interaction of extrusion and formulation significantly ($p<0.05$) affected the phytate and tannin contents of the blended flours (Figures 3 and 4). The phytate contents of cereal flour with the higher proportions of sorghum than maize was found to reduce whereas those with more maize than sorghum recorded lower tannin contents on extrusion at $p<0.001$. In all the formulations, the tannin content reduced when extrusion was done. Zinc was positively correlated with the phytate content in the cereal flours ($p<0.05$) as

shown in Table 8.

Table 6. Effect of extrusion and formulation of cereal flours with baobab, orange-fleshed sweet potato and grain amaranth powder on their micronutrient content (mg/ 100 g dwb)

Formulation		Micronutrient content (mg/100g)		
		Beta carotene content	Iron content	Zinc content
A	Extruded	1074.4 ± 1.0^{Ah}	7.35 ± 0.10^{Da}	1.88 ± 0.12^{Ge}
	Control	1301.5 ± 13.6^{Ahi}	5.26 ± 0.01^{Da}	2.06 ± 0.00^{Gf}
B	Extruded	385.8 ± 12.0^{Ab}	14.40 ± 0.09^{Da}	1.46 ± 0.09^{Ga}
	Control	1277.8 ± 20.8^{Ah}	4.25 ± 0.10^{Da}	1.68 ± 0.16^{Gbcd}
C	Extruded	280.3 ± 1.3^{Aa}	14.35 ± 0.02^{Da}	1.89 ± 0.05^{Ge}
	Control	1324.7 ± 20.5^{Ai}	5.15 ± 0.13^{Da}	1.62 ± 0.07^{Gbcd}
D	Extruded	693.1 ± 1.2^{Ad}	22.12 ± 0.07^{Da}	1.59 ± 0.03^{Gabc}
	Control	1320.8 ± 13.1^{Ai}	6.70 ± 0.02^{Da}	1.68 ± 0.04^{Gbcd}
E	Extruded	1043.9 ± 12.5^{Ag}	15.64 ± 0.07^{Da}	1.68 ± 0.03^{Gcd}
	Control	772.3 ± 1.4^{Ae}	4.29 ± 0.02^{Da}	1.86 ± 0.08^{Ge}
F	Extruded	601.9 ± 19.2^{Ac}	19.88 ± 0.09^{Da}	1.76 ± 0.02^{Gde}
	Control	885.0 ± 0.7^{Af}	6.35 ± 0.06^{Da}	1.89 ± 0.05^{Ge}
G	Extruded	577.9 ± 1.3^{Ac}	11.49 ± 0.02^{Da}	1.88 ± 0.04^{Ge}
	Control	1003.1 ± 7.0^{Af}	28.19 ± 2.67^{Da}	1.49 ± 0.02^{Gab}
%CV		38.7%	82.3	10.2
p-value		<0.001	0.219	<0.001

Values with a similar uppercase letter followed by different lowercase letters in the superscript are statistically different at $p<0.05$ whereas values with a similar uppercase letter followed by similar lowercase letters are statistically similar at $p<0.05$. Values are means of triplicates for a sample.

Table 7. Main effect of extrusion and formulation on tannins and phytate contents of composite flour

Formulation	Phytates mg/100g	Tannin mg/100g
A	12.85 ± 1.70^{Aab}	757.7 ± 149.4^{Aa}
B	6.25 ± 2.26^{Aabc}	873.3 ± 385.6^{Aa}
C	8.61 ± 1.04^{Abcd}	1109.0 ± 381.7^{Abc}
D	10.07 ± 1.61^{Abcd}	1078.8 ± 213.5^{Ac}
E	7.57 ± 1.91^{Aa}	570.7 ± 63.0^{Aab}
F	11.47 ± 2.17^{Ad}	1329.9 ± 265.2^{Ad}
G	8.01 ± 1.08^{Acd}	1163.0 ± 225.0^{Ab}
%CV	18.8	26.8
LSD	2.56	387.9
Extrusion		
Extruded	8.74 ± 3.21^{Ba}	775.0 ± 215.3^{Ba}
Control	9.78 ± 1.98^{Bb}	1191.5 ± 314.6^{Bb}
%CV	28.8	27.4

Values with a similar uppercase letter followed by different lowercase letters in the superscript in a column are statistically different at $p<0.05$ whereas values with a similar uppercase letter followed by similar lowercase letters are statistically similar at $p<0.05$. Values are means of triplicates for a sample and all the results are on a dry weight basis. The error bars represent the standard error.

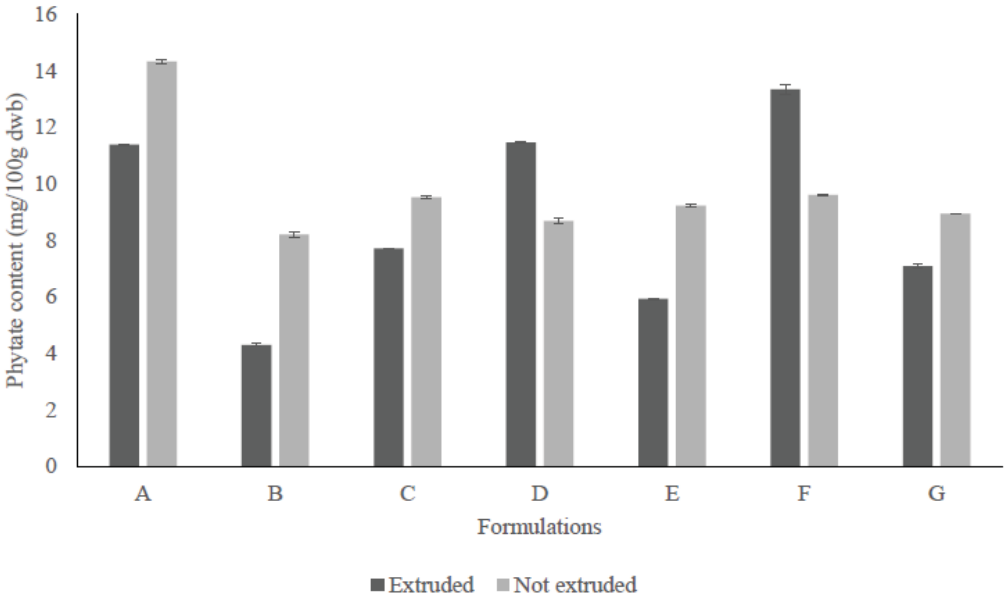

Figure 3. Effect of the interaction between extrusion and formulation on the phytate content of blended flours Values are means of triplicates for a sample. The error bars represent the standard error.

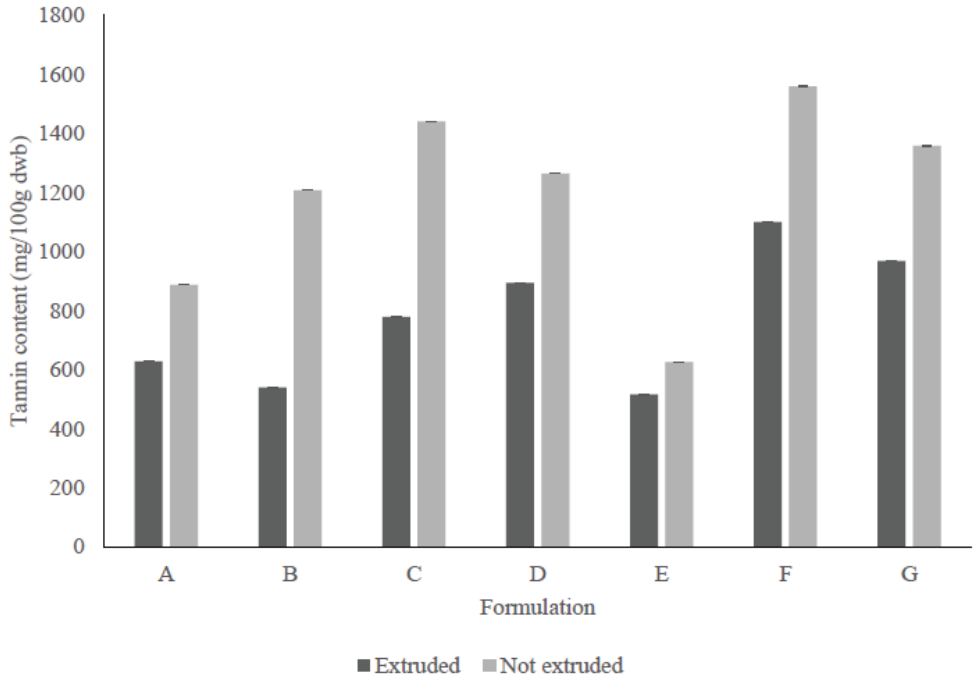

Figure 4. Effect of the interaction between extrusion and formulation on the tannin content of blended flours Values are means of triplicates for a sample. The error bars represent the standard error.

Table 8. Correlation between micronutrients and anti-nutrients in cereal flours

Micronutrient	Anti-nutrient	
	Phytates	Tannins
Iron	-0.04	-0.03
Zinc	0.45*	-0.10

*Value significant at $p<0.05$

4. Discussion

4.1 Nutrition Composition of Raw Material

The findings in the present study showed amaranth and OFSP are the most nutritious fortificants of protein, beta-carotene and zinc in the formulations. The protein content for the amaranth grain found in this study was comparatively within the range of 13.37 to 23.28% reported by Kachiguma et al (Kachiguma et al., 2015) for various accessions grown in Malawi. However, Ayo, (2001) reported slightly a higher figure of 13.65% for the protein content. The range for the protein level reported by that particular study was 13.37% to 21.50%. In proximate composition, baobab had the highest ash content pointing to higher mineral composition. OFSP was the only fortificant that had beta-carotene. In as much as drying of the OFSP roots deteriorates the level of beta-carotene through oxidation (Owade et al., 2018), the level of beta-carotene found in the current study was still higher than the levels reported by Aywa et al (Away et al., 2013) in some of the raw roots of some ascensions grown in Kenya. An earlier study by {Formatting Citation} found that maize flour had undetectable levels of beta-carotene, thus the 1987±0.05 µg RAE/100 g of vitamin A in OFSP flour makes it ideal as a fortificant. The two kinds of in this study had the highest contents of carbohydrates and energy values. Additionally, the drying of the fresh OFSP roots and grinding into flour has been shown to increase the carbohydrate contents of these ingredients seven-fold (Hacineza et al., 2007).

Considering that cereal flours are high in carbohydrate, this finding justifies the need to fortify the blended flours to increase their protein content.

4.2 Anti-nutrient Content of Raw Materials

Fortification of cereal flours that targets to increase their mineral content seeks to limit the anti-nutrient content of the specific flours. This is because of anti-nutrients such as phytic acid form complexes with micronutrients making them less bioavailable (Coulibaly et al., 2011). Evaluation of the cereal grains have shown that the content of anti-nutritional factors could be as high as 40-60% of the total caloric intake among the populations in SSA (Gupta et al., 2015); posing the risk of low bioavailability of micronutrients in the food taken. Sorghum and maize flours were found to have a higher content of tannin and phytate, respectively, than the fortificants. Increasing the tannin content of the cereal flour blend resulted in declining zinc content. This implies that improving the micronutrient content should not just be limited to the formulation of cereal flour blends with ingredients rich in zinc, additional treatment to address the anti-nutritional factors has to be applied.

4.3 Proximate Composition of Blended Flours

Incorporation of amaranth into the blended flours significantly improved the protein and ash content of the flours. The high protein content of the amaranth grain has been a selling point for its incorporation in food and feed whereby the augmentation of the feed and the food is the target (Ayo, 2001; Pisarikova et al., 2006). However, increasing the maize content had a reverse effect on the fibre content as it was decreasing. This is explained majorly by the low content of fibre in the maize grain compared to the other ingredients. The whole grain cereals are adjudged to be rich in fibre (Sarwar, 2013). Subsequently, incorporation of the fortificants into cereal flours resulted in the decreased energy density of the flours. The average energy values achieved in the extruded cereal flour blends was higher than the average energy values per gram of carbohydrate of 4 KCal. Most flour manufacturers usually add fats to increase the energy density of the flours (Okoth et al., 2017); however, in the present study, there was no addition of fat to the flour blends. Addition of fat in flour blends has a deleterious effect on the keeping quality of the flour as it decreases the shelf-life of the flour.

Extruded flour had a higher content of fat whereas reducing the ash, protein, carbohydrates and fibre contents. In their evaluation of flour fortification for the preparation of breakfast cereal, Santos et al (Santos et al., 2019) reported a threefold increase in the fat; increase that is higher than what was reported in the current study. This is attributed to the utilization of legumes which are known to be richer in oils in their study. The greatest decline in the protein content due to extrusion was seen in treatments with the highest amaranth content whereas the carbohydrates and energy values increased in samples with higher proportions of sorghum. The impact of extrusion on the proximate composition of extruded cereal flour has varied depending on the ingredients incorporated into the cereal flour. Whereas Yusuf et al (Yusuf et al., 2018) reported a decline in fibre as reported in this study, he reported contrary findings of an increase in the carbohydrate content of an extruded groundnut-sorghum flour blend. On the other hand, Tadesse et al., (2019) reported a decline in the carbohydrate content of an extruded soy-sorghum flour blend.

4.4 Micronutrient and Anti-nutrient Content of Blended Flours

In both extruded and non-extruded blended flours, the minimum beta-carotene level for fortified flours, 500

mg/100g (Owade et al., 2018), was achieved. Incorporation of amaranth into cereal flour was reported to improve the iron and zinc whereas not achieving significant levels of vitamin A and its equivalents (Akande et al., 2017). Beta carotene was significantly reduced as a result of the extrusion process. Similar trends were reported by Akande et al., (2017) in their study that evaluated the effect of extrusion conditions on the nutrient composition of cereal flours. With increasing temperature of extrusion, it was reported that the vitamin A levels of the extruded flour declined. Cereal flour blends with OFSP rather than amaranth grain powder had higher degradation of the beta-carotene due to extrusion. Exposure of the OFSP to thermal treatment has been shown to result in degradation of the beta-carotene content through oxidation (Owade et al., 2018). Additionally, reduction in the moisture contents of the cereal flour blends resulted in a decline in the beta-carotene contents. This can be explained by the first order-kinetics of reduction of beta-carotene in dehydrated foods (Neto et al., 1981), whereby in low moisture beta-carotene deteriorates due to discolouration (Chou & Breene, 1972; Pénicaud et al., 2011).

One of the greatest limitations of cereal-based flours in SSA is the bioavailability and content of the micronutrients (Tadesse et al., 2015). Utilization of locally available ingredients to develop nutrient dense formulations of the of cereal-based composite flour has been recommended as one of the affordable strategies to fight malnutrition especially among the under five years old children to whom porridge constitutes a great part of the diet (Akande et al., 2017). The levels of iron, zinc and beta-carotene achieved in the formulations were 4.29 ± 0.02 to 28.19 ± 2.67**,** 1.46 ± 0.09 to 2.06 ± 0.00 and 280.3±1.3 to 1320.8±13.1 mg/100g dwb, which were higher than some of the levels reported for most rich sources including the indigenous vegetables such as cowpea leaves vastly consumed in SSA (NutriSurvey, 2007b, 2007a; Owade et al., 2019). The formulations in the present study thus serve as major food vehicles for the respective micronutrients especially to the most vulnerable population in SSA which are the children under the age of five years.

Extrusion lowered the anti-nutrient contents of the cereal-based composite flours by 10.6-35.0%. The findings in this current study lend support to previous works by Gürbilek (Gürbilek, 2016) who reported a 16.55 –50.85% decline in the anti-nutritional factors in sorghum flour blended cereal foods. Extrusion results in the destruction of inhibitory anti-nutritional factors such as phytic acids which lower the bioavailability of micronutrients in the cereals blended foods (Omosebi et al., 2018). On the other hand, increasing the sorghum ration in the cereal flours resulted in higher anti-nutrient content. The phytate content of the cereal flour blends with sorghum significantly reduced on extrusion. Phytate contents reported in cereals has been estimated at 0.18 to 6.39% with higher intake among those consuming whole wheat cereal; for phytic acid has a higher concentration in the bran (Gupta et al., 2015). These levels are higher than those in the formulation of the cereal flour blends which ranged between 0.006 and 0.012%. Extrusion of the flour lowered the level of the phytate content by a further percentage. This is because extrusion hydrolyses phytic acid to phosphate molecules, thus destroying it (Wani & Kumar, 2016). In the formulation of the cereal flour blends, precooking is thus recommended as a measure of improving the bioavailability of the zinc and iron (Gupta et al., 2015).

The extrusion also achieved a decline of 35.0% in the tannin content of the composite flours and an average tannin content of 0.78% was achieved. In animal studies, it has been established that serially increasing the tannin content in feeds from 0.00% to 0.02% showed a linear reduction in the haemoglobin and hepatic iron concentration (Delimont et al., 2017). Fortification seeks to minimize the anti-nutritional factors while increasing the micronutrient contents of the composite flours. With the increasing zinc contents, the phytic acid contents of the cereal flour blends also increased.

5. Conclusion

Incorporation of OFSP, baobab and amaranth in the maize-sorghum cereal flour blend increases the zinc, iron, protein and beta-carotene contents while reducing the tannin content of the composite flours. However, the phytic acid content of these flours increases with increasing proportions of the fortificants and reduction of the cereal flours. Fortification aims to improve the overall bioavailability of the nutrients in the formulation. To this end, extrusion of the flour blends reduces the level of anti-nutritional factors. In as much as the beta-carotene is degraded on extrusion, the minimum content of 500µg/100g for fortified foods is still achieved. This study provides input to the nutritional programmes and the ever-evolving dietary practices on the most cost-effective ways to alleviate micronutrient and protein deficiencies in SSA. Considering the value-chains of the ingredients used as fortificants in this study, the output of this study can be promoted as one of the possible ways for commercialization of these value chains.

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Conflict of Interest

The authors declare that they have no conflict of interest concerning this research.

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Greener Analytical Method for Determination of Iodine Number of Edible Oils

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Abstract

A greener analytical method for determination of iodine number (IN) of oils is presented. As per the AOAC standard method, a large amount of solvent and reagent was used, and long incubation time was required. This research is aimed at using less amount of solvent and reagent, less sample weight, and shorten the analysis time by using the modified titrimetric AOAC standard method. The study showed that by reducing the sample size, the amount of reagent could be decreased to 1.00 mL and the reaction time of 1 min is enough for completion of the reaction. The amount of reagent used was at least 25 times less than that of the classical method. There was no significant difference at 95% confidence level between the results obtained by the proposed method and the standard method, and both results correlated well. The present method can be applied to edible oils commonly found in the market (iodine number range of 6.0 to 130).

Keywords: iodine number, edible oil, green analytical method, modified titrimetric method

1. Introduction

Nowadays, iodine number (IN) is one of the great interest parameters for measuring the degree of unsaturation of oil or fat and quickly indicating the group to which the oil belongs to. It is defined as the number of halogen in centigram, expressed as iodine, which is absorbed by one gram of oil sample (percent iodine absorption). The higher iodine number, the more unsaturated fatty acid parts are present in oil. The interesting application of iodine number is its use as a parameter in process control as well as a quality parameter in traded palm oil product. It is used as a guidance tool for the purchasing of raw materials and controlling hydrogenation reaction (Weiss, 1970). It is also used to investigate the properties of vegetable oil that used to produce biodiesel that will affect some properties of biodiesel, such as viscosity, cloud point, and storage stability which are changed due to the oxidation and polymerization reactions of double bonds in the oil. Moreover, since oil of high iodine number will contain high content of unsaturated bonds in fatty acids, it indicates the nutritional value (Knothe, 2002).

Several analytical techniques for determination of iodine number of edible oils have been reported. Some studies are undertaking by measuring the amount of remaining reagent from the reaction by using ion selective electrode (ISE) in potentiometric method (Honda & Kashimoto, 1978; Mahapatra, 2011) or measuring the absorbance of the remaining reagent (Kamson, 1986; Lee & Pollard, 1984), which is simple but still using large amount of reagent and solvent. Some papers also introduced flow injection techniques (FI) to make a more comfortable and faster analysis (Thomaidis & Georgiou, 2000). However, though the method provided automatic measurement the continuous flowing of reagent still generated large amounts of waste solution. Furthermore, nuclear magnetic resonance(NMR), near infrared(NIR), and Furior transform infrared(FT-IR) spectroscopic techniques have been proposed (Baeten & Aparicio, 2000; Che Man & Setiowaty, 1999; Foca et al., 2016; Hendl, 2001; Yang et al., 2005). The gas chromatographic method is an alternative method for determination of IN as per AOCS Cd1c-85 (Firestone, 1989). By this method, IN is calculated from the fatty acid composition in the oil sample. With this technique, quantification of different free fatty acids can be made simply. However, it involves

a complicated mathematical calculation, high-cost instrument, and well operation skill.

The determination of iodine number of edible oils according to the AOAC standard methods (Helrich, 1990) is carried out by using the titration method. An oil sample is analyzed by reacting with Hanus solution (iodine monobromide in glacial acetic acid) or Wij's solution (iodine monochloride), and then the amount of the remaining reagent is determined. This titrimetric method is simple and requires only general laboratory skills and apparatus. However, as per the AOAC titrimetic method, 25 mL of IBr reagent and 10 mL of chlorinated solvents per analysis is required with 30 to 60 min incubation time.

For many years, the green chemistry movement has been promoting ways to reduce the risks of chemical use to humans and the environment**.** The main concept of green chemistry is the application of chemistry skills and knowledge to reduce or eliminate the use and/or generation of hazardous substances (Anastas, 1999; De Marco et al**.**, 2019).

In this research, it is aimed to modify the titrimetric AOAC standard method for the determination of iodine number of edible oils by using less amounts of sample and reagent, and shorter incubation time. The experimental conditions were studied for reducing the amount of reagent and solvent, using short analysis time, and avoiding the usage of chlorinated solvent**.**

2. Method

2.1 Materials and Reagents

All chemicals, i.e., glacial acetic acid, potassium iodide, starch soluble, dichloromethane (as per AOAC titrimetric method), n-propanol, iso-octane, and oleic acid were analytical reagent grade. Standard solutions of 0.1 M and 0.005 M sodium thiosulfate were prepared by dissolving in deionized (DI) water and standardized against potassium iodate. Hanus solution (iodine monobromide, IBr) concentration of 0.10 M was prepared with glacial acetic acid according to the AOAC method (Helrich, 1990). The Hanus solution is stable for at least 1 month.

2.2 Sample Preparation

Nine samples of commercially available edible oil were purchased from local stores in Chiang Mai, Thailand, with IN range from 5 to 130. These included coconut, palm, olive, rice bran, sesame, canola, corn, sunflower, and soybean oils. Weighed 0.10 g of sample and dissolved it with iso-octane to 10 mL. One-mL aliquot of this sample solution (0.010 g sample) is used for analysis.

2.3 Determination of IN by AOAC *Titrimetric Method (Helrich, 1990)*

The AOAC titrimetric method was slightly modified. An aliquot of 0.25 g oil sample was dissolved in 10 mL of dichoromethane (instead of chloroform), follows by adding 25 mL of IBr solution. Shook it and incubated in the dark for 30 min. Then, added 15% w/v potassium iodide solution 10 mL. Titrated with 0.1 M sodium thiosulfate to pale yellow. Added few drops of starch indicator and continued titrating until the blue color entirely disappears. Blank determination was conducted similarly but without oil. The iodine number was then calculated using equation (1).

$$IN = [(VB\text{-}VS) \times M \times 12.69] / W \quad (1)$$

Where V_B = mL $Na_2S_2O_3$ used for blank determination, V_S = mL $Na_2S_2O_3$ used for sample determination, M = molarity of $Na_2S_2O_3$, W = weight of sample (g)

2.4 Greener Titrimetric Method for Determining Iodine Number

This method was modified from the AOAC titrimetric method to be a small scale titration. Weighed 0.10 g sample and dissolved it with iso-octane to 10 mL. One-mL aliquot of this solution (0.010 g sample) was mixed with IBr solution 1 mL. Shook it and incubated in the dark for 1 min. Then, added 6 % w/v potassium iodide solution 1 mL. Titrated with 0.005 M sodium thiosulfate to pale yellow. Added few drops of starch indicator and continued titrating until blue entirely disappears. Conducted blank determination. The iodine number was calculated using equation (1).

3. Results and Discussion

3.1 Optimization Studies

The parameters which affected analytical performance and the optimum condition are presented in Table 1.

Table 1. Optimization of the conditions of downscaled titrimetry

Parameters	Condition
Sample weight	0.010 ± 0.001 g
IBr solution	
- Concentration	0.10 M
- Volume	1.00 mL
Incubation time	1 min
Solvent	Iso-octane 1.0 mL

3.1.1 Effect of Type and Amount of Solvent

Chloroform has long been used for dissolving oil sample and its use has been banned in some countries. n-Propanol was used for an online spectrophotometric approach (Thomaidis & Georgiou, 2000) since it readily mixed with IBr solution. However, when this mixture is mixed with an aqueous solution of potassium iodide, the emulsion is formed. In this work, dichloromethane, n-propanol, and iso-octane (Takeshita et al., 1994) were compared for use in the determination of IN. Although dichloromethane is a chlorinated solvent, it was used for comparison purpose. The results as shown in Table 2 indicated that there was no significant difference in the iodine number by using these solvents. Iso-octane was readily mixed with IBr solution, did not form an emulsion with oil and aqueous solution. To avoid emulsion forming and to eliminate the usage of chlorinated solvent, iso-octane was chosen for dissolving oil samples.

Table 2. The determination of IN using different solvents (n=3)

Type of solvent	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
Dichloromethane	53.6	2.6	131.7	2.2
n-Propanol	58.5	0.7	136.5	1.1
iso-Octane	55.4	0.3	125.6	0.9

It was also attempted to reduce the volume of solvent used for dissolving oil samples. By utilizing the procedures described in section 2.4 but using the different volumes of solvent, iso-octane, i.e., 1.00, 5.00, and 10.00 mL, the resulting iodine numbers of oil samples are shown in Table 3. It was found that there was no significant difference observed in the IN of these oil samples by using 1.00, 5.00, and 10.00 mL of solvent. Therefore, 1.00 mL of iso-octane was selected for dissolving 0.01 g of oil.

Table 3. Effect of the amount of solvent for IN determination of palm and soybean oils. (n=3)

Volume of solvent (mL)	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
1.00	55.6	0.5	127.7	0.9
5.00	56.6	1.0	129.6	1.2
10.00	56.2	1.1	128.2	1.1

3.1.2 Effect of Incubation Time

The rate and extent of the halogenation reaction depend on incubation or reaction time (Earle & Milner, 1939; Markley, 1947). As suggested in the AOAC standard method, the higher iodine number of oil has required the longer the incubation time to complete the reaction. The effect of incubation time ranges from 1 to 30 min was investigated as shown in Table 4. The results were shown that there was no significant difference in iodine number of both palm oil and soybean oil for the analysis employing the incubation time of 1, 5, 15, and 30 min (one-way ANOVA test; F-cal = 0.074, F-crit = 6.59 for palm oil, and F-cal = 0.013, F-crit = 6.59 for soybean oil). Therefore, the incubation time of 1 min was chosen. It should be noted that this incubation time is appropriate for 0.01 g of oil having iodine number equal or less than that of soybean oil.

Table 4. The effect of reaction time for IN determination of palm and soybean oils. (n=3)

Incubation time (min)	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
1	57.6	0.5	127.9	1.2
5	57.5	0.2	128.4	0.8
15	57.4	0.2	128.9	1.0
30*	56.8	1.3	129.7	0.9

* as per AOAC method

3.1.3 Effect of the Weight of Sample

As per AOAC method, 25.00 mL of IBr solution is recommended for 5.00 g of oil sample, therefore, for 1.00 mL of this reagent should be reacted quantitatively with 0.100 g of sample. The weight of soybean oil ca. 0.01, 0.02, and 0.05 g were studied for its effect on IN. The IN (mean ± S.D., n=3) of 131.8 ± 3.3, 102.1± 0.2, and 45.9 ± 0.1 were obtained with the sample weight of 0.01, 0.02, and 0.05 g, respectively. The reaction of IBr with sample requires 50-60% excess of this reagent as mentioned in AOAC method (Helrich, 1990). Therefore, sample weight of 0.010 g of soybean oil and other oils with IN less than that of the soybean oil is appropriate for reacting with 1.00 mL of 0.10 M IBr solution.

3.1.4 Effect of Concentration and Volume of IBr Solution

Though the concentration of IBr solution of 0.10 M is stated in AOAC method, it was investigated whether or not the lower concentration of this reagent can be used. The effect of concentration of IBr solution was investigated by analysis of soybean oil with different concentrations of IBr solution ranged from 0.025 to 0.100 M, IN (mean ± SD, n=3) obtained were 44.5 ± 1.1, 82.5 ± 0.8, 109.5 ± 1.5, and 127.3 ± 1.3, respectively. This results are also in consistency with the analysis of palm oil and sunflower oil at IBr concentration of 0.050 and 0.10 M. The volume of IBr solution was also investigated and the results are shown in Table 5.

Table 5. The effect of volume of IBr solution for determination of IN (n=3)

Sample	Volume of Hanus solution (mL)			IN
	1.00	1.50	2.00	by AOAC method
Coconut	5.6 ± 1.4	6.6 ± 1.3	3.4 ± 0.7	6.5 ± 0.7
Oleic acid	90.2 ± 1.3	91.1 ± 2.4	92.8 ± 4.5	90.3 ± 1.1
Linolenic acid	143.1 ± 1.0	153.3 ± 3.5	157.8 ± 6.0	154.9 ± 1.0
Soybean	121.7 ± 4.2	132.1 ± 3.5	135.0 ± 3.7	124.6 ± 1.5
Ricebran	97.7 ± 3.2	98.5 ± 3.6	104.8 ± 4.2	100.1 ± 1.2
Sunflower	116.5 ± 1.6	119.5 ± 2.3	135.2 ± 1.7	126.2 ± 1.1
Palm	59.1 ± 2.0	58.7 ± 2.6	61.3 ± 2.4	58.8 ± 1.0

It is shown in Table 5 that the volume of reagent had affected on the IN of linoleic acid, soybean oil, rice bran oil, and sunflower oil. For oil samples having IN less than 100, 1.00 mL of reagent is appropriate. However, for oil having IN more than 100, 2.00 mL of reagent should be used.

3.2 Real Sample Analysis

The proposed greener titrimetric method was applied for determination of IN of real oil samples. Oleic acid and nine vegetable oil samples were subjected to the analysis. The analytical results are shown in Table 6. To test whether or not the results obtained by both methods were different, the paired t-test was performed. The observed t-value, t_{cal} = 0.011, was less than the critical t-value, t_{crit} = 2.10, therefore, there was no significant difference between the results obtained by the proposed method and the AOAC standard method. The developed method has superior advantages on at least 25 folds reducing the amounts of reagent and analysis time, and consequently producing very small amounts of waste solution.

Table 6. Analytical results for IN determination of edible oils by greener titrimetric method (n=3)

Oil	AOAC standard method		Proposed method		% Difference
	IN	%RSD	IN	%RSD	
Coconut	7.5	17.1	6.5	11.1	14
Palm	59.0	0.3	58.8	1.7	1
Olive	82.1	0.6	84.4	0.9	3
Rice bran	100.1	0.5	101.9	2.0	2
Sesame	108.8	0.4	106.2	1.3	2
Canola	109.9	0.6	106.5	2.3	3
Corn	112.3	0.7	110.2	2.1	2
Sunflower	126.2	0.5	127.3	0.4	1
Soybean	126.8	0.8	124.6	1.2	2
Oleic acid	85.9	0.5	90.3	1.3	5

4. Conclusions

The greener titrimetric method for determination of iodine number of edible oils provided various advantages such as short analysis time, low reagent and solvent consumption. By reducing the sample size, reagent, solvent, and reaction time could be dramatically reduced. The amount of solvent and reagent used were at least 25 times less than that of the classical standard method. By using simple titration method, there is no significant difference between the results obtained by the proposed method and the standard method for the IN in the range of 6.0-130.

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Attitude towards Food Associated with Food Preferences in Japanese Elementary and Junior High School Students

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Abstract

Food-related preferences and practices are formed in early childhood. Our prior study suggested that children's food preferences were related to their attitude towards food, especially "concern about food" and "respect for food." In this study, we investigated the association of the high and low level of "attitude towards food" to food preferences of 6–16-year-old students. In 2017, a questionnaire was given to 1,658 students and guardians who attended public school and junior high school in the Hyogo prefecture of Japan. A total of 497 (29.9%) completed questionnaires were returned. The Kruskal–Wallis, Mann–Whitney U, and Jonckheere–Terpstra tests were employed to assess any associations between the independent variables and three levels of "concern about food" and "respect for food" with significance being at $p < 0.05$. The number of foods disliked by the students significantly decreased with increasing levels of "attitude towards food". The present study suggests that the students' "attitude towards food" was associated with their food preferences.

Keywords: food preference, attitude towards food, elementary school children, junior high school students

1. Introduction

Food preferences are very complicated. Taste preferences are created in early childhood when children are predisposed to prefer high-energy, sugary, and salty foods, and also tend to reject new foods (Schwartz et al., 2011; Cosmi, Scaglioni & Agostoni, 2017). As noted above, these appear to be intimately related to children's behavior and development. Food neophobia is generally regarded as the reluctance to eat, or the avoidance of new foods. In contrast, picky or fussy eaters are usually defined as children who consume an inadequate variety of foods through rejection of foods that are familiar, as well as unfamiliar, to them (Dovey, Staples, Gibson & Halford, 2008). Picky eating among children is a common concern, yet little is known about how these behaviors develop in early childhood. Picky eaters consume fewer total fats, less energy, and less protein than children without picky eating behaviors (Dubois, Farmer, Girard & Peterson, 2007). Picky eating can affect children's nutritional intake and dietary quality, and negatively impact their growth and development (Dubois et al., 2007; Cano et al., 2016). It is important to improve children's food neophobia and/or picky or fussy eating habits. In this study, all of these behaviors are defined as food preferences, as teachers and parents were unable to distinguish among these behaviors. Eating habits learned in early childhood often continue throughout adulthood (Ventura & Worobey, 2013). Some researchers found that limited food preferences relate to children's human environment, with preferences resembling those of their mother, father, and siblings (Birch, 2013; Pliner & Pelchat, 1986). Parental food habits and feeding strategies are the most important determinants of a child's eating behavior and food choices (Scaglioni et al., 2018). A preference for fat taste in mothers correlates with overweight and obesity in their children (Sobek et al., 2020). Parents of picky eaters were more likely to report that their children consumed a limited variety of food (Mascola, Bryson & Agras, 2010; Osera, Tsutie, Kobayashi & Kurihara, 2016). It is important to increase a child's eating experience. Cosmi suggested that while genetically determined individual differences exist, repeated offerings of food can modify innate preferences

(Cosmi, Scaglioni & Agostoni, 2017). Early-life experiences with various tastes and flavors have a role in promoting future healthy eating (Scaglioni et al., 2018).So, the role of parents for children during childhood is important.

While the role of parents in the development of their children's food preferences is important, the specific method is not known. Our previous study suggested that 3–5-year-old children's "concern about food" and "respect for food" were associated with a decrease in the number of foods they disliked (Osera, Tsutie, Kobayashi & Kurihara, 2016). These concepts are reported in Japan to be important in the development of mind and body during childhood in "*shokuiku"* (a type of nutritional education) during kindergarten (MEXT, 2018). "Concern about food" and "Respect for food" are classified as attitude. In the KAB model, attitude is regarded as a leading factor of behavior.

In addition "Respect for food" important for not only food preferences but also Sustainable Development Goals (SDGs), there are connected with good health and well-being and quality education (United Nations, 2015). Our previous study suggested that the relationship between food preferences and their attitude especially "Respect for food" during childhood (Osera, Tsutie, Kobayashi & Kurihara, 2014). In the present study, we aimed to determine whether the same concepts could be found in children of 6–16 years of age in elementary and junior high school.

2. Method

This was a cross-sectional study. From May to September 2017, a questionnaire was given to 1,658 mothers and their children, 6–16 years of age, who lived in Hyogo prefecture. In addition, we suggested that the 6-9 year old students answer the questionnaire with their guardians.

2.1 Questionnaire

Anthropometric measurements (weight and height) were self-reported. Body mass index (BMI) was used to indirectly assess adiposity and was calculated as weight/height^2 (kg/m^2). BMI cutoffs for the children were generated from pooled international data for BMI and were linked to BMI cutoff points used in adults (Kazmarzyk, Janssen, Morrison & Tremblay, 2007). The Japan Obesity Society's BMI cutoff points for adults were used in the current analyses to classify weight status in Table 1 (Miyazaki, 2018).

Student's food habits and lifestyle, including behaviors and attitudes, are shown in Tables 2 and 3. The questionnaire contained nine questions on food habits and attitudes toward food which referred to our previous study's questionnaire (Osera, Taniguchi, Hashimoto & Kurihara, 2018; Osera et al., 2017a). The questionnaire comprised one questionnaire on "concern about food", "respect for food", self-rated health (SRH), "talk about food", "talk about taste", "help set the table", "help cooking", "liking school lunch", "liking home meals". Both 5 point rating scales were used, with higher scores indicating more positive food habits. For example, questions regarding 'concern about food' utilized a 5-point rating scale (5 = high concern, 4 = middle concern, 3 = concern, 2 = little concern, 1 = no concern). In addition, we analyzed student's preferences at each of the three levels of "respect for food (respect)" and "concern about food (concern)". We grouped scores of the "concern" and "respect" variable into three categories: the 'high' included the responses 'higher', 'middle' included the responses 'high' whereas 'low' includes the responses 'medium', 'low', and 'none' according to the result of Table 2 and our previous study (Osera, Tsutie, Kobayashi & Kurihara, 2016).

In addition, questions about SRH measures were included with five response categories (Joffer, Jerden, Ohman & Flacking, 2016; Warnoff et al., 2016). In this study, we grouped scores of the SRH variable into two categories: the 'excellent' included the responses 'excellent' whereas 'other' includes the responses 'very good', 'good', 'fair' and 'poor' in Table 3.

Student's likes and dislikes were included in the questionnaire with the response to each being either "yes" or "no." If students answered "yes", students also chose the foods they disliked by themselves from a list of the following 55 foods: noodles, rice, bread, konjac, sweet potatoes, potatoes, azuki beans, soybeans, freeze-dried tofu, tofu, deep-fried tofu, sesame, pumpkin, peas, string beans, carrots, leeks, green peppers, broccoli, spinach, Japanese mustard spinach, cabbage, cucumber, burdock, Japanese radish, onions, corn, eggplant, Chinese cabbage, tomatoes, cherry tomatoes, bananas, tangerines, apples, pineapple, Enoki mushrooms, Shimeji mushrooms, dried shitake, toasted laver, Hijiki, seaweed, squid, shrimp, fish paste cake, Spanish mackerel, salmon, liver, beef, chicken, pork, cheese, yogurt, milk, eggs, and quail eggs. The foods on the list were selected from what is available at regular school lunches and often disliked by children as noted in our previous study (Osera, Tsutie, Kobayashi & Kurihara, 2016).

2.2 Statistical Analysis

We compared the student's food behaviors, including preferences, likes and dislikes, based on the three levels of respect and concern. In addition, we noted the top 10 disliked foods listed by the guardians or themselves. We compared the ratio of children who disliked each of the top 10 foods by the three levels of "respect" and "concern." The Kruskal–Wallis, Mann–Whitney U, and Jonckheer–Terpstra tests were used to compare the results using SPSS version 25.0 J (IBM, New York, NY, USA).

2.3 Ethics Statement

The students and their guardians were fully informed about the objectives and methods of this study. They voluntarily answered the questionnaire without any compulsion and with the understanding that they could withdraw from the study at any time. Individual privacy was strictly protected throughout the investigation. Signed consent was obtained from the guardians of each child. This study was approved by the Kobe Women's University Ethics Committee regarding Human Subjects (approval number H29-1).

3. Results

3.1 Characteristics of the Answers for the Questions related to Food Habits

Responses for questionnaire items related to food habits and lifestyle are shown in Tables 1 to 3. A total of 497 questionnaires were analyzed, which included 233 children of 6–12 years of age and 153 children of 13–16 years of age. There was no significant relationship among age, sex, and BMI (Table 1). Table 2 showed that there was a significant relationship between food preferences and "concern" or "respect."

Table 1. Baseline characteristics classified by food preferences

	Food preferences	**Presence**		**Absence**		**P value***
Items	Category	N	%	N	%	
Age	6–12	233	(58.0)	58	(58.0)	N.S.
(y.o.)	13–16	153	(39.6)	42	(42.0)	
Sex	Boy	166	(43.1)	54	(54.5)	N.S.
	Girl	219	(56.9)	45	(45.5)	
BMI	<18.5	290	(76.3)	66	(67.3)	
(kg/m^2)	18.5–24.9	85	(22.4)	32	(32.7)	N.S.
	>=25.0	5	(1.3)	0	(0.0)	

* Fisher's exact test.

Table 2. Association between food preferences and "Concern about food"/ "Respect for food"

Food preferences		**Presence**		**Absence**		**P value***
Items	Category	N	%	N	%	
Concern about food	None	16	(4.1)	0	0.0	0.000
	Low	37	(9.6)	2	2.0	
	Medium	73	(18.9)	9	9.0	
	High	150	(38.9)	39	39.0	
	Higher	110	(28.5)	50	50.0	
Respect for food	None	2	(0.5)	0	0.0	0.000
	Low	17	(4.4)	2	2.0	
	Medium	43	(11.2)	4	4.0	
	High	155	(40.4)	23	23.0	
	Higher	167	(43.5)	71	71.0	

* Fisher's exact test.

3.2 Disliked Foods Stratified by "Concern about Food" and "Respect for Food" Levels

Of all the children, 79.4% had some food disliked. The average number of disliked foods, of the 55 items listed, was 5.3 ± 5.0. The quartiles are shown in Table 3.

Children in the low "Concern" level group had significantly more disliked foods compared with the middle and high "Concern" level groups (6.0 ± 5.2, 5.1 ± 5.6, and 4.6 ± 3.8 items, respectively, $p < 0.05$). Children in the low "Respect" level group had significantly more disliked foods compared with the middle and high groups as

noted in Tables 4 and 5 (7.0 ± 6.7, 4.9 ± 4.4, and 4.9 ± 4.7 items respectively, $p < 0.05$). The number of foods that children disliked decreased significantly as the level of "Concern about food" and/or "Respect for food" increased.

Children chose the foods their children disliked from a list of 55 foods. The ratio of children who disliked each of the top 10 foods was investigated. Figure 1 showed that when children who disliked each of some of the top 10 disliked foods significantly or tendency reduced. This was similar to the number of foods children disliked relative to "Respect" and "Concern" shown below in Tables 4 and 5.

Table 3. Number of disliked food

Count	N	%
1 to 2	116	(32.8)
3 to 4	93	(26.3)
5 to 7	68	(19.2)
8 to 42	77	(21.8)

Table 4. Average number of disliked foods among three levels of "Concern about food"

	N	Mean		SD		
Low Concern	116	6.0	±	5.2	#	# *
Middle Concern	138	5.1	±	5.6		
High Concern	100	4.6	±	3.8	N.S.	

* $p<0.05$ by Kruskal-Wallis test.

# $p<0.05$ by Mann–Whitney U test.

SD: standard deviation N.S.: not significant

Table 5. Average number of disliked foods among three levels of "Respect for food"

	N	Mean		SD		
Low Respect	62	7.0	±	6.7	#	## *
Middle Respect	149	4.9	±	4.4		
High Respect	142	4.9	±	4.7	N.S.	

* $p<0.05$ by Kruskal-Wallis test.

# $p<0.05$, ## $p<0.01$ by Mann–Whitney U test.

SD: standard deviation N.S.: not significant

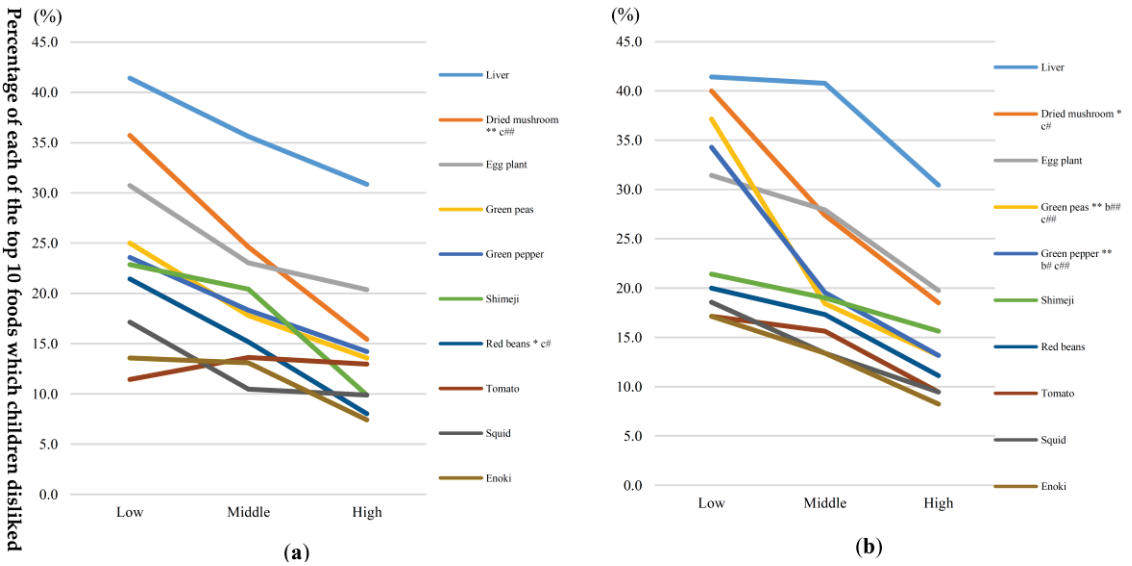

Figure 1. Percentage of children who disliked each of the top 10 disliked foods in the high, middle, and low groups stratified by (a) concern about food and (b) respect for food

* $p < 0.05$, ** $p < 0.01$ by Jonckheere–Terpstra test; # $p < 0.05$, ## $p < 0.01$ by Mann–Whitney *U* test; a, High vs. Middle; b, Middle vs. Low; c, High vs. Low.

3.3 Relationship between Presence or Absence of Food Preferences and Attitudes toward Food based on high to low "Concern about food" and "Respect for food"

As shown in Table 2, we classified concern and respect into the following five levels: none, low, medium, high, and higher. The individual questions from the low and middle groups were subsequently stratified by "other" and "higher." We combined three groups to determine the relationship between the presence or absence of food preferences and concern/respect for food (data not shown). There was a significant relationship between food preferences and "Concern"/ "Respect" ($p < 0.001$).

There was a significant relationship between food preferences and children's attitudes toward food without "help set the table" in "Concern." In the low and high groups, there was no significant relationship between food preferences and attitude toward food. In the middle group, there was no significant relationship between food preferences and attitude toward food except for talk about taste noted in Table 6 and Appendix Table A1.

In "Respect for food," there was a significant relationship between food preferences and attitude toward food except for "talk about food" and "help set the table." In the high and middle groups, there was no significant relationship between food preferences and attitude toward food. In the high group, there was a significant relationship between food preferences and attitude toward food except for "SRH," "talk about food," and "help set the table." In "respect for food," there was a significant relationship with "liking school lunch" and food preferences noted in Table 6.

Table 6. Association between food preferences and food habits based on high to low "Respect for food"

Respect for food		**Low**					**Middle**				
Food preferences		**Presence**		**Absence**		**P value***	**Presence**		**Absence**		**P value***
Items	Category	N	%	N	%		N	%	N	%	
SRH	Other	7	29.2	0	0	N.S.	13	36.1	1	8.3	N.S.
	Very good	17	70.8	2	100		23	63.9	11	91.7	
Talk about food	Other	54	87.1	6	100	N.S.	131	85.6	19	82.6	N.S.
	Higher	8	12.9	0	0		22	14.4	4	17.4	
Talk about taste	Other	50	80.6	5	83.3	N.S.	122	78.7	17	73.9	N.S.
	Higher	12	19.4	1	16.7		33	21.3	6	26.1	
Help set the table	Other	39	88.6	4	80	N.S.	81	81	13	86.7	N.S.
	Higher	5	11.4	1	20		19	19	2	13.2	
Help Cooking	Other	42	95.5	5	100	N.S.	92	92	14	93.3	N.S.
	Higher	2	4.5	0	0		8	8	1	6.7	
Liking School Lunch	Other	28	65.1	2	40	N.S.	62	62	9	60	N.S.
	Higher	15	34.9	3	60		38	38	6	40	
Liking Home meals	Other	40	64.5	4	66.7	N.S.	63	40.9	11	47.8	N.S.
	Higher	22	35.5	2	33.3		91	59.1	12	52.2	

* Fisher's exact test.

Respect for food		**High**					
Food preferences		**Presence**		**Absence**		**P value***	**P value***
Items	Category	N	%	N	%		
SRH	Other	9	27.3	2	14.3	N.S.	0.048
	Very good	24	72.7	12	85.7		
Talk about food	Other	121	72.5	44	62	N.S.	N.S.
	Higher	46	27.5	27	38		
Talk about taste	Other	106	63.9	34	47.9	0.03	0.002
	Higher	60	36.1	37	52.1		
Help set the table	Other	61	69.3	24	63.2	N.S.	N.S.
	Higher	27	30.7	14	36.8		
Help Cooking	Other	80	90.9	29	76.3	0.044	0.044
	Higher	8	9.1	9	23.7		
Liking School Lunch	Other	46	52.3	8	21.1	0.002	0.000
	Higher	42	47.7	30	78.9		
Liking Home meals	Other	50	29.9	11	15.5	0.023	0.011
	Higher	117	70.1	60	84.5		

4. Discussion

In this study, we found that Japanese elementary and junior high school students' food preferences were associated with their "Attitude towards food." This is evidence for continuing to teach "Concern about food" and "Respect for food" during childhood and school age (Osera, Tsutie, Kobayashi & Kurihara, 2016).

Picky children displayed more problem behaviors, both internalizing and externalizing, than non-picky eaters (Jacobi, Schmitz & Agras, 2008). On the other hand, early childhood eating problems were not associated with later eating problems at 16 years of age (Hafstad, Soest & Torgersen, 2013). Dovey et al. found that food neophobia peaked at the ages of 2 to 6 years (Dovey, Staples, Gibson & Halford, 2008). A previous study of ours found that neophobia disappeared in 10% of children aged 4 to 6 years (Osera, Tsutie, Kobayashi & Kurihara, 2014). Additionally, another previous study of ours suggested that although children may dislike some types of food, these dislikes may disappear as they become adolescents (Osera et al., 2017 b). In addition, our previous study had a cross-sectional design, so we could not establish a clear causal relationship. However, the KAB model has shown that knowledge is a key factor in behavior (Ren et al., 2020).

The three levels of "concern about food" and "respect for food" are not significantly different among the levels as noted in Tables 6, and A-1. "Respect for food" was significantly related to "liking school lunch." The aim of Japanese public kindergarten guidelines is attention to "concern about food" (MEXT, 2018). As well, the aim of Japanese public primary school is attention to feelings for food (MEXT Course of home economics, 2018), which is similar to "respect for food" in this study. Additionally, our previous study suggested that changes in food preferences among 4 to 6-year-old children were related to their food habits. Enjoying school lunches and respect for food had a significant relationship with changes in food preferences among 4 to 6-year-olds within four groups (Osera, Tsutie, Kobayashi & Kurihara, 2014). "Respect for food" in the high group had a significant relationship between food preferences and enjoying school lunch. This result supports the aim of the Japanese public primary schools of teaching attention to feelings for food (MEXT Course of home economics, 2018). In addition, Akamatsu & Iuchi (2009) have performed research related to "Gratitude for food" . Their research revealed that children scoring higher in "Gratitude for food" ate vegetables every day compared with other children in grades 5 and 6 who hardly ate any vegetables. Based on these results, the presence or absence of food preference is likely to differ according to children's attitude toward food.

This study's top five disliked foods were liver, dried mushrooms, eggplant, green peas, and green peppers. These were the same as the most disliked foods as in our previous study (Osera, Tsutie, Kobayashi & Kurihara, 2016). We found the same most disliked foods among high school students in this study as in our previous study (Osera et al., 2017). The same five foods were the most disliked in three different stages of life, but this is not representative of all Japanese, and we would like to repeat the study in different aged populations. In part, the results of this study may have been due to the presence of supertasters, those able to detect 6-n-propylthiouracil (PROP) (Fox, 1932; Cohen & Ogdon, 1949; Harris & Kalmus, 1949). Kosugi & Horio (2005) found that 28% of the population were supertasters, and this taste sensitivity to PROP may be related to food preferences. As such, children's food preferences may be determined not only psychologically but also by physiology of taste.

Rahill et al. (2018) found that age affects the level of food fussiness in children, with younger children (5–8 years old) having higher levels of food fussiness than older children (9–12 years old). This finding is similar to those observed in earlier studies (Powell, Farrow & Meyer, 2011; Hursti & Sjoden, 1997; Ashcroft et al., 2008). Older children are more likely to have had greater exposure to various foods, and they will become less neophobic as fewer foods are novel them (Cooke & Wardle, 2005). In a future study, we will assess subjects of different ages. In a previous study, 74.6% of the mothers noted that their children disliked one or more foods (Osera, Tsutie, Kobayashi & Kurihara, 2016). In this study, 79.4% of the students answered that they disliked one or more foods. Additionally, the average number of disliked foods was the same as that of our previous study (Osera, Tsutie, Kobayashi & Kurihara, 2016).

In addition "Respect for food" important for not only food preferences but also SDGs, there are connected with good health and well-being and quality education (United Nations, 2015). We think that the "Respect for food" is linked to sustainability.

Limitation of this study is that it cannot make clear the causal relationship. In the near future, we would test the hypotheses by interventional studies on the basis of the result of this study and our previous study (Osera, Tsutie, Kobayashi & Kurihara, 2016) Based on behavior science, "attitude" is the leading factor of behavior. In addition, our previous children's retrospective cohort study suggested the same factors shown as this study's result; "Enjoying school lunch" and "Respect for food" (Osera, Tsutie, Kobayashi & Kurihara, 2014). On the other hand, there study was small number and groups of different ages. It is necessary to continue the same survey in the future and confirm whether the same results can be obtained. Larger number of the study population with varied age distribution will be needed. However, it is an advance that similar trends in attitudes about food preferences were made clear in early childhood and elementary school and junior high school students. In addition, self-reported anthropometric measurements in young adults used to calculate BMI has its limitations. However, this will have potential impact on the results especially with the presence of the sample PROP. To future understand this phenomenon, we will do an interventional study on the basis of the result of this study in the near.

In this study, we found that student's food preferences were associated with their "Attitude towards food". These findings may provide important information for improving food preferences not only in young children but also in elementary school and junior high school students. In addition, this is the evidence for the importance of teaching "Respect for food" in schools.

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Appendix A

Table A-1. Association between food preferences and food habits based on high to low "Concern about food"

Concern about food		**Low**					**Middle**				
Food preferences		**Presence**		**Absence**		**P value***	**Presence**		**Absence**		**P value***
Items	Category	N	%	N	%		N	%	N	%	
SRH	Other	14	36.8	0	0	N.S	10	29.4	2	22.2	N.S
	Very good	24	63.2	5	100		24	70.6	7	77.8	
Talk about food	Other	113	90.4	11	100	N.S	128	85.3	29	74.4	N.S
	Higher	12	9.6	0	0		22	14.7	10	25.6	
Talk about taste	Other	106	85.5	11	100	N.S	112	74.7	21	53.8	0.017
	Higher	18	14.5	0	0		38	25.3	18	46.2	
Help set the table	Other	68	86.1	5	83.3	N.S	70	72.2	16	64	N.S
	Higher	11	13.9	1	16.5		27	27.8	9	36	
Help cooking	Other	76	96.2	6	100	N.S	85	87.6	20	80	N.S
	Higher	3	3.8	0	0		12	12.4	5	20	
Liking school lunch	Other	54	68.4	2	33.3	N.S	60	62.5	10	40	N.S
	Higher	25	31.6	4	66.7		36	37.5	15	60	
Liking home meals	Other	60	47.6	4	36.4	N.S	67	45.0	15	38.5	N.S
	Higher	66	52.4	7	63.6		82	55.0	24	61.5	

* Fisher's exact test.

Concern about food		**High**					
Food preferences		**Presence**		**Absence**		**P value***	**P value***
Items	Category	N	%	N	%		
SRH	Other	5	22.7	1	7.1	N.S	0.048
	Very good	17	77.3	13	92.9		
Talk about food	Other	67	61.5	29	58	N.S	0.021
	Higher	42	38.5	21	42		
Talk about taste	Other	61	55.5	24	48	N.S	0.002
	Higher	49	44.5	26	52		
Help set the table	Other	44	77.2	20	74.1	N.S	N.S
	Higher	13	22.8	7	25.9		
Help cooking	Other	54	94.7	22	81.5	N.S	0.043
	Higher	3	5.3	5	18.5		
Liking school lunch	Other	23	40.4	7	25.9	N.S	0.000
	Higher	34	59.6	20	74.1		
Liking home meals	Other	27	24.5	7	14.0	N.S	0.011
	Higher	83	75.5	43	86.0		

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A Novel Dairy Fermented Frozen Dessert with Honey and Pomegranate Juice: Physicochemical, Rheological and Sensory Properties

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Abstract

The effect of fat content and added yoghurt, honey and pomegranate juice concentration on the overrun, and the physicochemical, rheological and sensory properties of frozen yoghurt samples was investigated, aiming in the production of a novel low-fat and functional dairy fermented frozen dessert. For this purpose, the methodology of mixture experiment was applied to twenty samples, while a control sample (without using honey and pomegranate juice) was also produced. According to the results, the increase in yoghurt concentration resulted in increasing pH, overrun, brightness, elastic modulus (G´) determined at -2 °C and sensory hardness of the samples, while it reduced color parameters a* and b*, creaminess, sweetness and fattiness. Increasing honey concentration reduced lactic acid concentration, G´ at -2 °C, hardness, sensory acidity, and to a lesser extent pH, while increased color parameter b*, overrun, sensory color intensity, creaminess, sweetness, and fattiness of the samples. The increase in pomegranate juice concentration resulted in decreasing pH, brightness and b*, as well as increasing a*, color intensity, creaminess and sensory acidity. Finally, fat, by interacting with one or two of the three constituents (yoghurt, honey and pomegranate juice), decreased pH, creaminess and fattiness, while increased lactic acid concentration, b*, color intensity and to a lesser degree the overrun level of the samples. Overall acceptability of the samples indicated that it is possible to use honey and pomegranate juice in the production of low-fat frozen yoghurt with favorable sensory properties.

Keywords: fat content, frozen yoghurt, honey, pomegranate juice, sensory properties

1. Introduction

Yoghurt, a fermented dairy product, is known for its health promoting properties and its high nutritional value. The increased value of yoghurt, when compared to milk, is due to the presence of beneficial bacteria and specific bioactive compounds. These ingredients, together with the nutritional profile of yoghurt, make it a particularly important dairy product, which is widely accepted worldwide and associated with a healthy diet (Tamime & Robinson, 2007; Fernandez et al., 2017).

Ice cream is a dairy product that is consumed as an ice cream dessert and is prepared by freezing the ice cream mixture, with constant stirring (Deosarkar et al., 2016). It contains dairy and non-dairy ingredients (including sweetening agents) (Goff & Hartel, 2013) and is proposed as an effective means of growth of probiotic organisms (Cruz et al., 2009). It is of great value, as it contains high quality protein and calcium, which is easily assimilated by the body. Today, ice creams of a wide variety of flavors are available (Deosarkar et al., 2016).

Frozen yoghurt is a complex dairy fermented frozen dessert. The increasing interest in frozen yoghurt is due to its desirable properties, as well as its nutritional value. It combines the cooling effect of ice cream with the sensory and nutritional properties of fermented milk products (Tamime & Robinson, 2000; Pinto et al., 2012).

Consumers trends for a healthier diet has turned researchers interest in developing functional dairy desserts with low-fat content that are sugar-free and are further enriched with nutritional-functional additives.

Honey is a natural sweetener that can substitute sugar in many dairy products (Dimitreli et al., 2019; Sarkar & Chandra, 2019), including frozen yoghurt, the use of which has not been reported so far. Honey has been used since ancient years due to its nutritional and therapeutic properties. Among other things, honey plays an

important role as an antioxidant, anti-inflammatory and antibacterial agent (Meo et al., 2017).

Frozen yoghurt may contain numerous flavoring agents including fruit juices. Pomegranate is very popular, due to its biological effects, as it contains phenolic compounds, which account for strong antioxidant activity and specific physiological functions (antitumour, anti-inflammatory) (Gil et al., 2000; Karimi et al., 2017). Pomegranate juice have been used in many dairy and no-dairy beverages (Dimitreli et al., 2019), however, its use in frozen yoghurt has not been reported yet.

Therefore, the aim of the present work was to evaluate the effect of yoghurt concentration, honey and pomegranate juice addition, as well as fat content on the physicochemical, rheological and sensory properties of frozen yoghurt samples. The idea for this project came from the need to develop a functional low-fat, sugar-free, dairy dessert, which would combine the high nutritional value of yoghurt and the appealing characteristics of ice cream. For this reason, honey was used, instead of sugar, as a sweetening agent, while the nutritional value of the product was further enriched with the addition of pomegranate juice.

2. Materials and Methods

2.1 Materials

Semi-skimmed (1.5% fat) pasteurized and homogenized bovine milk, milk cream, milk powder, pine honey, pomegranate juice and bottled water were purchased from the local market. For the preparation of pomegranate juice, the fruits are washed, drained, cut up and finally pressed. The extracted juice is filtered, pasteurized at 72 °C for 15 s and then packaged. Commercial Direct Vat Set type starter culture, consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* και *Streptococcus thermophilus* (Jointex X3, Dosi 4; CSL Centro Spermentale, de Latte S.P.A, Zelo Buon Persico, Italy), and stabilizer (Cream Gold 50, Technoblend, Zona industrial JESCE sn, Matera, Italy) were also used for yoghurt and frozen yoghurt samples production, respectively.

2.2 Preparation of Yoghurt

The milk was heat treated at 95 °C for 5 min with constant stirring, cooled to 42 °C, inoculated with the starter culture (according to the manufacturer's instructions) and incubated at 42 °C for about 6 h until the pH reached 4.6. Following fermentation, the yoghurt was cooled to room temperature (approximately 20 °C), stirred and placed at 4 °C for 24 h before further using.

2.3 Frozen Yoghurt Samples Production

The chemical composition of the raw materials used for frozen yoghurt production is given in Table 1. The preparation of the frozen yoghurt samples was based on the design of the mixture experiment, presented in Table 2. The amounts of the ingredients added (per 110 g of sample) ranged from 57 to 80 g for yoghurt, 6 to 18 g for honey and 0 to 15 g for pomegranate juice. Fat content was adjusted at two different percentages (levels) 1% and 3%. The final proportion of the ingredients of the ice cream mixture (Table 3) was obtained by taking into account the chemical composition of the raw materials (Table 1) (yoghurt, milk cream, skimmed milk powder, honey) and the following equation that calculates the milk solids-not-fat content of each sample (honey concentration was expressed in dry basis):

$$\text{Milk solids-not-fat (\%)} = [100 - (\text{Fat \%} + \text{Honey \%} + \text{Stabilizer \%})] / 7 \quad (1)$$

where Fat % is the final fat content of the samples, Honey % and Stabilizer % are the percentages of the ingredients added in the ice cream mixture. Equation 1 calculates the milk solids-not-fat that contribute to the freezing point depression, as a function of fat and non-dairy ingredients (sweetening agents, stabilizers) concentration, so as a balance of the ice cream mixture to be achieved.

For the needs of the experiment an additional sample (control) was prepared using sugar, as a sweetening agent, instead of honey (Table 3). Stabilizer was added at a percentage of 2.5% to all frozen yoghurt samples.

The ingredients of the ice cream mixture were mixed in the following order: water and milk cream were first mixed and heated at 50 °C. The milk powder and the stabilizer were then added with simultaneous stirring until dissolve. The honey was incorporated into the mixture, which was then heat treated at 72 °C for 10 min and cooled to ambient temperature before yoghurt and pomegranate juice addition. The ice cream mixture was then placed at 4 °C for 24 h. Following ripening, the mixture was frozen at −6 °C under continuous stirring, using a laboratory ice cream maker (Grand Gelato GIRMI). The frozen yoghurt samples were packaged into 200mL plastic cups and stored at −18 °C until further analysis. For oscillatory testing, the samples were placed into special made containers (see 2.6 subsection). Frozen yoghurt samples were produced in duplicate.

2.4 Physicochemical Analysis

Physicochemical analysis of the frozen yoghurt samples involved pH (using a laboratory pH meter, GP353 ATC, EDT Instruments, Kent U.K.), acidity (expressed as lactic acid concentration %, AOAC, 1998) and color determination using a tristimulus colorimeter (Micro Color LMC, Dr. Bruno Lange GmbH, Dusseldorf, Germany). The color components evaluated were L* (brightness), a* (+ red to – green component) and b* (+ yellow to – blue component) of the CIE Lab scale (Hunter Lab format). Physicochemical measurements were conducted in triplicate.

Table 1. Chemical composition of the raw materials used for the production of frozen yoghurt

Raw materials	Dry matter (%)	Fat (%)
Yoghurt	10.10	1.4
Milk cream	40.50	35.0
Skimmed milk powder	82.10	0.8
Honey	82.74	-
Pomegranate juice	13.30	-

Table 2. The mixture experiment design

Sample	Yoghurt (g)*	Honey (g)*	Pomegranate juice (g)*	Fat (%)
1	80.0	6.0	4.0	1.0
2	69.0	6.0	15.0	1.0
3	57.0	18.0	15.0	1.0
4	80.0	10.0	0.0	1.0
5	72.0	18.0	0.0	1.0
6	71.6	11.6	6.8	1.0
7	75.8	8.8	5.4	1.0
8	70.3	8.8	10.9	1.0
9	64.3	14.8	10.9	1.0
10	71.8	14.8	3.4	1.0
11	80.0	6.0	4.0	3.0
12	69.0	6.0	15.0	3.0
13	57.0	18.0	15.0	3.0
14	80.0	10.0	0.0	3.0
15	72.0	18.0	0.0	3.0
16	71.6	11.6	6.8	3.0
17	75.8	8.8	5.4	3.0
18	70.3	8.8	10.9	3.0
19	64.3	14.8	10.9	3.0
20	71.8	14.8	3.4	3.0

* The amount of each component (except fat) is expressed as g / 110 g of sample

2.5 Overrun Determination

The overrun of the frozen yoghurt samples was calculated, as described in Goff and Hartel (2013), according to equation 2.

$$\text{Overrun } \% = [(W_1 - W_2) / W_2] \times 100 \quad (2)$$

where W_1 is the weight of the ice cream mixture and W_2 the weight of the same volume of ice cream.

2.6 Rheological Measurements

Rheological measurements of the frozen yoghurt samples were performed, using a DMA rheometer (Bohlin C-VOR 150, Malvern Instruments, Ltd, Worcestershire, UK), over a temperature range of –2 °C to 30 °C, stimulating the conditions that the sample melts into the mouth. A Peltier plate system (–30 to + 180 °C), adapted to the rheometer, was used for temperature control. Rheological measurements were conducted in triplicate.

For oscillatory testing a serrated plate and plate geometry was used in order to avoid slip effects. The upper plate that had a diameter of 40 mm was lowered toward the upper surface of the sample which was placed into

specially made aluminium containers (10 mm height and 40 mm diameter). The height of the samples inside the containers was approximately 2000 μm. A strain deformation of 1.5×10^{-5}, which was within the linear viscoelastic region, at a frequency of 1 Hz was applied and the elastic (G′) and viscous (G″) moduli of the samples over the set temperature range were recorded. From the derived mechanical spectra, the G′ and the loss tangent (tan δ) at −2 °C and 28 °C were obtained.

The apparent viscosity of the samples was determined using a plate and cone geometry. Particularly, the instrument was equipped with a 4° stainless steel cone that was lowered to the measuring position set at a 150 μm gap, after the sample was placed between the cone and the parallel plate-base of the rheometer. The applied strain was 40 s^{-1} (corresponding approximately to the strain applied to the sample during swallowing) (Omar et al., 1995). The apparent viscosity at −2 °C and 28 °C was obtained.

Table 3. The final ratio of the ingredients of the ice cream mixture (in g), corresponding to each sample (total mass: 100 g)

Sample	Milk cream	Yoghurt	Water	Honey	Pomegranate Juice	Skimmed milk powder	Stabilizer
1	−	72.8	8.6	6.6	3.6	5.9	2.5
2	0.3	62.8	7.4	6.6	13.7	6.7	2.5
3	0.9	51.9	5.3	19.7	13.7	6.0	2.5
4	−	72.8	8.3	10.9	−	5.5	2.5
5	0.3	65.5	7.0	19.7	−	5.0	2.5
6	0.3	65.2	7.3	12.7	6.2	5.8	2.5
7	−	69.0	8.1	9.6	4.9	5.9	2.5
8	0.3	64.0	7.4	9.6	9.9	6.3	2.5
9	0.6	58.5	6.4	16.2	9.9	5.9	2.5
10	0.3	65.3	7.2	16.2	3.1	5.4	2.5
11	5.7	72.8	3.4	6.6	3.6	5.4	2.5
12	6.0	62.8	2.4	6.6	13.7	6.0	2.5
13	6.6	51.9	0.3	19.7	13.7	5.3	2.5
14	5.7	72.8	3.3	10.9	−	4.8	2.5
15	6.0	65.5	1.9	19.7	−	4.4	2.5
16	6.0	65.5	2.2	12.7	6.2	5.2	2.5
17	5.7	69.0	3.0	9.6	4.9	5.3	2.5
18	6.0	64.0	2.4	9.6	9.9	5.6	2.5
19	6.3	58.5	1.3	16.2	9.9	5.3	2.5
20	6.0	65.3	2.2	16.2	3.1	4.7	2.5

Sample 21/ Control sample: Milk cream: 0.3 g; Yoghurt: 65.5 g; Water: 10.3 g; Sugar**:** 16.4 g; Pomegranate juice: 0.0 g; Skimmed milk powder: 5.0 g; Stabilizer: 2.5 g.

2.7 Sensory Analysis

The sensory analysis design was a randomized balanced incomplete block design (BIB), which is characterized by the following parameters: t = 21 treatments (samples), k = 5 treatments assessed by each panelist, b = 21 panelists, n = 5 replicates of each treatment in the design and λ = 1 pair of similar treatments. The design in question was carried out twice, so that totally 42 samples (including the control sample) were assessed by the panelists.

The sensory profile of the samples was evaluated by determining seven objective sensory variables (intensity of color, hardness, creaminess, intensity of typical yoghurt taste, sweetness, acidity and fattiness) and one subjective / "hedonic" variable (overall acceptability of the product). Trained panelists were asked to express their opinion for each sample and variable by ticking on an unstructured linear scale, whose length was 15 cm and whose left end (0 cm) was assigned as "not at all intensive" and the right end (15 cm) was assigned as "very intensive", except for the intensity of color (whose left end was assigned "very pink", its center was assigned "no color-white" and its right end was assigned as "very yellow-brown"). Both the order of the panelists and the sequence of the samples on each plate were previously randomized to avoid statistical bias.

Prior to the analysis, the samples were placed into cups made of Styrofoam, at a temperature range between −10 °C and −6 °C. Additionally, bottled water in a plastic glass was provided to each panelist in order to clean his

palate during the time period between the samples.

2.8 Statistical Analysis

In the context of the statistical analysis, a mixture experiment design was utilized. Thirteen statistically significant response variables were analyzed and additionally three design variables / factors (yoghurt, honey and pomegranate juice) and one process variable (fat) were included in the design. Each response variable was regressed against the design variables and the process variable by applying the special cubic polynomial model, which is the most suitable model regarding the mixture experiment designs. All the terms were subjected to the forward selection model and only the statistically significant terms (including the factors which are always used) were included in the final equations. Reliability and validity of the regression equations were evaluated by observing the determined (R^2), the adjusted (R^2_{adj}) and the predicted regression coefficients (R^2_{pred}) and the lack of fit test value ($P > 0.05$), respectively. It should be noted that the coefficients R^2 and R^2_{pred} should differ by a percentage lower than 20 % in order to ensure an adequate reliability of the model.

In addition, main effect plots (corresponding to the process variable), contour plots and Cox response trace plots corresponding to the response variables were utilized to examine the particular trends that are developed between the response variables and the factors.

Finally, the method of MaxDiff was used, so as to find the most acceptable samples according to panelists' preference, and a proportion preference (Best-Worst scaling) for all treatments was derived.

3. Results and Discussion

The mean values of the physicochemical characteristics and overrun are shown in Table 4, while the mean values of the rheological and sensory properties are presented in Tables 5 and 6, respectively. Based on the results of the forward selection, reducing models in the form of polynomial equations were formed (Table 7).

Table 4. Mean values of the physicochemical properties and overrun of the frozen yoghurt samples

Sample	pH	Acidity (% lactic acid)	L*	a*	b*	Overrun (%)
1	4.96	1.075	78.745	0.270	8.920	33.75
2	4.68	1.180	73.115	3.145	7.370	35.75
3	4.76	1.000	67.100	3.120	10.100	40.60
4	4.99	0.850	88.355	− 2.490	13.045	37.70
5	5.11	0.495	84.070	− 1.940	15.210	39.95
6	4.86	0.920	74.280	1.220	9.230	36.55
7	4.97	0.720	77.295	0.810	9.080	35.35
8	4.57	1.005	74.645	1.850	8.210	36.40
9	4.75	1.045	72.820	1.900	9.625	38.00
10	4.94	0.920	75.185	0.260	10.850	37.15
11	5.01	0.925	79.975	0.110	9.560	37.10
12	4.67	1.125	72.025	2.740	8.190	41.65
13	4.52	1.115	64.675	3.560	10.830	44.40
14	4.92	0.890	86.515	− 2.020	14.500	40.75
15	4.85	0.875	82.205	− 1.700	16.955	42.45
16	4.78	1.015	74.630	0.880	10.905	40.90
17	4.83	0.985	77.110	0.405	10.280	35.35
18	4.81	1.080	73.320	1.570	9.595	37.90
19	4.65	1.015	69.875	2.125	10.980	41.70
20	4.82	1.050	75.650	0.480	12.005	36.80
21*	5.12	0.700	89.240	− 4.420	13.665	54.20

*Sample 21 is the control frozen yoghurt sample

3.1 Physicochemical Properties

The pH values of the samples ranged from 4.52 to 5.12, while their acidity expressed as lactic acid concentration %, ranged from 0.495% to 1.180% (Table 4). In general, samples with increased percentage of added yoghurt or/and increased pomegranate juice concentration exhibited lower pH values and increased lactic acid concentration, due to the low pH values of yoghurt and pomegranate juice (the pH values for yoghurt and pomegranate juice were 4.6 and 3.1, respectively).

Table 5. Mean values of the elastic modulus (G´), tanδ and apparent viscosity of the frozen yoghurt samples at two different temperatures (−2 °C and 28 °C).

Sample	G´ (–2 °C) (Pa)	G´ (28 °C) (Pa)	tanδ (–2 °C)	tanδ (28 °C)	Viscosity (–2 °C) (Pa · s)	Viscosity (28 °C) (Pa · s)
1	81.7	8.0	0.593	0.599	0.354	0.100
2	73.7	7.4	0.683	0.700	0.145	0.081
3	36.3	5.7	0.619	0.663	0.109	0.059
4	66.5	10.6	0.721	0.728	0.122	0.075
5	42.8	5.0	0.651	0.685	0.118	0.062
6	63.0	10.4	0.691	0.700	0.217	0.076
7	67.5	6.7	0.692	0.699	0.124	0.068
8	68.4	6.9	0.706	0.717	0.121	0.062
9	49.3	8.6	0.705	0.713	0.164	0.093
10	50.3	8.9	0.678	0.684	0.169	0.081
11	70.6	7.6	0.779	0.790	0.266	0.084
12	68.9	8.2	0.703	0.720	0.238	0.103
13	36.4	5.3	0.677	0.694	0.129	0.067
14	63.0	9.5	0.708	0.723	0.123	0.066
15	35.4	6.0	0.620	0.660	0.120	0.063
16	64.6	9.8	0.700	0.714	0.129	0.068
17	70.1	8.2	0.663	0.673	0.128	0.066
18	67.4	9.1	0.686	0.845	0.125	0.078
19	49.7	7.7	0.574	0.600	0.158	0.082
20	53.2	9.2	0.616	0.626	0.132	0.080
21	69.6	13.3	0.880	0.901	0.229	0.093

*Sample 21 is the control frozen yoghurt sample

Table 6. Mean values of the sensory (objective) variables of the frozen yoghurt samples

Sample	Color	Hardness	Creaminess	Yoghurt taste	Sweetness	Acidity	Fattiness
1	6.7	9.6	3.0	4.6	3.8	6.7	3.1
2	2.5	10.8	3.8	7.5	3.8	8.4	5.0
3	5.9	1.6	10.8	5.8	10.2	6.7	7.6
4	8.0	7.8	7.0	5.5	4.7	3.9	4.8
5	8.2	4.2	11.5	4.9	10.9	4.2	11.7
6	5.5	9.8	8.2	10.6	7.0	8.7	9.4
7	8.4	11.0	5.2	6.9	5.2	7.7	5.4
8	6.7	8.3	6.9	4.8	5.0	7.1	4.9
9	4.9	4.7	11.5	5.4	8.5	9.5	6.1
10	7.4	5.0	11.6	6.1	7.8	4.5	9.6
11	7.4	11.9	5.5	6.3	1.8	6.8	1.5
12	5.6	10.8	3.8	7.5	2.9	9.1	2.4
13	12.5	2.4	6.4	4.3	10.6	6.1	7.0
14	8.1	13.1	5.3	7.0	2.8	4.7	3.0
15	8.6	3.3	8.4	6.8	11.1	4.2	7.7
16	8.4	9.0	4.7	7.2	6.0	7.3	5.9
17	7.1	11.0	4.1	7.6	4.7	8.1	5.0
18	5.5	12.3	4.2	6.5	2.3	7.4	3.4
19	8.7	5.7	8.3	6.9	8.1	7.3	5.8
20	8.2	7.2	9.1	6.9	10.2	4.7	6.5
21	7.7	6.7	10.7	7.0	11.0	3.6	8.9

*Sample 21 is the control frozen yoghurt sample

As it can be seen in Cox response trace plots for pH shown in Figure 1 (A and B), fat significantly affected the change in pH, regulating the action of yoghurt and honey. Particularly, the increase in fat content from 1% to 3%

followed by the simultaneously increase in the concentration of added yoghurt resulted in increasing the pH of the samples. Furthermore, increasing fat and honey concentration resulted in reducing samples pH, however this change was weaker than the change caused by the yoghurt-fat interaction (Table 7). At an individual level, yoghurt increased samples pH regardless fat content, but this happened more strongly at 3% fat concentration. Pomegranate juice had a permanent negative effect, which was strong in both 1% and 3% fat content. Finally, honey raised the pH at 1% fat content but when the amount of the latter was increased the change was reversed, resulting in a pH decrease. The effect of honey, yoghurt, pomegranate juice and fat on the pH of the samples depends on the pH value of these ingredients, their concentration in the ice cream mixture, as well as the concentration of the other ingredients that also affect pH. The pH values of all ice cream mixture components were: yoghurt 4.6, honey 5.0, pomegranate juice 3.1, cream 6.7 and water 7.8. Regarding the effect of fat, it is worth noting that the increase in its concentration, which was achieved by the addition of milk cream, reduced the amount of water required. As water has a fairly high pH value (the largest of all the ingredients) reducing its percentage in the ice cream mixture resulted in reducing the pH value of the samples.

Figure 1 (C and D) shows a clear increase in acidity when the amount of pomegranate juice increases and the concentration of yoghurt and honey decreases. Pomegranate juice affected samples acidity the most (Table 7), while the change caused by it was of the same intensity in both 1% and 3% fat content. In addition, increasing the percentage of fat and reducing the percentage of yoghurt and honey caused an increase in acidity and, if they were accompanied by an increase in the pomegranate juice concentration, led to quite high levels of lactic acid concentration. Yoghurt slightly reduced acidity at 3% fat content, but practically did not change it at all when the fat content was reduced (1%). Finally, increasing honey concentration reduced samples acidity regardless of the fat content, however, the change was relatively stronger at 1% fat content. The acidity of the samples was affected by their pH value. Therefore, reduced pH values led to an increase in samples acidity. However, the action of the various components on the acidity of the samples did not correspond exactly to the action on their pH, due to the presence of milk proteins into the ice cream system that possess strong buffering capacity (Salaün et a., 2005).

Table 7. The polynomial equations and the R^2 and R^2_p coefficients that correspond to the statistically significant response variables

Response variable	Polynomial equation	R^2 (%)	R^2_p (%)
pH	$5.06x_1 + 4.48x_2 + 3.09x_3 + 0.07x_1V - 0.80x_2V$	91.83	89.34
Acidity (% lactic acid)	$0.93x_1 + 0.14x_2 + 2.63x_3 + 0.53x_1x_2V$	65.52	56.82
L*	$87.49x_1 + 54.39x_2 - 2.24x_3$	85.07	82.51
a*	$-1.54x_1 - 0.87x_2 + 27.05x_3$	92.77	91.61
b*	$9.32x_1 + 37.12x_2 - 17.81x_3 + 0.77x_1V$	84.97	81.20
Overrun (%)	$33.02x_1 + 68.32x_2 + 44.43x_3$	63.29	55.80
G′(–2 °C)	$96.30x_1 - 177.10x_2 + 71.90x_3$	93.11	91.85
Hardness	$18.14x_1 - 55.08x_2 + 8.50x_3$	85.10	82.86
Creaminess	$1.85x_1 + 44.58x_2 + 0.46x_3 - 9.98x_2V$	73.03	65.72
Color	$7.30x_1 + 16.65x_2 - 5.02x_3 + 36.05x_2x_3V$	41.51	23.19
Sweetness	$-1.90x_1 + 59.97x_2 + 1.21x_3$	86.90	84.78
Sensory Acidity	$7.32x_1 - 12.92x_2 + 28.99x_3$	61.52	54.58
Fattiness	$0.59x_1 + 39.20x_2 + 1.82x_3 - 9.73x_1x_2V$	80.26	71.73

G′(–2 °C) is the elastic modulus measured at – 2 °C; R^2_p is the predicted R^2

According to the results shown in Table 4, the brightness of the samples ranged from 64 to 89. In general the samples could be characterized from moderate to quite white. As it concerns the color parameter a*, its values ranged from negative (–4.42) (increased green color intensity) to positive (3.56) (increased red color intensity) values. Finally, all samples showed positive values of the parameter b*, which ranged from 7.3 to 16.9. This implies that all samples exhibited yellow color.

As shown in Figure 2A, the increase in the amount of added yoghurt significantly increased samples brightness, while a reverse change of slightly greater intensity was observed with increasing pomegranate juice concentration and reducing yoghurt and honey concentration (Table 7). In addition, by consulting the Cox response trace plot of Figure 2A and the regression coefficients of Table 7 for L*, honey and fat had no effect on samples brightness. The increase in white color intensity with increasing yoghurt concentration is due to the presence of caseins, which are present in the frozen yoghurt system in the form of casein micelles. Increasing the

concentration of casein micelles increases the reflection of light (Walstra et al., 2006) resulting in increased samples brightness. The red color of the pomegranate juice was responsible for reducing samples brightness. Reduced values of brightness in kefir-type products with increasing pomegranate concentration were also reported by Dimitreli et al. (2019).

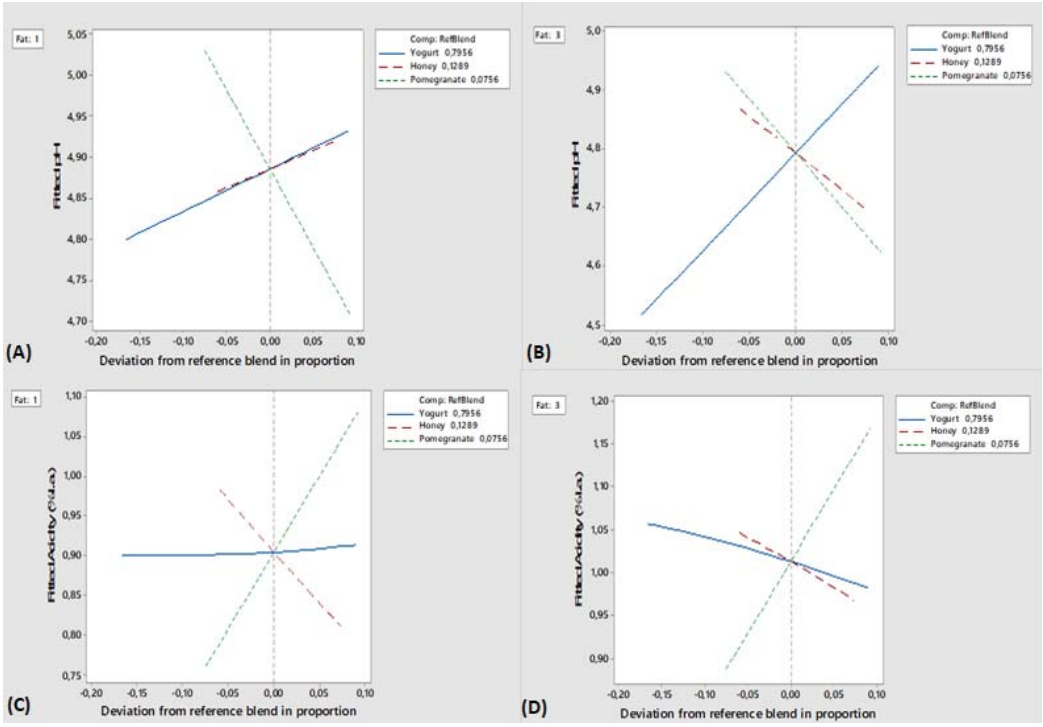

Figure 1. The Cox response trace plots at 1% and 3% fat content levels corresponding to pH (A and B, respectively) and acidity (% lactic acid) (C and D, respectively). The effect of yoghurt is marked in blue, the effect of honey is marked in red and the effect of the pomegranate juice is marked in green

Pomegranate juice was almost entirely responsible for the change of a* (Figure 2B, Table 7), the increase of which significantly increased the variable, enhancing the presence of red color in the samples. On the other hand, honey practically did not affect the response variable at all, while the increase in yoghurt concentration and the corresponding reduction of pomegranate and honey slightly reduced the value of a*. This resulted in reducing red color intensity of the samples and enhancing green color intensity (leading the variable in negative values). The red color of pomegranate juice is responsible for increasing the intensity of the red color of frozen yoghurt samples. Similar results concerning pomegranate juice were reported by Dimitreli et al. (2019) for kefir-type products. The decrease in the values of a* to negative values with the increase in yoghurt concentration, is due to the presence of riboflavin in milk. Riboflavin is a pigment, which has a yellow-green color (Walstra et al., 2006).

As shown in Figure 2 (C and D) and Table 7, the interaction of yoghurt with fat (the increase in fat content from 1% to 3% in combination with yoghurt increase) had a positive effect on b*. The yellow color intensity was also enhanced by the increase in honey concentration and the parallel decrease in the percentages of the other ingredients. On the contrary, the increase in pomegranate juice concentration resulted in reduced values of b*, and the same change but of lesser intensity, was observed when the concentration of yoghurt increased. The increase in yellow color intensity with the interaction of fat and yoghurt can be attributed to the presence of carotenes into the milk, which are yellow in color and are found in fat globules (Walstra et al., 2006). The milk cream, which increases the fat content of the samples, contains higher concentration of carotenes than milk and therefore has a more significant effect on the yellow color intensity of the samples when compared to yoghurt. This means that the decrease in yoghurt concentration in combination with the decrease in the percentage of pomegranate juice (which also affects negatively the intensity of yellow color) and the simultaneous increase in the percentage of honey increase the intensity of the yellow color of yoghurt samples. Honey increases yellow color intensity due to the increased values of b* that exhibits (Karabagias et al., 2014).

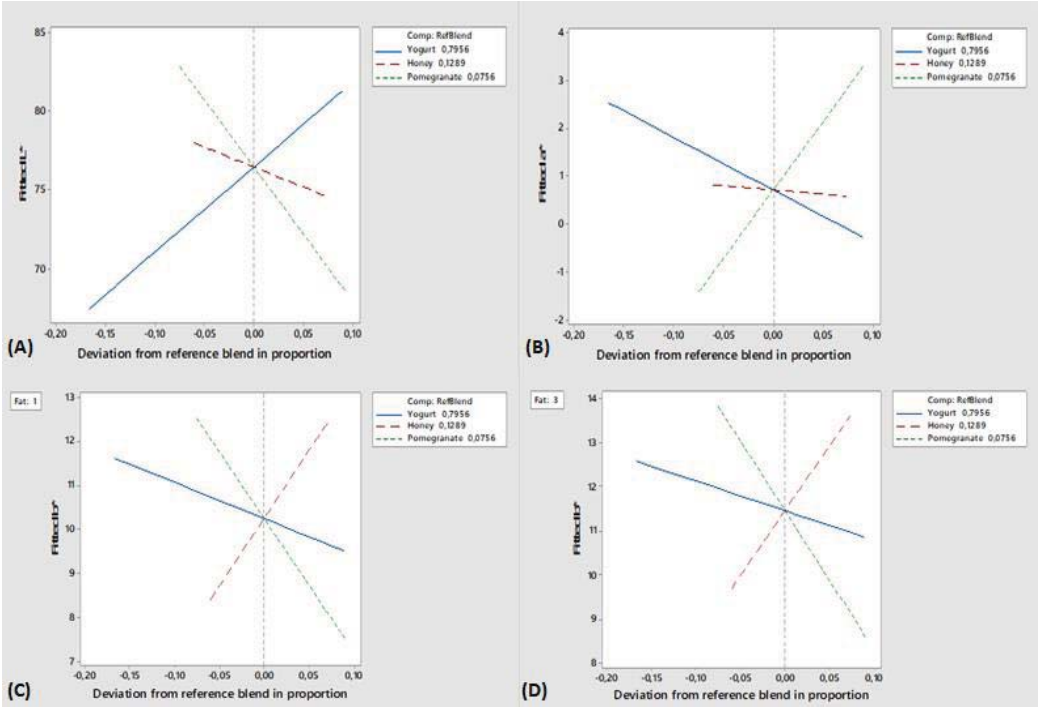

Figure 2. The Cox response trace plot corresponding to L* (A) and a* (B), as well as the Cox response trace plots at 1 % and 3 % fat content levels corresponding to b* (C and D, respectively). Graphs A and B present the same effect of the factors on each response variable at both the fat levels (1% and 3%). The effect of yoghurt is marked in blue, the effect of honey is marked in red and the effect of the pomegranate juice is marked in green

3.2 Overrun

As it can be seen in Table 4, the control sample showed the highest value of overrun (54.20%). In general, the overrun level of frozen yoghurt samples ranged from 33.75% to 44.40%. Similar overrun levels (49.5 ± 1.5%) are reported in the literature for frozen yoghurt samples made with caprine milk (Martinou-Voulasiki & Zerfiridis, 1990).

The interaction of yoghurt with pomegranate and fat benefited the slightly increase of the overrun levels, especially when the fat content increased from 1% to 3% and the concentration of pomegranate juice was also increased (Figure 3A and B). Increasing the amount of honey led to a definite increase in overrun, especially at 1% fat concentration. Yoghurt sharply reduced overrun when the percentage of fat increased to 3%, while its effect was less at 1% fat content. Finally, pomegranate juice reduced the overrun at 1% fat concentration and increased it at 3%. The increase was aided by the interaction with fat, which exerts a positive effect on the response variable. The positive effect of fat on the overrun levels of the samples may be due to the presence of proteins into the membrane of fat globules, which have the ability to incorporate air bubbles into the mixture (Walstra et al., 2006). The increase in overrun values with increasing honey concentration might be probably also attributed to the presence of proteins into the honey. As it concerns the effect of yoghurt, by reducing its percentage into the ice cream mixture, an additional amount of skimmed milk powder is required to be used, which in turn increases the percentage of proteins into the ice cream system, thus resulting in increased air bubbles incorporation. Pomegranate juice affects the overrun in different ways depending on the fat content of the samples. This is probably due to the variation in the concentration of the other ingredients that affects overrun levels, due to the addition of juice.

3.3 Rheological Properties

As it can be seen in Table 5, G′ at −2 °C ranged from 81.7 Pa to 35.4 Pa, while temperature increase (28 °C) resulted in decreasing values of elasticity for all frozen yoghurt samples. This is due to the melting of the samples resulting in increasing their viscous behavior. As for tanδ, its values at −2 °C, ranged from 0.570 to 0.880, indicating that frozen yoghurt samples can be described as elastic, since they exhibited tan δ values lower than one. However, with temperature increase, the values of tanδ did not show a significant increase, as would be expected. This can be probably attributed to the presence of proteins and stabilizers into the ice cream system

that form strong intermolecular interactions and help maintain the elasticity of the samples regardless temperature increase.

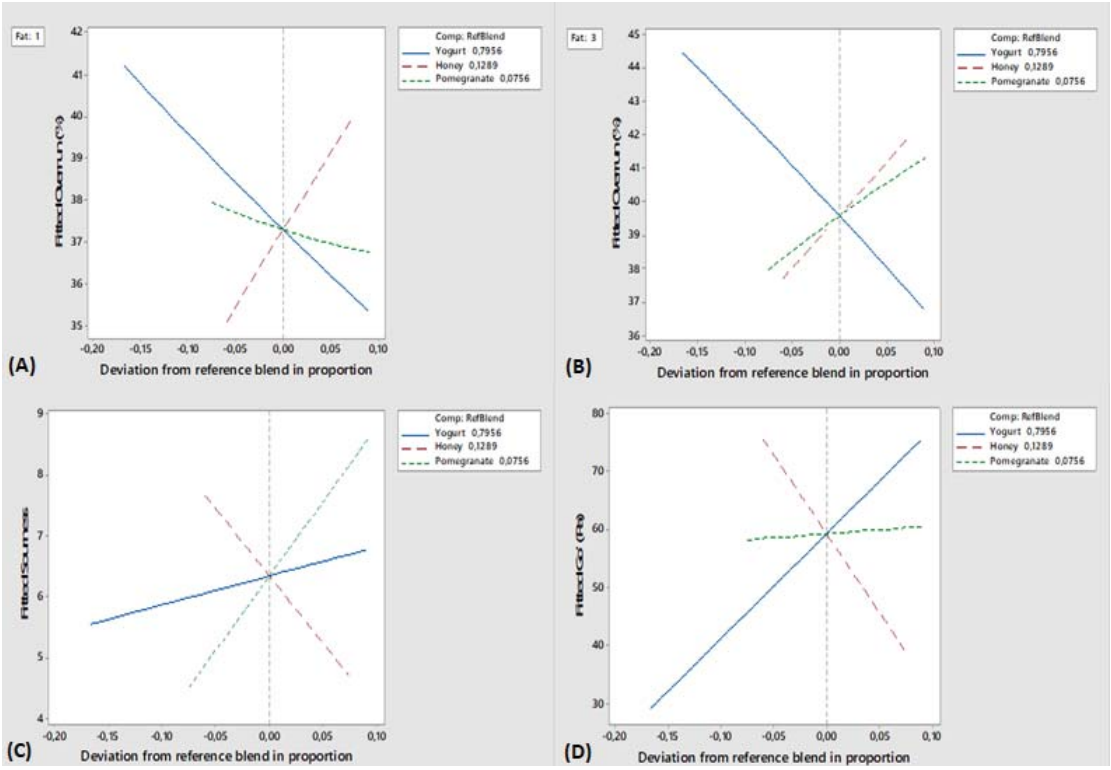

Figure 3. The Cox response trace plots at 1% and 3% fat content levels corresponding to overrun (A and B, respectively), as well as the Cox response trace plot corresponding to sensory acidity (C) and G′(D). Graphs C and D present the same effect of the factors on each response variable at both the fat levels (1% and 3%). The effect of yoghurt is marked in blue, the effect of honey is marked in red and the effect of the pomegranate juice is marked in green

G′ is the elasticity modulus (G') at – 2 °C

The apparent viscosity of the samples at –2 °C ranged from 0.354 Pa · s to 0.109 Pa · s, while temperature increase resulted in decreasing viscosity values for all frozen yoghurt samples (Table 5). With the temperature increase, the mobility of the molecules also increases, as a result of which they show reduced resistance to flow and thus reduced viscosity.

Among rheological properties only G′ at –2°C exhibited a reliable model and was further analyzed (in order for the specific cubic equation to fit the elements of a dependent variable, R^2 must be at least 40 %).

The most important role in the change of G′ was played by honey, the increase of which led to a sharp decrease of the variable response (Figure 3D, Table 7). The effect of yoghurt was smaller, the increase of which resulted in increasing G′, when its concentration was increased at the expense of the amounts of other ingredients. The increase in pomegranate concentration had a negligible effect in G′, in relation to yoghurt and honey. Fat, as a process variable, did not affect the change of G′ at all, since no interaction of it with any of the components was found to be statistically significant. The increase in honey concentration causes the decrease in the freezing point of the ice cream mixture, and thus results in the formation of reduced number of ice crystals. The ice cream in this case appears soft with reduced hardness-consistency (Walstra et al., 2006). The formation of increased number of ice crystals contributes to an increase in the hardness-consistency of the samples. On the contrary, the increase in consistency caused by the increase in yoghurt concentration can be attributed to the decrease in honey concentration, which as mentioned above plays the most important role in the change of G′.

3.3 Sensory Properties

The complete analysis of the model, regarding the change of sensory acidity (Table 7, Figure 3C), is mainly based on the fact that yoghurt, had little effect on the acidity of the product, when compared to honey and

pomegranate, while pomegranate exerted the greater action. Fat did not interact with any of the components and had no effect on samples acidity. Particularly, honey reduced samples acidity, pomegranate drastically increased their acidity and yoghurt increased acidity in a lesser degree. Panelists could distinguish to a significant extent the positive contribution of pomegranate juice to the acidity of the samples and to a lesser extent the decrease in acidity caused by the increase in the concentration of honey.

Regarding the individual action of the ingredients and that of the interaction in the color of the samples (Table 7, Figure 4A and B), honey increased color intensity of the samples to yellow-brown, while pomegranate contributed in increasing pink color intensity. Yoghurt had a limited effect, in relation to pomegranate and honey, on color change, and at the lower level of fat content slightly increased the color, while at the upper level of its addition it decreased it more strongly. Honey, at 3% fat concentration, increased the color more intense when compared to 1% fat content. On the other hand, the effect of increasing pomegranate concentration was practically similar between the two fat levels, reducing the color by a large step. Among other things, changing the amount of fat affects the color change and, when it interacts with honey and pomegranate, increases it. In addition, it affects the action of honey and pomegranate, since, at 1% fat content, honey mainly determines the color change, while when added at 3%, pomegranate assumes this role. In addition, the positive effect of the interaction leads to increased color values, as shown in the Cox trace, especially in the maximum amount of honey and pomegranate added to frozen yoghurt samples. This effect is so important that gives the impression to the panelists that e.g. two samples with 15% pomegranate juice concentration and different fat content (1% and 3%) will be rated as "pink" and "white", respectively, as well as two samples with 18% honey concentration and different fat content (1% and 3%), may be rated as "white" and "yellow-brown", respectively. The results of the sensory evaluation are in agreement with those of the color measurement, in terms of the individual action of the different components. The interaction of fat with pomegranate and / or honey indicates the important role of fat in color. In particular, when fat content is high in the presence of honey, the carotenes that fat globules contain will increase the intensity of the yellow color. Conversely, when the fat content is high in the presence of pomegranate juice (which exhibits red color) it will increase the brightness of the samples due to increased light reflection caused by fat globules. Fat globules reflect light, increasing this way the intensity of white color of the samples (Walstra, et al., 2006).

Examining and describing the model that focuses on the variable of creaminess (Table 7, Figure 4C and D), one concludes that honey sharply increased the variable, when its concentration increased and those of pomegranate and yoghurt decreased. Pomegranate and yoghurt (pomegranate at a slower rate) reduced the creaminess and even faster at 1% fat content. In addition, at 3% fat concentration, honey increased creaminess at a slower rate than that at 1% fat content. Fat had a significant effect on the change of creaminess, as at 3% concentration the maximum value of creaminess (at 18% honey concentration) was significantly reduced (8.5 points) compared to the maximum value corresponding to 1 % fat content (12.7 points). The decrease in fat content results in increasing the amount of the skimmed milk powder to be added in the ice cream mixture, as it can be seen in Table 3, and thus increasing the protein content of the samples. The proteins are well known for their ability to contribute to a smooth texture at the ice cream, through emulsification of the fat, foam formation and stability of the air bubbles (Goff & Hartel, 2013). On the other hand, fat is responsible for a solid structure to be formed during freezing and therefore for consistency, appearance (dryness), and melting resistance. Thus, the increased fat content can results in the appearance of a granular texture in the ice cream, while low fat content contributes to a uniform creamy texture (Walstra et al., 2006). The simultaneously decrease in fat content and increase in protein concentration resulted in increasing the perception of samples creaminess by the panelists. As it concerns honey, the reduction of its addition rate leads to the formation of an increased number of ice crystals, and the possibility of creating a sandy texture that the panelists perceived as a lack of creaminess is increased. The decrease in creaminess with increasing yoghurt concentration is due to the possible decrease in honey concentration.

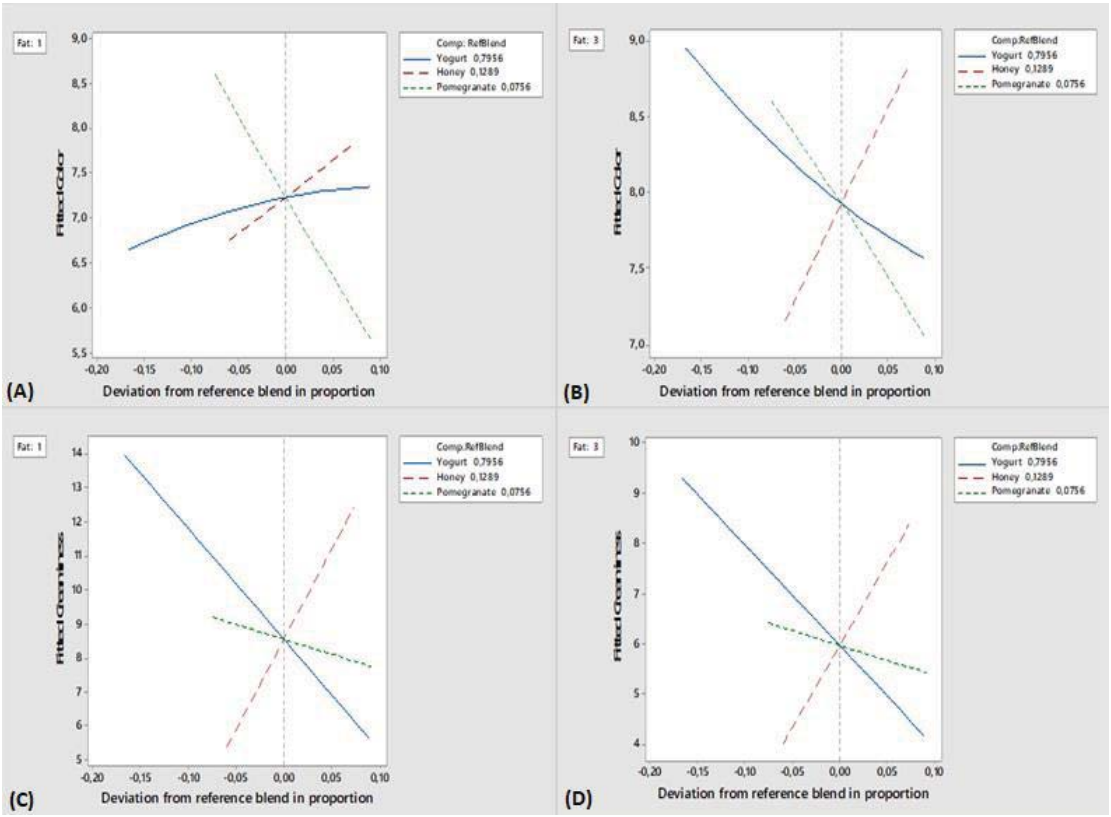

Figure 4. The Cox response trace plots at 1% and 3% fat content levels corresponding to color (A and B, respectively) and creaminess (C and D, respectively). The effect of yoghurt is marked in blue, the effect of honey is marked in red and the effect of the pomegranate juice is marked in green

The typical yoghurt taste of frozen yoghurt samples did not exhibit a reliable model and was not further analyzed.

By consulting Figure 5A, it becomes apparent that yoghurt increased the hardness of the samples, while honey reduced it (when the amount of yoghurt and pomegranate were reduced). Pomegranate, despite the size of the partial regression coefficient, had a negligible effect on hardness. Fat, as a process variable, did not affect the change in the variable response, as it did not interact with any of the components of the linear model (Table 7). The results of the sensory evaluation regarding the effect of the ice cream mixture ingredients on the hardness of the frozen yoghurt samples are in agreement with those of the rheological measurements.

As it can be seen in Figure 5B and by consulting the polynomial equation shown in Table 7, honey played a dominant role in increasing frozen yoghurt samples sweetness, while the action exerted by yoghurt (negative) and pomegranate (positive) in it was small to minimal, compared to that of honey. The fat did not affect the change in sweetness or the action of the terms of the linear model, as it did not interact with them and was not statistically significant. Based on the above, the panelists distinguished almost completely the change in sweetness depending on honey concentration. On the contrary, they did not discriminate the effect of pomegranate addition on samples sweetness, while they could notice the small reduction in samples sweetness caused by yoghurt. The sweet taste of frozen yoghurt samples is mainly due to the presence of honey. The slight decrease in sweetness intensity with yoghurt increase can be attributed to its acidic taste.

According to the Cox traces shown in Figure 5 (C and D) honey sharply increased the fattiness of the samples, with a relatively lower rate at 3% fat content. On the other hand, fattiness decreased when the amount of the added yoghurt was increased. Pomegranate caused a slight reduction in fattiness at both fat levels. The interaction of yoghurt with honey and fat had a negative effect on fattiness, which is indicated by the negative regression coefficients (Table 7), and this happens when fat content increases from 1% to 3%, leading to reduced fattiness of the samples. This means that panelists evaluated a sample, with 1% fat content, as more fatty, while the sense of fattiness was reduced, when it had 3% fat. Since the effect of pomegranate on fattiness was very low

compared to that of the other ingredients, increasing honey and decreasing yoghurt concentration increases the fattiness of the samples and the reverse change occurs when the percentage to yoghurt increases and that of honey decreases. The increase in fattiness with decreasing fat content may be attributed to the increase in creaminess caused by fat content reduction, as already explained. The same stands for yoghurt and honey effect on fattiness. In particular, the increase in the honey concentration and the decrease in the percentage of yoghurt led to an increase in creaminess and thus in fattiness.

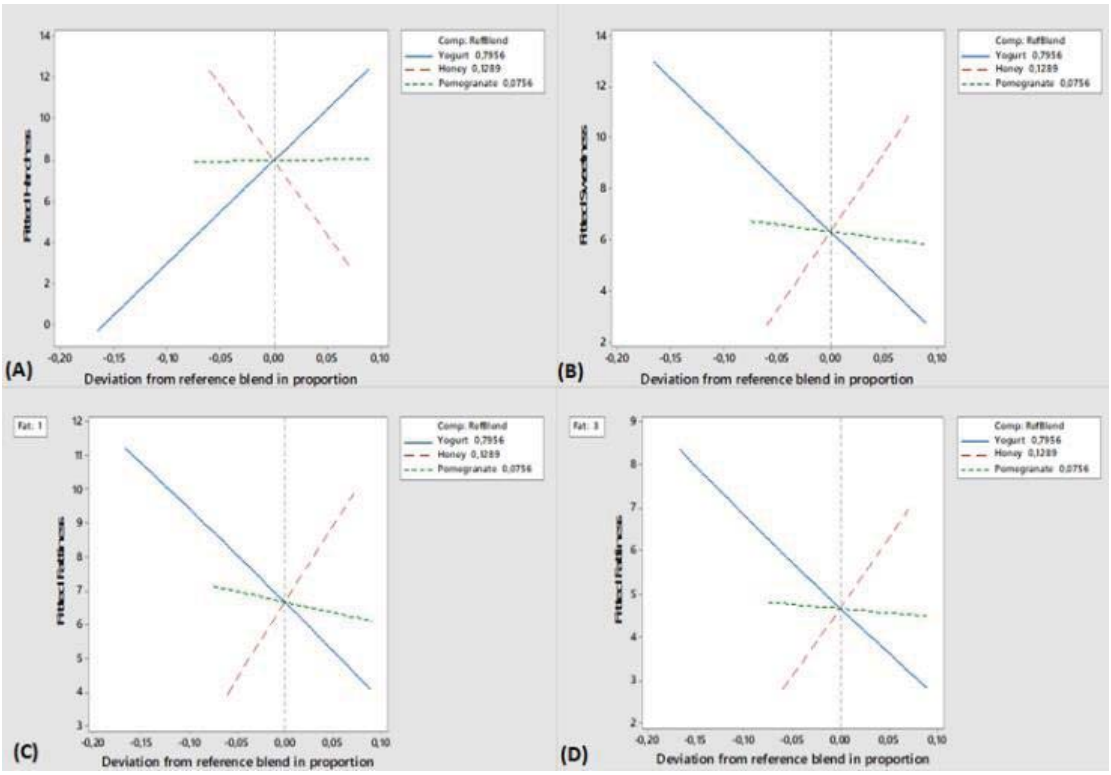

Figure 5. The Cox response trace plot corresponding to hardness (A) and sweetness (B), as well as the Cox response trace plots at 1 % and 3 % fat content levels corresponding to fattiness (C and D, respectively). Graphs A and B present the same effect of the factors on each response variable at both the fat addition levels (1 % and 3 %). The effect of yoghurt is marked in blue, the effect of honey is marked in red and the effect of the pomegranate juice is marked in green

The proportion preference (Best-Worst scaling) for all treatments is shown in Figure 6. As it can be seen, sample 5 and control were the most preferable samples followed by samples 20, 9, 13, 10, 6, 3 and 8. These results indicate that frozen yoghurt samples with honey and pomegranate juice addition were acceptable by the panelists. Furthermore, it seems that fat reduction did not affect panelists' preference, since the most acceptable frozen yoghurt sample (5) had 1% fat content and most of the preferred samples had low fat content (9, 10, 6, 3, 8). However, further statistical analysis is required so as to find the optimum concentration of the ice cream mixture ingredients, according to panelists' preference.

Marginal Utility	Marginal Probability	Treatments
2.2539	0.2376	21
2.2053	0.2263	5
1.0807	0.0735	20
1.0436	0.0708	9
0.9570	0.0649	15
0.7671	0.0537	10
0.7309	0.0518	6
0.6886	0.0497	3
0.2212	0.0311	8
-0.265	0.0191	7
-0.324	0.0180	4
-0.369	0.0172	2
-0.614	0.0135	16
-0.691	0.0125	18
-0.753	0.0117	19
-0.764	0.0116	13
-1.033	0.0089	12
-1.097	0.0083	14
-1.244	0.0072	17
-1.257	0.0071	11
-1.537	0.0054	1

Figure 6. The proportion preference per treatment (Best-Worst scaling). The positive values of the first column (Marginal Utility) correspond to the most preferable samples

4. Conclusion

Yoghurt, honey, pomegranate juice and fat content significantly affected frozen yoghurt properties. There was a good agreement between sensory evaluation results and instrumental measurements. Panelists' preference revealed that frozen yoghurt samples with honey and pomegranate juice addition exhibited favorable sensory properties. Fat reduction, followed by an increase in the amount of added skimmed milk powder and thus protein content, contributed in increasing samples creaminess and perception of fattiness, and at the same time did not affect panelists' preference. Further statistical analysis is required so as to find the optimum concentration of yoghurt, honey, pomegranate juice and fat in the novel dairy fermented frozen dessert.

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Biochemical Characterization and Nutritional Profile of Jam and Syrup from *Saba senegalensis* fruit in Côte d'Ivoire

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Abstract

Saba senegalensis is a plant to the family of Apocynaceae and its fruit called Saba is mainly used as food. For better valorisation, this study aimed to evaluate the nutritional potential of jam and syrup derived of this fruit. The study was carried out on the fruit of *Saba senegalensis* harvested in the north of Côte d'Ivoire. After jam and syrup formulation, pH, dry matter, ash, macronutrients, vitamins, minerals, phytonutrients, anti-nutritionals factors and nutritional profile have been determined. The results showed that jam and syrup of Saba were acidic with respective pH of 3.11 ± 0.01 and 3.65 ± 0.05. They contented higher in carbohydrates with respective rates of 56.53 ± 0.24 % and 66.27 ± 1.08 %. Vitamin C rate in jam and syrup was respectively about 20.01 ± 0.01 mg/100 g and 18.33 ± 2.22 mg/100 g. The most important mineral was potassium which rate is 136.71 ± 4.08 mg/100 g and 241.76 ± 5.9 mg/100 g in jam and syrup respectively. They also contain phytonutrients such as polyphenols (respectively 103.18 ± 0.69 mg/100 g and 3.29 ± 0.02 mg/100 g) and antinutritional factors such as oxalates (respectively 102.01 ± 6.93 mg/100 g and 19.96 ± 0.01 mg/100 g). Nutritional profile has classified *Saba Senegalensis* jam and syrup to the group 4 of foods, foods that must be eaten occasionally. The transformation of Saba in jam and syrup could be a good way to valorise this fruit and also ensuring its consumption through the year.

Keywords: jam, syrup, nutritional profile, nutritional value, *Saba senegalensis*

1. Introduction

Fruits are essential for the alimentation. They have high nutritional value and serve to supplement the nutrients provided by cereals, starchy foods, vegetables (Ojure & Quadri, 2012). Fruits have generally low content of lipids, sodium, calories and are an important source of many important nutrients such as dietary fibber, vitamin C, minerals and phytonutrients such as phenols and flavonoids. These compounds protect the body against oxidative stress and degenerative diseases by developing the body's ability to defend against external attacks (Yoshikawa, Toyokuni, Yamamoto & Naito, 2000; Ena, Shalini, Pragati, Reena, & Rai, 2016). However, the fruits have higher water content, which makes them susceptible to biological and chemical degradation agents such as microorganisms and oxidation reactions (Nout, Hounhouigan, & Boekel, 2008). The fruits are therefore very perishable and can only be kept fresh for a few days at room temperature, hence the need to transform them. It is in this context that our work is based on the fruit *Saba senegalensis* better known under the name of côcôta in Côte d'Ivoire, Wèda in Burkina Faso, Madd in Senegal or Saba in French.

Saba senegalensis is a wild liana which pushes in African savannahs (Diabagaté, Traoré, Cissé, Soro, & Brou 2019). The effects of *Saba senegalensis* in local traditional medicine are varied. From fruit, to roots, to leaves and latex, everything is usable (Burkill, 2000; Bandoma, 2009; Dari 2013; Sarr *et al*., 2018). According to Sarr *et al*. (2015), the crushed leaves of Saba are used for the care of wounds and the roots against female sterility. According to Bâ, Dalpé, and Guissou (1996), the latex of *Saba senegalensis* combats cough and tuberculosis. In addition, its coagulation gives a natural rubber for various local uses. Nutritionally, the pulp of fruit of *Saba*

senegalensis is a true source of β-carotene (189.62 ± 1.33 µg/100g) which would play a significant role in the prevention of cancer (Diabagaté *et al*., 2019). These authors also showed that the pulp was acidic (pH = 3.03 ± 0.01) and rich in total phenolic (264.76 ± 4.54 g/100g), vitamin C (36.67 ± 2.22 g/100g), and minerals like potassium (116.96 ± 2.06 mg/100g) and calcium (36.61 ± 2.79 mg/100g). Moreover, according to Attah *et al.* (2012) and James, Rotimi, and Bamaiyi (2015), the indigenous fruits are appropriate to be transform into products like juice, jam, syrup and wine that could ameliorate not only the nutrition and health of population, but also livelihoods. Indeed, the processing of these fruits could improve the financial incomes of poor families.

The fruit of *Saba senegalensis* is a seasonal fruit and therefore not always available. The necessity to transform this fruit into jam and syrup could be one of the ways to valorise the fruit of *Saba senegalensis,* improve the acceptability of this astringent fruit and ensure its consumption through the year in Côte d'Ivoire. The aim of this study was to determine the nutritional potential of jam and syrup of *Saba senegalensis* for better valorisation in Côte d'Ivoire.

2. Material and Methods

2.1 Material

The plant material used in this study is the fruit of *Saba senegalensis* harvested in the village of Waraniéné located about 5 km from the city of Korhogo. Waraniéné is located at the North of the Côte d'Ivoire between the parallels 8°26 and 10°27 of Northern latitude and 5°17 and 6°19 of longitude Western. The ripe fruits of *Saba Senegalensis* were harvested between May and July 2019 from lianas in the region of Korhogo. The fruits were identified and monitored until the maximum maturity stage. About 10 and 15 healthy orange-yellow fruits were harvested at randomly from each tree.

2.2 Methods

2.2.1 Jam Preparation

The extraction of raw pulp of Saba was carried out according to the method of **Diabagaté *et al*. (2019)**. The *Saba Senegalensis* fruits (10 kgs) were cutted into half. The pulp with seeds was removed with stainless spoon and were put into the mixer of mark ilux (NO: LX-176P/AC: 220-240V 50Hz 350W) for mixing. The mixture was put into the stainless-steel sieve and blended manually to separate the seeds from the pulp. The pulp obtained was stored at -20°C for analysis. About 500 g of raw pulp of *Saba Senegalensis* were mixed with 400 g of white sugar. The whole was brought to cooking until thickening of the mixture (105°C about 20 min). After cooking, the jam was stored in airtight containers.

2.2.2 Syrup Preparation

The seeds covered of pulp were soaked for 1 hour in water then kneaded and sieved. The sugar (750g) was added to 500 ml of the juice. The mixture was boiled approximately for 10 min until the sugar was completely dissolved. After cooling, the mixture was stored in airtight containers.

2.2.3 Chemical Composition of Products Derived from Saba

pH measurement

The pH was determined according to Le Coque (1955) method. It was carried out on the filtrate obtained by grinding 2g of sample with 28 ml of distilled water and centrifuged at 3000 rpm for 10 min. The pH is determined using a pH meter (Consort pH meter P 107).

Titratable acidity

Titratable acidity was determined according to Kimaryo, Massawi, Olasupo, and Holzapfel (2000) method. Sample (2 g) was mixed in distilled water (28 ml) and centrifuged at 3000 rpm for 10 min. The filtrate (10 ml) was dosed with a 0.1 N sodium hydroxide (NaOH) solution and 2 to 3 drops of phenophthalein (φφ) were added until it turns pink.

Dry matter content

Dry matter was determined according to the method of Association of Official Analytical Chemists (AOAC) (1990). The sample was dried with the drying oven (Memmert 854 Schwabach) during 24 h, to 105 °C and was cooled in a desiccator during 1 h, then weighed and the dry matter was determined.

Ash content

Ash content was obtained according to **AOAC (1990)** method. Each jam and syrup (about 5 g) was introduced into porcelain capsules and placed in a muffle furnace (JP Sélecta SA 313066) for electric heating for 6 h at 550 °

C. After heating, the samples was cooled in a desiccator for 2 h and the ash were expressed as a percentage of the initial weight of the samples.

Total carbohydrate content

Total carbohydrate content was determined by difference method:

$$[100\ \% - (\%\ \text{moisture} + \%\ \text{ash} + \%\ \text{fat} + \text{protein}\ \%)] \quad (1)$$

Determination of total sugars

The total sugars were determined according to the method of Dubois, Gilles, Hamilthon, Rebers, and Smith (1956). One hundred (100) µl of the juice extract were placed in a test tube. Two hundred (200) µl of phenol (5%, w / v) and one (1) ml of concentrated sulfuric acid were added successively to the reaction medium. After homogenization of the reaction medium, the optical density is determined using a spectrophotometer (GENESYS 5) at 490 nm against a control containing no sweet extract. Optical densities were converted into the amount of total sugars using a calibration line obtained from a glucose solution (1 mg / ml).

$$\%\ \text{total sugar} = D0490 / a \quad (2)$$

a: director coefficient of the calibration line = 9.525

Determination of reducing sugars

Reducing sugars were determined by the method of Bernfeld (1955) using dinitrosalicylic acid (DNS). In this method, pentose and hexose, under the effect of heat, transform into furfural compounds. These compounds, in the presence of DNS, produce a specific staining with reducing sugars. The reaction medium was composed of:

- 0.1 ml of sugar extract solution;
- 0.9 ml of distilled water;
- 0.5 ml of DNS.

The mixture was heated in a boiling water bath for 5 min, then allowed to cool for 10 min at room temperature. Then, 3.5 ml of distilled water are added to the reaction medium. The optical density is read at 540 nm in the presence of a control. This value is converted into mg of reducing sugars using a calibration curve obtained from a solution of glucose at 1 mg / ml.

$$\%\ \text{reducing sugars} = Do540 / a \quad (3)$$

a: director coefficient of the calibration line = 10.056

Quantitative determination of Vitamin C

The method used for the determination of vitamin C was described by Pelletier (1985). The samples (10g) (ME) were diluted in 40 ml of metaphosphoric acid-acetic acid (2 %; w / v). The mixture was centrifuged at 3000 rpm for 20 minutes and the supernatant was made up to 50 ml with boiled distilled water. A 10 ml sample placed in a vial was titrated with 2.6 DCPIP at 0.5 g / L until a persistent pink color was obtained for 30 s. The vitamin C content was given as a percentage according to the following expression:

$$\text{Vitamine C}\ (\%) = \frac{(0{,}5 \times V \times 10^{-3}) \times 5}{ME} \times 100 \quad (4)$$

Beta-carotene Content

Total carotenoids were extracted according to Rodriguez-Amaya (1999) method. Samples were crushed and homogenized. About 5 g of sample was homogenized with 100 ml of methanol: petroleum ether (1: 9, v / v), the mixture was transferred to a separatory funnel. The petroleum ether layer was filtered through sodium sulfate, transferred to a volumetric flask to a volume of 100 ml, made up with petroleum ether. Finally, the total carotenoid content was measured by a spectrophotometer at the wavelength of 450 nm.

The results were expressed in β-carotene equivalents (µg / 100 g of fresh material).

Fat content

The fat was extracted according to the method of AOAC (1995). Five grams (5 g) of sample was added to a Whatman cartridge and the whole placed in the extractor of soxhlet. The fat was extracted with 60 ml of hexane at reflux for 6 hours at the boil. The hexane was evaporated using a rotary evaporator. The previously tared extraction flask was dried in an oven at 60 ° C. for 30 min and was cooled in desiccator. The fat content was

expressed as a percentage of the initial weight of the sample.

Fibbers Content

Crude fibbers were determined according to AOAC (1990). In 2g of sample, were added 50 ml of 0.25 N sulfuric acid and the mixture was boiled for 30 min under reflux. About 50 ml of 0.31 N sodium were added and the whole brought to the boil for 30 min under cooling agent. The extract obtained was filtered through Whatman filter paper and the residue washed until complete elimination of the alkalis. The residue obtained was incinerated in an oven at 550 ° C for 3 h, cooled in a desiccator and the ash obtained were weighed.

Mineral content

Ash (0.1g) was weighed in platinum crucibles to which was added 1 ml of distilled water. In each crucible, 5 ml of hydrofluoric acid 50 % and 2 drops of sulphuric acid (v / v) were added, homogenized and heated at 100°C until fully evaporated. Residue obtained was dissolved in 10 ml of 50 % hydrochloric acid. Solution was left to stand for 10 minutes on the bench and the final volume was brought to 100 ml (Diabagaté *et al*., 2019). The contents of sodium, magnesium, phosphorus, potassium, calcium and iron were determined by atomic absorption spectrophotometry at their specified wavelengths at 589.3 nm; 285.2 nm; 410 nm; 766.5 nm; 422.7 nm and 510 nm.

2.2.4 Phytonutrients

Polyphenols content

Total polyphenols content were estimated by the Folin-Ciocalteu method (Scalbert, Monties, & Janin, 1989). About 200 µl of sample were mixed with 800 µl of Folin-Ciocalteu reagent. The mixture was kept for 2 minutes in the dark at room temperature and 1 ml of sodium carbonate (75 g.l-1) was added. The mixture was placed in a water bath maintained at 50 ° C for 15 minutes and cooled. The absorbance was measured at 760 nm using of a UV 1205 spectrophotometer and the results expressed in mg / 100 g.

Flavonoids content

The flavonoid content was determined according to Meda, Lamien, Romito, Millogo and Nacoulma (2005). In a flask contening 0.5 ml of sample, were successively added 0.5 ml of 10 % aluminum chloride, 0.5 ml of 1N potassium acetate and 2 ml of distilled water. The mixture was left for 20 minutes in the dark. Optical density was read at 415 nm against a reference.

2.2.5 Antinutritionals Factors

Tannin content

The tannin content was determined by the method described by Bainbridge, Tomlins, Wellings, and Westby (1996). One 1 ml of sample was added 5 ml of vanillin reagent. The mixture was left for 20 min in the dark and the optical density (OD) was read at 500 nm against a blank. The amount of tannins was determined using a standard range established from a stock solution of tannic acid (2 mg / ml) under the same conditions as the test.

$$\text{Tannins (mg/100)} = \frac{\text{DO500 x 103}}{\text{3,11 x me}} \quad (5)$$

Calibration line: OD500 = 3.11 mass (mg) Tannic acid

me: mass (g) of the sample.

Phytate content

The phytate content was determined by the method described by Mohammed, Ponnamperuma, and Youssep (1986). About 0.5 g of the sample was homogenized in 25 mL of 3 % (w / v) trichloroacetic acid (TCA). The mixture was centrifuged at 3500 rpm for 15 min. The supernatant was mixed with 3 mL of 1 % (w / v) ferric chloride. The solution obtained was heated in boiling water bath for 45 min. After cooling, the solution was centrifuged at 3500 rpm for 10 min. The supernatant was mixed with 5 mL of hydrochloric acid (0.5 N). To the mixture obtained, 5 ml of sodium hydroxide (1.5 N) were added and the whole was taken to a boiling water bath for 15 min. The solution obtained was centrifuged at 3500 rpm for 10 min. To the supernatant (1 mL) , 4.5 mL of boiled, air-cooled distilled water and 4.5 mL of orthophenantroline reagent were added. The mixture was left for 1 hour before reading the optical density (OD) at 470 nm against a blank. A standard range was established from a stock solution of Mohr's salt (10 µg iron / mL) under the same conditions as the test for the determination of the amount of phytate-ferric in the sample.

$$\text{Phytates (mg/100)} = \frac{DO_{470} \times 4}{0{,}033 \times m_e} \quad (6)$$

Calibration line: OD (470) = 0.033 mass (μg) Phytate sodium

me: mass (g) of the sample.

Oxalic acid

The oxalic acid (OA) content was determined according to the method described by AOAC (1995). About 0.5 ml of sample was added to 100 ml of potassium hydroxide (0.1N KOH). The mixture was boiled for 30 min at 80°C. After cooling, the solution was filtered and 5ml of concentrated sulfuric acid (H2SO4) was added. The filtrate is heated ta the temperatures at 60 ° C to 70 ° C for 10 min then titrated with 0.1N potassium permanganate solution (KMnO4) until a persistent pink color. The content of oxalic acid was determined by the following relationship:

$$\%\ OA = \frac{V \times 0{,}45 \times 2}{Pe} \quad (7)$$

Pe: The test sample (0.5 g)

V: burette drop (KMnO4) or volume of KMnO4 poured

0.45 = quantity of oxalic acid corresponding to 1 liter of 0.1N solution of KMnO4

2.2.6 Nutritional Profile

Food nutrient profile was calculated according to Darmon, Vieux, Maillot, Volatier, and Martin (2009) system. This system is based on two (2) indicators: the SAIN score, based on qualifying nutrients (ie, positive nutrients), and the LIM score, based on disqualifying nutrients (ie, the nutrient to be limited).

The SAIN score was an unweighted arithmetic mean of the percentage adequacy for Five (5) positive nutrients. It was calculated for 100 kcal of food, as follows:

$$SAIN = \frac{\frac{\frac{\text{Vitamin C}}{\text{RNI Vitamin C}} + \frac{\text{Iron}}{\text{RNI Iron}} + \frac{\text{Calcium}}{\text{RNI Calcium}} + \frac{\text{Protein}}{\text{RNI Protein}} + \frac{\text{Fiber}}{\text{RNI Fiber}}}{5} \times 100}{\text{Energy}} \times 100 \quad (8)$$

RNI (Recommended Nutritional Intake)

The LIM score was the mean percentage of the maximal recommended values for 3 nutrients, the intakes of which should be limited in a healthy diet.

The LIM score was calculated for 100 g of food as follows:

$$LIM = \frac{\frac{Na}{3153} + \frac{SFA}{22} + \frac{\text{Added sugar}}{50}}{3} \times 100 \quad (9)$$

SFA= Saturated Fatty Acid

The recommended nutritional intake are based on French and European nutritional recommendations. In particular, the daily maximal recommended value for SFAs and added sugars corresponded to 10 % of 2000 kcal, ie, 22 and 50 g, respectively, and that of sodium corresponded to a daily intake of 8 g NaCl (ie, 3153 mg Na). The 2000-kcal value was chosen as a reference for energy intake because it is close to the mean observed energy intakes in the French population. Overall, the SAIN, LIM system was based on 8 basic nutrients (5 included in the SAIN plus 3 included in the LIM) (Darmon *et al*., 2009).

Based on reference daily energy intake of 2000 kcal, the optimum value for the SAIN was 100 % for 2000 kcal, which was equivalent to 5 % for 100 kcal food. The SAIN value ≥5 indicated, therefore, a good nutrient density. Unlike the SAIN, the LIM was calculated for 100 g. Thus, the reference value used to derive the threshold value for the LIM score was based on food intake rather than on energy intake. Because the mean daily food intake (including solid foods only) observed in the French population was ≈1330 g/d, the maximal value for the LIM score was 100 % for 1330 g, which was equivalent to 7.5 % for 100 g food. A LIM value <7.5 indicated, therefore, a low content of limited nutrients (Darmon *et al*., 2009).

Based on SAIN and LIM score values, each food was classified into 1 of 4 possible SAIN and LIM groups (Darmon *et al*., 2009):

Group 1: SAIN ≥ 5 and LIM < 7.5 (Foods recommended for health)

Group 2: SAIN < 5 and LIM < 7.5 (Neutral foods)

Group 3: SAIN ≥ 5 and LIM ≥ 7.5 (Foods recommended in small quantities or occasionally)

Group 4: SAIN < 5 and LIM ≥7.5 (Foods to limit).

2.2.7 Statistical Analysis

Results made in triplicate measurements were expressed as means with standard deviation. A one-way ANOVA was performed and means were separated using Tukey test ($p \leq 0.05$) with Statistica 7.1 software.

3. Results

3.1 Biochemical Composition of Jam and Saba Syrup

3.1.1 pH, Titratable Acidity, Ash and Dry Matter

The pH, titratable acidity, ash and dry matters contents of jam and syrup of Saba were given in Table 1. Results showed that pH, titratable acidity, ash and dry matters differ significantly to the jam and syrup of Saba. The jam and the syrup have low pH values (respectively 3.11 ± 0.01 and 3.65 ± 0.05) with a titratable acidity respectively of 0.23 ± 0.01 % and 0.09 ± 0.01 %. On the other hand, the dry matter contents was higher than 50 %. Also, they contained respectively 0.63 ± 0.05 % and 1.06 ± 0.09 % of ash.

Table 1. Compositions in pH, titratable acidity, ash and dry matter

	Contents	
	Jam	**Syrup**
pH	3.11 ± 0.01^{b}	3.65 ± 0.05^{a}
Titratable acidity	0.23 ± 0.01^{a} %	0.09 ± 0.01^{b} %
Ash	0.63 ± 0.05^{b} %	1.06 ± 0.09^{a} %
Dry matters	58.08 ± 0.37^{b} %	67.47 ± 1.45^{a} %

Values are means ± standard deviations of three measures (n = 3). The same letter subscripted in the same line indicates that there is no significant difference between samples for the parameter concerned ($p<0.05$).

3.1.2 Macronutrient Content

Macronutrient content were significantly different ($P<0.05$) in the jam and the syrup of *Saba Senegalensis* (Table 2). Results showed that the jam and the syrup had low lipid contents (respectively 0.92 ± 0.06 % and 0.13 ± 0.01 %). On the other hand, the syrup (66.27 ± 1.08 %) was higher total carbohydrate content than the jam (56.53 ± 0.24 %). The total sugars contents of the jam and the syrup were respectively 13.36 ± 0.14 % and 17.35 ± 0.30 %.

Table 2. Macronutrient compositions of Jam and Saba Syrup

	Contents (%)	
	Jam	**Syrup**
Lipids	0.92 ± 0.06^{a}	0.13 ± 0.01^{b}
Total carbohydrates	56.53 ± 0.24^{b}	66.27 ± 1.08^{a}
Total sugar	43.36 ± 0.14^{b}	47.35 ± 0.30^{a}
Reducer sugar	3.05 ± 0.34^{a}	0.28 ± 0.02^{b}

Values are means ± standard deviations of three measures (n = 3). The same letter subscripted in the same line indicates that there is no significant difference between samples for the parameter concerned ($p<0.05$).

3.1.3 Vitamins and Minerals

The Table 3 presented the vitamin C, β-carotene and minerals contents of jam and syrup of *Saba senegalensis*. Results showed the significant difference between the parameters studied. The vitamin C rate was highest in the jam (20.00 ± 0.01 mg/100 g) that in the syrup (18.33 ± 2.22 mg/100 g). In addition, β-carotene rate of the jam was 174.34 ± 3.01 µg/100 g while its content in the syrup was in the traces form.

Concerning minerals contents, potassium was the most abundant mineral in jam and syrup with respective values of 136.71 ± 4.08 mg/100 g and 241.76 ± 5.9 mg/100 g. The formulated products contained a significant amount of calcium (53.46 ± 3.96 mg/100 g and 87.49 ± 5.42 mg/100 g). Nevertheless, they were low in iron contents (1.01 ± 0.21 mg/100 g and 3.11 ± 0.87 mg/100 g).

Table 3. Vitamins and minerals contents

	Contents	
	Jam	**Syrup**
Vitamins		
Vitamin C	20.00 ± 0.01^{a} mg/100 g	18.33 ± 2.22^{b} mg/100 g
β-carotene	174.34 ± 3.01^{a} µg/100 g	Traces
Minerals	**Jam (mg/100 g)**	**Syrup (mg/100 g)**
Sodium (Na)	5.25 ± 0.31^{b}	18.80 ± 1.08^{a}
Magnesium (Mg)	16.19 ± 0.21^{b}	34.49 ± 0.54^{a}
Phosphorus (P)	14.19 ± 0.44^{b}	40.46 ± 1.32^{a}
Potassium (K)	136.1 ± 4.08^{b}	241.76 ± 5.90^{a}
Calcium (Ca)	53.46 ± 3.96^{b}	87.49 ± 5.42^{a}
Iron (Fe)	1.01 ± 0.21^{b}	3.11 ± 0.87^{a}

Values are means ± standard deviations of three measures (n = 3). The same letter subscripted in the same line indicates that there is no significant difference between samples for the parameter concerned ($p<0.05$).

3.2 Phytonutrients and Anti-nutritional Factors

The phytonutrients content and antinutritionals factors content were shown in Table 4. Results were significantly different ($p<0.05$) on phytonutrients and anti-nutritional factors in each formulated products. Polyphenols were more abundant in *Saba Senegalensis* jam (103.18 ± 0.69 mg/100g) than in syrup (3.29 ± 0.02 mg/100g). In addition, phytates were more abundant in Saba jam (102 ± 6.93 mg/100g). Results also showed that flavonoids content were lower in jam and syrup of Saba with respective values of 19.00 ± 0.99 mg/100g and 0.20 ± 0.02 mg/100g.

Table 4. Phytonutrients contents and antinutritionals factors

Contents (mg/100g)		
	Jam	**Syrup**
Phytonutrients		
Total polyphenols	103.18 ± 0.69^{a}	3.29 ± 0.02^{b}
Flavonoids	19.00 ± 0.99^{a}	0.20 ± 0.02^{b}
Antinutritionals Factors		
Tannins	78.51 ± 0.14^{a}	1.08 ± 0.04^{b}
Phytates	102 ± 6.93^{a}	19.96 ± 0.01^{b}
Oxalates	19.87 ± 0.01^{a}	6.62 ± 0.21^{b}

Values are means ± standard deviations of three measures (n = 3). The same letter subscripted in the same line indicates that there is no significant difference between samples for the parameter concerned ($p<0.05$).

3.3 Nutritional Profile

The Figure 1 showed the SAIN and LIM scores for jam and syrup of *Saba Senegalensis*. The SAIN score of the jam was 2.88 and LIM score was 54.70. The SAIN score of the syrup was 3.85 and LIM score was 97.39.

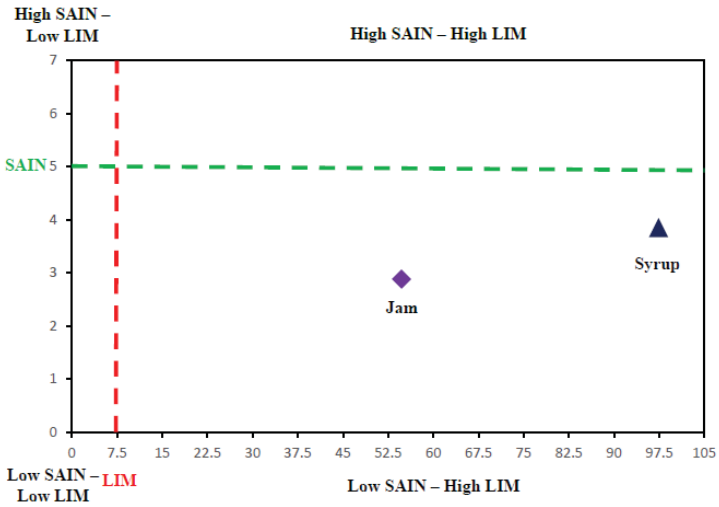

Figure 1. Nutritional profile of jam and syrup of Saba

4. Discussion

The aim of this study was to evaluate the nutritional potential of jam and syrup of *Saba senegalensis* for better valorisation of this fruit. The pH value showed that jam and syrup was acidic. It was established that acidity is one of the main parameters that determine the quality of food and it is due to the presence of organic acids (Ouchemoukh, Louaileche, & Schweitzer, 2007). The acid content of the jam and the syrup of *Saba Senegalensis* obtained in this result indicated that *Saba Senegalensis* fruit could contain organic acids. Due to the high acidic content, jam and syrup of *Saba senegalensis* could be able to tolerate microbial activities, especially pathogenic germs and will have a long shelf life. According to Vondruskova, Slamova, Trckova, Zraly, and Pavlik (2010), organic acids have the ability to lower pH and thereby reduce the growth of certain pathogenic bacteria. The dry matter corresponds to the mass of sample after complete evaporation of the free water. The dry mater content in jam and syrup of *Saba Senegalensis* was higher than 50 %. This property would indicate that the jam and the syrup could resist a long time against the microbial growth. Dry matter contents was higher than those obtained by Badarou and Sanni (2014) in tamarind jam ≈ 54 ± 1.79 % and pineapple syrup ≈ 21.40 %. Carbohydrates content in this result were higher than 50 %. The high carbohydrate content in jams can be associated to the large presence of sugar (> 50 g/ 100g) as observed from the nutrition labelling on its packaging (Whitney & Rolfes, 2005; Naeem *et al*., 2017). The total sugars contents (43.36 ± 0.14 %) obtained in this result were lower than those of Parkouda, Oboulbiga, and Sawadogo-Lingani (2015) in the Saba jam in Burkina Faso (65.36 ± 0.92 %). The difference of sugars obtained in this result and those of Parkouda *et al*. (2015) could be explained either by the different varieties of the fruit or by the technological treatments applied.

According to Khalil and Saleemullah (2004), carotenoids is the biological antioxidants and play an important role in human health. β-carotene was converted into retinol (vitamin A) in the human body with a better yield estimated at 1/6 (Favier *et al*., 1993; Diabagaté *et al*., 2019). Vitamin A was involved in growth, vision, and resistance to infection and its deficiency is a major contributor to infant and child mortality (Tee, 1992; Diabagaté *et al*., 2019). *Saba Senegalensis jam* could thus contribute to the recommended nutritional intake of carotenoids, especially in children. The β-carotene content of Saba syrup was in the form of traces. The great loss of carotenoids in the syrup could be explained by the method of manufacture of the Saba juice used for the formulation of the syrup and by the cooking method. Indeed, according to Alvarez-Jubete and Tiwari (2013), cooking influences the carotenoid content with varying degrees of stability between the different compounds. Saba *Senegalensis* jam and *Saba Senegalensis* syrup have high content of vitamin C. According to the Institute of Medicine (2005) for the consumption of vitamin C, the Accepted Daily Intake (ADI) permitted is 110 mg/days. Due to the high content of vitamin C, the consumption of jam and syrup of Saba could be contribute to attain the requirement daily of vitamin C. In this study, the ash content obtained was higher than that reported by Parkouda *et al*. (2015) in Saba jam from Burkina Faso, which was 0.35 ± 0.01 %. The difference of ash contents obtained in this result and those of the literature could be explained either by the different varieties, the geographical locations of Saba studied or by the technological treatments applied. The ash contents in food explain that it contain a significant amount of minerals.

Minerals are extremely important because they are responsible to several metabolic reactions in body (Traoré, Assemand, Digbeu, Kouadio, & Brou, 2018). This result showed that potassium was abundant in jam and syrup of *Saba Senegalensis*. As far as potassium, the potassium content of Saba jam was higher than that obtained by Ajenifujah-Solebo and Aina (2011) in plum jam (90.42 + 0.01 mg/100g). Moreover, the potassium content of *Saba senegalensis* syrup (241.76 ± 5.90 mg/100g) were higher than those obtained by Agence Nationale de Sécurité Sanitaire, alimentation, Environnement et travail (ANSES) (2013) in pineapple syrup (105 mg/100 g). Potassium is well known for its important role in regulating heart rate and neurotransmission (Alinnor & Akalezi, 2010. As the importance of these potassium contents, jam and syrup of Saba could be appropriate to help solve the problems of sodium-related diseases. The calcium contents in Saba jam (53.46 ± 3.96 mg/ 100g) and the Saba syrup (87.49 ± 5.42 mg/100 g) were respectively higher than those of papaya jam (36.00 ± 1.00 mg/100 g) reported by Ena, Shalini, Pragati, Reena, and Rai, (2016) and date syrup (44.41 mg/100g) reported by Farahnaky, Mardani, Mesbahi, Majzoobi, and Golmakani (2016). The relatively high levels of calcium in the two products suggest that they could have therapeutic value in a hypocalcaemia state such as osteoporosis. Indeed, calcium is a major factor in ossification and plays a role in muscle contraction, and absorption of vitamin B12 (Mensah, Okoli, Ohaju-Obodo, & Eifediyi 2008). The studies carried out by Onwuliri and Obu (2002) have showed that the minerals were important for their absolute involvement in vital physiological functions such as the regulation of osmotic pressure, regulation of electrolyte flows between the intra-and extracellular medium (by Na / K pump) (Traoré *et al*., 2018). Due to the high potassium content, jam and syrup of *Saba senegalensis* could be recommended in diet.

As for as the antinutritional factor, results showed the high level of phytates and tannins in jam and syrup of *Saba senegalensis*. Indeed, the high content of phytates and tannins in diet could be responsible for the unavailability of certain minerals such as iron, Zinc, magnesium and calcium (Traoré *et al*., 2018). In addition to the antinutritional factor, the jam and syrup of *Saba senegalensis* contained phytonutrients, which play the role of antioxidants. In previous studies, Weiguang, Joan, and Casimir (2005) demonstrated that polyphenols was considered as powerful antioxidants against radical phenomena leading to tissue or cellular degeneration. Furthermore, Sarni-Manchado and Cheynier (2006) showed that they are able to activate the natural anti-cancer defences. The flavonoid content of Saba jam (19.00 ± 0.99 mg/100g) was higher than that of strawberry jam (14.08 ± 0.99 mg/100g) reported by Branka, Danijela, Martina, and Dragović-Uzelac (2012). Moreover, the total phenolic content (103.18 ± 0.69 mg/100g) of the jam was higher than that obtained by Patras, Brunton, Tiwari, and Butler (2011) for strawberry jam (83.71 ± 0.90 mg/100g). The total phenolic and flavonoid contents of jam and syrup of Saba could give those potential therapeutic or preventive properties.

With regard to nutritional profile, results showed that jam and syrup of Saba have low score SAIN (SAIN <5) and high LIM score (LIM> 7.5). The SAIN and LIM scores have classified the jam and syrup of *Saba senegalensis* belong to the group 4 of foods, foods that must be consumed with moderation and therefore occasionally. For a better consumption of the syrups and jams of *Saba senegalensis*, it will be interesting to produce them without added sugar. The results were in agreement with those of Darmon *et al*. (2004) who classified products rich in sugar as foods to limit in consumption. For a better consumption of foods from this group, it will be interesting to produce jams and syrup of *Saba senegalensis* without added sugar. Contrary to these results, several studies classified cowpea seeds in food of group 1, recommended foods for health because cowpea seeds have high score SAIN higher than 5 and low score LIM lower than 7.5 (Traoré *et al*., 2018; Traoré *et al.,* 2020).

5. Conclusion

The present study showed that jam and syrup of *Saba senegalensis* were an important source of minerals such as magnesium, potassium, calcium and phosphorus. In addition, jam and syrup of Saba contained high rate of carbohydrates, total sugars, phenolic compounds and vitamin C whose presence facilitates the absorption of minerals. The nutritional profile showed that jam and syrup of Saba belong to the group of foods who must be consumed moderately. For a better consumption, it is desirable to produce jam and syrup of *Saba Senegalensis* fruit without added sugar. This study has shown that Saba jam and Saba syrup have a high nutritional value and could thus contribute to the improvement of the population's diet and these products would offer interesting possibilities of the economic market.

Statement of competing interests

The authors have no competing interest in relation to their work.

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