Tropical Agricultural Research & Extension 27 (3): 2024

RESEARCH ARTICLE

PHYSICOCHEMICAL AND ORGANOLEPTIC PROPERTIES OF GREEK-TYPE YOGHURT FORTIFIED WITH OR WITHOUT OMEGA-3 FATTY ACID AND HONEY

Yusuf IO, Adediran OA*, Awodoyin RO, Olusola OO, and Omojola AB

Department of Animal Science, University of Ibadan, Ibadan, Nigeria

Received: 02 November 2023, Accepted: 09 July 2024, Published: 30 September 2024

ABSTRACT

This study was designed to determine the physicochemical and organoleptic properties of Greek yoghurt fortified with or without honey and Omega-3 fatty acids at different inclusion levels. Greek–type yoghurt was prepared in seven formulations thus: No addition of honey and Omega-3 fatty acid (Treatment 1), honey at 5% inclusion level (Treatment 2), honey at 7.5% inclusion level (Treatment 3), honey at 10% inclusion level (Treatment 4), honey at 5% inclusion and Omega-3 fatty acid at 2% inclusion level (Treatment 5), honey at 7.5% inclusion level and Omega-3 fatty acid at 2% inclusion level (Treatment 6) and honey at 10% inclusion level and Omega-3 fatty acid at 2% inclusion level (Treatment 7). The organoleptic properties, physicochemical properties, Thiobarbituric Acid Reactive Substances (TBARS) and microbial loads of yoghurts were determined using standard procedures on days 0, 7 and 14 of storage in a completely randomized design. Data were subjected to ANOVA at α-0.05. There were significant differences among the microbial population of the treatments (P<0.05). Formulated yoghurt samples revealed T7 as having the highest microbial load (5.54± 0.21 CFU/mL) while T5 had the lowest (0.22± 0.03 CFU/mL). Treatment 4 was significantly higher (P<0.05) in fat (0.94± 0.02%), WHC (74.14± 0.08 g/kg^{-1}) and titratable acidity (0.89± 0.00 g/L) and had the lowest value in pH (4.08± 0.02), total solids (33.50± 0.05%) and crude protein (7.06± 0.04%) w. In comparison, treatment 7 was significantly higher (P<0.05) in syneresis (35.96± 0.13%) and lactose (3.08± 0.12). Fat, total solids, pH and WHC, declined with storage days while titratable acidity, moisture content and syneresis increased with storage days. The yoghurts were generally well accepted and rated by the panellists in this trend: T2>T3>T4>T6>T5>T7>T1.

Keywords: Greek-type yoghurt, Microbial load, Milk, Organoleptic properties, Physico-chemical, Thiobarbituric acid reactive substances

INTRODUCTION

The demand for protein in the human diet is increasing daily, which has resulted in human sourcing for animal protein such as eggs, meat and milk, which could also help combat the problem of malnutrition. Dairy products are thought to provide several health advantages due to their nutritional richness and high digestibility (Sadeghi, 2016). Yoghurt is regarded as the most nutritional product among all fermented dairy foods and, over the years, has been brought into the category of foods that contribute to human health (Behare, 2016).

Corresponding author: dimu4ever@yahoo.com

In contemporary times, yoghurt is one of the most popular fermented milk beverages, with consumers appreciating its flavour, nutritional content, and healthful benefits. Furthermore, yoghurt possesses functional properties that modern consumers have well sought after (Solowiej *et al*., 2018; Sarkar, 2019).

Human health and general well-being are essential; as a result, consumers are always looking for more health-friendly alternatives, attempting to adapt their lifestyles and seeking out more health-friendly foods. (Granato *et al*., 2020). In this regard, food by-products'

nutritional and bioactive components can be good sources for generating novel food items to improve consumer health and well-being. (Gullón *et al*., 2020; López-Pedrouso *et al*., 2020).

Omega-3 fatty acids are examples of bioactive molecules whose interest in scientific inquiry has grown over time due to their established wellness benefits. The human body cannot produce these fatty acids, so they must be obtained through food and supplements (Amegovu *et al*., 2014). As a result, fortifying foods with Omega-3 fatty acids has been recommended as a pragmatic strategy for boosting the intake of these fatty acids (Metcalf *et al*., 2003).

Fish oil is one of the highly concentrated and natural sources of long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic acid (EPA) and decohexanoic acid (DHA) in the human diet, whose deficiency can have a significant impact on human health (Karr *et al*., 2011; Siegel *et al*., 2012).

Fish oil has been added to yoghurt to increase its Omega-3 fatty acid content. Let *et al*. (2007) stated that yoghurt was suitable for delivering fish oil. Nevertheless, the inclusion of these fatty acids into diets, as well as their processing, pose unique nutritional hurdles for their optimal delivery (Huber *et al*., 2009).

The rapid oxidation rate of fish oil can be mitigated by including synthetic or natural antioxidants (Huber *et al.,* 2009). However, natural antioxidants are favoured over synthetic antioxidants owing to health hazards associated with the synthetic ones (Stip and Bels, 2009).

Natural honey offers a variety of beneficial qualities, including anti-bacterial, anti-oxidant, anti-inflammatory, antibiotic, antiviral, and wound healing capabilities (Seema and Simon, 2013). Honey, because of its beneficial properties, can also be employed as a sweetener in producing yoghurt (Roumyan *et al*., 1996; Chick *et al*., 2001). It can also be deployed to inhibit oxidation of Omega-3 polyunsaturated fatty acids.

Honey's anti-oxidative potential is attributed to its high contents of beneficial carotenoids, phenolics, and flavonoid components (Ivarez *et al.,* 2010). The amount and type of antioxidants in honey are greatly influenced by the source, type, geographical conditions, and processing techniques (Mohammed *et al*., 2013). This experiment aimed to determine the physicochemical properties, consumer preference, microbial load and level of oxidation indicators (TBARS) of Greek yoghurt fortified with honey and Omega-3 fatty acid over a 14-day storage period.

MATERIALS AND METHODS

Experimental site

The experiment was done at the Animal Products and Processing Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Procurement of experimental materials

Powdered milk, starter culture, honey, and fish oil (a source of Omega-3 fatty acids) were obtained from a commercial store in Ibadan. The manufacturing details, such as the NAFDAC number, batch number, manufacturing date, expiry date, and ingredients, were checked at purchase. A total of 5 kg of powdered milk was used to prepare Greek yoghurt. The pH of the milk was read using a portable pH meter. The yoghurt starter culture was a 2:1 mix of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

Preparation of greek-type yoghurt

Five (5) kg of powdered milk was dissolved in 12 litres of water; homogenization was achieved by using a mechanical stirrer, after which the milk was heated at a temperature of 44 ^{o}C for 15 min. The milk was cooled to 43 ^{o}C and inoculated with starter cultures (2%, V/V) to produce 20 litres of Greek yoghurt. It was then incubated at 43 ^{o}C for 6 h. The resultant yoghurt obtained was poured inside a cheesecloth for straining and placed in a refrigerator at a temperature of 4 ^{o}C. This process was carried out for 4 h and stopped when the desired mass was achieved. Omega-3 fatty acid-rich fish oil was incorporated into the mixture; homogenization was achieved using a mechanical stirrer.

Experimental setup
Treatments were arranged as follows:
Control experiment (Treatment 1),
Greek yoghurt fortified with honey at a 5% level of inclusion (Treatment 2),
Greek yoghurt fortified with honey at a 7.5% level of inclusion (Treatment 3),
Greek yoghurt fortified with honey at a 10% level of inclusion (Treatment 4),
Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion (Treatment 5),
Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion (Treatment 6),
Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion (Treatment 7).

Determination of physicochemical parameters of greek – type yoghurt
The physical and chemical properties (titratable acidity, pH, Thiobarbituric Acid Reactive Substances (TBARS), Water Holding Capacity (WHC), syneresis, total solids, moisture content, lactose, fat, and protein) were determined using AOAC methods as described by Latimer (2023) on days 0, 7, and 14. The measurements of the parameters evaluated were carried out in triplicate.

Titrable Acidity
Ten millilitres of each sample was diluted to 100 mL. Twenty-millilitre aliquots of the diluent were pipetted into a 100-volumetric flask, and 1% phenolphthalein was added as an indicator. The mixture was titrated against 0.1 N NaOH to an end point of faint pink colour. Titrable acidity was determined using the equation:

$$TA\ (g/L) = (V \times 0.9)/m$$

where V = volume (mL); m = mass (g); 0.9 = correction factor for lactic acid.

pH was measured according to Gassem and Frank (1991).

Thiobarbituric Acid Reactive Substances (TBARS)
TBARS is used as an indicator of lipid oxidation. This was evaluated using the modified method described by Vernon *et al.* (1970). One gram of sample was weighed into a test tube and homogenized with 2 mL of distilled water. Trichloroacetic acid 2.5 mL was added into each test tube and centrifuged at 2000 rpm for 10 min. One milliliter of the centrifuged sample was decanted into a test tube, and 1 mL of Thiobarbituric acid was added to the test tubes. The mixture was further boiled for 35 min and poured into a quoit. A UV-VIS spectrophotometer was used to read the samples at 532 nm wavelength. The results were expressed as µg/g samples.

Water Holding Capacity (WHC)
This was determined using the method described by Kwasi Kpodo *et al.,* (2014). The yoghurt sample was mixed to ensure the homogeneity of the product. Two (2 mL) of distilled water was added to 5 g of yoghurt (Y) in 10 mL graduated centrifuge tubes. The mixture was stirred with a glass rod to disperse the sample in distilled water. After holding the mixture for 30 min, the mixture was centrifuged for 30 min at 1250 g at 20 °C. The whey expelled (WE) was removed and weighed. The excess water absorbed was expressed as the percentage of water bound by a 100 g sample. The density of the water was determined using the specific gravity bottle. The WHC (g/kg^{-1}) was calculated thus:

$$WHC = \frac{100x(Y - WE)}{Y}$$

Y = Yoghurt samples (g); WE = Whey expelled

Determination of Syneresis
A measuring cylinder was placed at room temperature. Twenty grams of yoghurt were poured into a funnel with filter paper to separate the whey. This was done for 3 h under room temperature for each treatment, as described by Latimer (2023). The syneresis was expressed as the percentage weight of whey over the initial weight of each sample.

$$\%\ Syneresis = \frac{Volume\ of\ Supernatant}{Weight\ of\ Sample} x\ 100$$

Determination of Total Solid

Ten grams of Greek yoghurt from each sample was weighed into the crucible of a known weight. The samples in each crucible were then transferred into the oven set at 65 ^{o}C to dry to a constant weight for 24 h. At the end of the 24 h, the crucibles were removed from the oven, transferred to a desiccator, cooled for ten min, and weighed. It was thus calculated:

$$\% \text{ Total Solid} = \frac{W3 - Wo}{W1 - Wo} x100$$

Wo – the weight of empty crucible
W_1 – the weight of crucible plus sample
W_3 – the weight of crucible plus oven-dried sample

Determination of lactose, fat, and protein

Lactose, fat, and protein were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (AOAC, 2005). Analyses were carried out in triplicate.

Determination of Microbial Load

The yoghurt samples based on individual treatment were divided into three equal parts. Twenty-eight grams (28 g) of Nutrient Agar was dissolved in 500 cm^3 of distilled water and boiled in a water bath for 30 min. The medium was then cooled to 45 °C before pouring into the plate under sterilized conditions for culturing bacteria. One gram of each sample was dissolved in 9 mL of sterilized water in test tubes and serially diluted up to 10^{-5}. Sterile pipettes were used to measure 1 cm^3 out of the 10^{-3} and 10^{-5} dilution fractions, and these were pipetted into two different labelled sterile Petri dishes, and molten agar at 45 °C was poured into them (using the pour plate method). It was swirled gently by hand for even distribution; the plates were inverted and incubated at 37 °C for 24 h. After this, they were examined under a microscope. Colonies at the end of the incubation period were counted, and the data was expressed as logarithms of the colonies forming unit (log_{10}CFU/g) sample. All analyses were done in triplicates following the procedures described by (Seydim and Sarikus, 2006).

Determination of sensory properties of Greek–type yoghurt

Ten trained panellists aged 25 – 45 were recruited and randomly allocated to freshly prepared samples of differently formulated Greek-type yoghurt. Equal quantities of the differently formulated samples (A, B, C, D, E, F, and G) were allotted to each panellist in a colourless disposable cup to assess the colour, flavour, taste, texture, and overall acceptability as described by Choi *et al.* (2016).

Statistical analysis

All data obtained was statistically analysed using the SAS (2012) programme, and means were separated using the Duncan Multiple Range Test. The statistical significance level was chosen at $P<0.05$. GraphPad Prism 5.0® software was used for all statistical analyses. The differences among the various groups were determined using one-way ANOVA.

RESULTS AND DISCUSSION

There are significant differences ($P<0.05$) among treatments and storage days (Table 1). There was no significant difference in the total fungi count, total bacteria count, and total coliform count value for T7 over the storage days.

The treatment's Total bacterial count (TBC), Total fungi count (TFC) and Total coliform count (TCC) values increased over the storage days. Bacteria and fungi cause objectionable changes that lower product quality, and their growth is favoured by low pH. There was a significant difference in the bacteria count of differently fortified Greek-type yoghurt evaluated. A range of 2.81 and 3.92 was obtained, with the least detected in Greek yoghurt fortified with honey at a 5% level of inclusion, while the highest was recorded in Greek yoghurt without honey and Omega-3 fatty acid; the presence could be a result of unsteady power supply and inadequate refrigeration in the storage environment (Lamye *et al.,* 2017).

Table 1: Evaluation of Microbial Population of Differently Fortified Greek-type Yoghurt

Days	Parameters	Treatment						
		T1	T2	T3	T4	T5	T6	T7
0	TBC (Cfu/ml) x10^3	2.34 ±0.07^{cd}	2.10 ±0.03^{e}	2.40 ±0.06^{bc}	2.28 ±0.03^{d}	2.14 ±0.06^{e}	2.44 ±0.04^{b}	2.54 ±0.03^{a}
7		3.02 ±0.24^{bc}	2.62 ±0.08^{d}	2.48 ±0.16^{d}	2.65 ±0.17^{d}	2.80 ±0.20^{cd}	3.36 ±0.21^{ab}	3.68 ±0.27^{a}
14		4.16 ±0.42^{c}	3.72 ±0.09^{c}	3.83 ±0.09^{c}	4.06 ±0.16^{c}	4.69 ±0.21^{b}	4.90 ±0.35^{b}	5.54 ±0.21^{a}
0	TFC (Cfu/ml) x 10^2	1.10 ±0.03^{cd}	1.05 ±0.03^{de}	1.26 ±0.03^{b}	1.12 ±0.04^{c}	1.01 ±0.03^{e}	1.36 ±0.04^{a}	1.40 ±0.04^{a}
7		1.09 ±0.18^{c}	1.58 ±0.21^{b}	1.07 ±0.13^{c}	1.28 ±0.13^{bc}	1.57 ±0.55^{b}	1.70 ±0.20^{b}	2.17 ±0.10^{a}
14		1.25 ±0.09^{c}	2.01 ±0.10^{ab}	1.32 ±0.06^{c}	1.74 ±0.19^{b}	1.96 ±0.39^{b}	2.10 ±0.30^{ab}	2.36 ±0.09^{a}
0	TCC (Cfu/ml) x 10^2	0.54 ±0.05^{c}	0.30 ±0.04^{d}	0.66 ±0.05^{b}	0.31 ±0.02^{d}	0.22 ±0.03^{e}	0.66 ±0.03^{b}	0.73 ±0.03^{a}
7		0.62 ±0.04^{b}	0.41 ±0.08^{cd}	0.74 ±0.08^{a}	0.47 ±0.06^{c}	0.32 ±0.07^{d}	0.79 ±0.07^{a}	0.80 ±0.06^{a}
14		0.74 ±0.13^{c}	0.59 ±0.07^{de}	0.890.06^{b}	0.65^{c} ±0.07^{d}	0.48 ±0.11^{e}	0.95 ±0.02^{ab}	1.09 ±0.07^{a}

Means along the same rows with different superscripts are significantly different (P<0.05).

TCC; Total coliform count, TBC; Total Bacteria count; TFC; Total fungi count

T1: Control, T2: Greek yoghurt fortified with honey at a 5% level of inclusion, T3: Greek yoghurt fortified with honey at a 7.5% level of inclusion, T4: Greek yoghurt fortified with honey at a 10% level of inclusion, T5: Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion, T6: Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion, T7: Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion

The population of bacteria increased with the increase in storage days, which corroborated the study of Sarkar *et al*. (2012), which reported that post–pasteurization containers and prolonged storage can result in an increase in bacteria count.

Thiobarbituric acid reactive substances of differently fortified Greek-type yoghurt during storage days (Table 2). There were significant differences in P<0.05 among the TBARS (an indicator of lipid oxidation) of the treatment.

Treatment 7 had the highest value among the treatments (5.89 µg/g), while T2 had the lowest value (5.20 µg/g) on day 0. There was no significant difference in the TBARS of T3, T5 and T7 over the storage days. The highest value (6.13 µg/g) over the storage days was recorded on day 14, while the lowest value (5.20 µg/g) was recorded on day 0. The TBARS values in honey and Omega-3 fatty acid-fortified yoghurt (treatment 7, day 14) were significantly higher than those of all other yoghurts during storage. This is in conformation with a study by Estrada (2011), which stated that there was a significant

Table 2: Thiobarbituric Acid Reactive Substances (µg/g) of differently fortified Greek-type yoghurt during storage days

Days	Treatments						
	T1	T2	T3	T4	T5	T6	T7
0	5.44±0.06^{e}	5.20±0.03^{f}	5.71±0.02^{c}	5.56±0.06^{d}	5.80±0.03^{b}	5.63±0.06^{cd}	5.89±0.05^{a}
7	5.76±0.08^{c}	5.36±0.06^{e}	5.77±0.02^{c}	5.66±0.04^{d}	5.88±0.03^{b}	5.74±0.07^{cd}	6.01±0.03^{a}
14	5.82±0.07^{c}	5.50±0.05^{d}	5.84±0.03^{c}	5.78±0.05^{c}	5.96±0.08^{b}	5.79±0.08^{c}	6.13±0.04^{a}

Means along the same rows with different superscripts are significantly different (P<0.05)

T1: Control, T2: Greek yoghurt fortified with honey at a 5% level of inclusion, T3: Greek yoghurt fortified with honey at a 7.5% level of inclusion, T4: Greek yoghurt fortified with honey at a 10% level of inclusion, T5: Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion, T6: Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion, T7: Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion

increase in TBARS values of functional yoghurt fortified with microencapsulated Omega-3 during 4 weeks of storage with refrigeration.

According to Al-Rowaily (2008), refrigerated storage of set yoghurt for seven days and strained yoghurt for fifteen days resulted in significant variation in oxidation parameters. The lipid oxidation values for the treatments were higher in Omega-3 fatty acid-fortified yoghurt. This showed that lipid oxidation progressed more rapidly in yoghurt with Omega-3 fatty acid addition than that without Omega-3 fatty acid, and this was in conformation with a study by Boran *et al*., (2006), who demonstrated that lipid oxidation could cause fish oils to degrade to unsatisfactory levels during refrigerated storage (4 ºC).

The result for the proximate composition of Greek-type yoghurt was differently fortified with varying levels of honey and Omega-3 fatty acids (Table 3). They all lie within acceptable ranges for yoghurt nutrient composition earlier reported by McKinley (2005) and Kibui *et al.* (2018). No significant difference was recorded in honey-fortified yoghurt samples T2 and T3 over the storage days. The protein values for treatment ranged from 8.08 – 11.65% on day zero. The protein

Table 3: Effect of different methods of fortification on physicochemical properties of Greek–type yoghurt

Treatment		T1	T2	T3	T4	T5	T6	T7
Protein (%)	0	8.66 ±0.07^{d}	11.65 ±0.13^{a}	9.50 ±0.14^{b}	8.08 ±0.10^{e}	8.73 ±0.06^{d}	9.09 ±0.13^{c}	8.99 ±0.10^{c}
	7	8.48 ±0.03^{d}	11.59 ±0.02^{a}	9.26 ±0.04^{b}	7.86 ±0.07^{e}	8.56 ±0.07^{d}	8.81 ±0.07^{c}	8.77 ±0.15^{c}
	14	7.91 ±0.03^{b}	13.8 ±5.21^{a}	8.78 ±0.04^{b}	7.06 ±0.04^{b}	7.99 ±0.08^{b}	7.96 ±0.05^{b}	7.90 ±0.02^{b}
Titratable Acidity (g/L)	0	0.86 ±0.00^{e}	0.89 ±0.00^{a}	0.88 ±0.00^{bc}	0.89 ±0.00^{a}	0.88 ±0.00^{b}	0.87 ±0.00^{d}	0.88 ±0.00^{c}
	7	1.20 ±0.01^{e}	1.06 ±0.01^{f}	1.30 ±0.01^{c}	1.39 ±0.02^{a}	1.23 ±0.00^{d}	1.32 ±0.01^{b}	1.29 ±0.00^{c}
	14	1.22 ±0.00^{e}	1.10 ±0.01^{f}	1.32 ±0.01^{b}	1.41 ±0.01^{a}	1.24 ±0.00^{d}	1.33 ±0.00^{b}	1.31 ±0.00^{c}
Fat (%)	0	0.69 ±0.02^{e}	0.86 ±0.02^{b}	0.73 ±0.04^{de}	0.94 ±0.02^{a}	0.76 ±0.03^{cd}	0.86 ±0.02^{b}	0.79 ±0.03^{c}
	7	1.15 ±0.06^{b}	1.27 ±0.03^{a}	1.14 ±0.06^{b}	1.29 ±0.02^{a}	1.13 ±0.06^{b}	1.27 ±0.03^{a}	1.14 ±0.05^{b}
	14	1.00 ±0.04^{b}	1.10 ±0.01^{a}	0.94 ±0.07^{bc}	1.09 ±0.01^{a}	0.92 ±0.03^{c}	1.08 ±0.03^{a}	0.97 ±0.03^{bc}
Moisture content (%)	0	71.16 ±0.05^{a}	67.03 ±0.12^{f}	68.92 ±0.16^{c}	70.91 ±0.11^{b}	69.09 ±0.11^{c}	68.03 ±0.13^{d}	67.61 ±0.06^{e}
	7	71.90 ±0.11^{a}	67.98 ±0.20^{g}	69.88 ±0.25^{d}	71.40 ±0.18^{b}	70.35 ±0.05^{c}	69.42 ±0.20^{e}	68.47 ±0.05^{f}
	14	73.87 ±0.08^{a}	69.96 ±0.12^{g}	71.88 ±0.25^{d}	73.36 ±0.16^{b}	72.30 ±0.05^{c}	71.47 ±0.08^{e}	70.43 ±0.04^{f}
Total solid (%)	0	47.36 ±0.07^{a}	46.82 ±0.04^{a}	44.24 ±0.06^{b}	35.85 ±1.19^{d}	35.91 ±0.16^{d}	38.50 ±0.11^{c}	38.66 ±0.03^{c}
	7	46.58 ±0.07^{a}	45.20 ±0.11^{b}	43.08 ±0.07^{c}	34.30 ±0.04^{g}	35.11 ±0.08^{f}	37.09 ±0.07^{e}	37.27 ±0.06^{d}
	14	45.79 ±0.07^{a}	44.41 ±0.11^{b}	42.30 ±0.07^{c}	33.50 ±0.05^{g}	34.34 ±0.06^{f}	36.34 ±0.05^{e}	36.48 ±0.03^{d}

Means along the same rows with different superscripts are significantly different (P<0.05)

T1: Control, T2: Greek yoghurt fortified with honey at a 5% level of inclusion, T3: Greek yoghurt fortified with honey at a 7.5% level of inclusion, T4: Greek yoghurt fortified with honey at a 10% level of inclusion, T5: Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion, T6: Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion, T7: Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion

content decreased with an increase in the percentage of honey, while that of Omega-3 fatty acid-fortified yoghurt increased. This is because the protein content of honey is negligible. The highest protein (12.35%) was observed in honey-fortified yoghurt at a 5% level of inclusion. Similar to observations in a related experiment by Rajunaik *et al*., (2017).

T4 and T2 had the highest value (0.89 g/L) for titratable acidity, while T1 had the lowest value (0.86 g/L). Significant differences occurred in all yoghurt samples. There was an increase in the titratable acidity values of the treatments over storage days. The titratable acidity of Greek yoghurt ranged from 0.86 to 1.41% lactic acid. The acidity of yoghurt increased during storage, irrespective of fortification with honey and Omega-3 fatty acids. The increased acidity is due to lactic acid bacteria continuing to ferment during storage because of post-acidification of products with lactic acid production (Aportela -palacios *et al*., 2005; Pereira *et al*., 2012).

Fat content varied significantly among the differently fortified Greek yoghurt, with the fat content recorded ranging from (0.69-0.94%). The fat content increased for all samples over storage days, and there was no significant variation in the fat content of T4 over the storage days.

Yoghurt fortified with honey only had a higher fat content due to the lower water content of honey and less lipase activity. Honey has a lower water content than Omega-3 fish oil, which can reduce the rate of fat degradation. It can also be attributed to the fact that honey does not contain lipases that break down triglycerides, resulting in less fat degradation.

Sodini *et al*., (2014) reported that the higher the protein content, the higher the total solids and their effect can sometimes be confounded because they are dependent variables. This aligns with observations in this study, where the protein content decreased as total solids decreased.

The decrease in protein during storage in yoghurt fortified with honey and Omega-3 fatty acids could be due to increased acidity during storage, which can denature proteins, making them more prone to degradation. It can also be due to temperature fluctuations during storage, which can activate proteases or cause protein denaturation.

The result for the proximate composition of Greek-type yoghurt differently fortified with varying inclusion levels of honey and Omega-3 fatty acid shows a significant difference in lactose, water-holding capacity, syneresis and pH of treatments (Table 4).

Table 4: Effect of different methods of fortification on physicochemical properties of Greek–type yoghurt

Treatment	Day	T1	T2	T3	T4	T5	T6	T7
WHC	0	71.35 ± 0.08^{d}	69.10 ± 0.15^{f}	73.06 ± 0.14^{b}	74.14 ± 0.08^{a}	70.81 ± 0.15^{e}	71.34 ± 0.08^{d}	72.10 ± 0.06^{c}
(g/	7	69.62 ± 0.04^{e}	67.39 ± 0.11^{g}	71.71 ± 0.06^{b}	72.91 ± 0.10^{a}	68.59 ± 0.05^{f}	69.83 ± 0.16^{d}	70.81 ± 0.18^{c}
kg^{-1})	14	65.78 ± 0.02^{e}	63.56 ± 0.07^{g}	67.86 ± 0.05^{b}	69.05 ± 0.06^{a}	64.76 ± 0.05^{f}	65.99 ± 0.12^{d}	66.94 ± 0.08^{c}
Lac	0	2.60 ± 0.03^{f}	2.76 ± 0.03^{c}	2.61 ± 0.04^{ef}	2.70 ± 0.02^{d}	2.66 ± 0.03^{de}	2.83 ± 0.04^{b}	2.91 ± 0.04^{a}
tose	7	2.75 ± 0.05^{d}	$2.89^{b}\pm0.01^{c}$	2.80 ± 0.03^{cd}	2.79 ± 0.03^{cd}	2.78 ± 0.03^{d}	2.96 ± 0.03^{b}	3.08 ± 0.12^{a}
	14	2.64 ± 0.04^{d}	2.76 ± 0.02^{c}	2.68 ± 0.03^{d}	2.67 ± 0.03^{d}	2.66 ± 0.02^{d}	2.84 ± 0.03^{b}	3.03 ± 0.05^{a}
Syn	0	14.63 ± 0.16^{f}	17.93 ± 0.09^{e}	9.97 ± 0.13^{g}	22.93 ± 0.17^{d}	29.58 ± 0.22^{b}	26.78 ± 0.32^{c}	33.00 ± 0.20^{a}
eresis	7	15.95 ± 0.13^{f}	19.14 ± 0.05^{e}	10.34 ± 0.06^{g}	24.10 ± 0.03^{d}	31.14 ± 0.14^{b}	27.96 ± 0.21^{c}	34.10 ± 0.14^{a}
(%)	14	17.84 ± 0.10^{f}	20.98 ± 0.07^{e}	12.19 ± 0.03^{g}	25.94 ± 0.04^{d}	32.97 ± 0.08^{b}	29.88 ± 0.16^{c}	35.96 ± 0.13^{a}
pH	0	4.83 ± 0.12^{c}	5.17 ± 0.12^{b}	4.87 ± 0.03^{c}	4.57 ± 0.15^{d}	4.83 ± 0.06^{c}	5.49 ± 0.10^{a}	5.00 ± 0.10^{bc}
	7	4.61 ± 0.04^{d}	4.87 ± 0.06^{b}	4.67 ± 0.06^{cd}	4.49 ± 0.04^{e}	4.59 ± 0.04^{d}	5.27 ± 0.06^{a}	4.74 ± 0.06^{c}
	14	4.21 ± 0.05^{de}	4.43 ± 0.04^{b}	4.26 ± 0.08^{cd}	4.08 ± 0.02^{f}	$4.15^{e}\pm0.04^{f}$	4.82 ± 0.04^{a}	4.30 ± 0.05^{c}

Means along the same rows with different superscripts are significantly different ($P<0.05$), WHC; water holding capacity

T1: Control, T2: Greek yoghurt fortified with honey at a 5% level of inclusion, T3: Greek yoghurt fortified with honey at a 7.5% level of inclusion, T4: Greek yoghurt fortified with honey at a 10% level of inclusion, T5: Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion, T6: Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion, T7: Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion

The water-holding capacity and lactose content increased from T2 to T4 and from T5 to T7, respectively. The highest value (74.14 g/kg^{-1}) for WHC was observed in T4, and the highest value (2.91 g/kg^{-1}) for lactose was observed in T7. The water holding capacity was reduced over storage days, while lactose content increased from day 0 to 7 and was reduced from day 7 to 14. The highest syneresis value (33.00%) was recorded in T7, while the lowest value (9.97%) was recorded in T3. Syneresis increased over the storage days for all treatments. In addition to the storage period, syneresis increases when the yoghurt recipe is devoid of preservatives (Vareltzis *et al*., 2016), and this could be one of the factors that caused the high degree of syneresis obtained in this study, as no method was formulated with preservatives. In general, the pH values in all treatments decreased slightly, and the pH value continued decreasing as the inclusion of honey increased. This is in conformation with a study by Rashid and Thakur (2012), which recorded a decrease in pH with an increase in the percentage of honey. This might be due to increased acidity because pH and acidity are inversely related. The highest pH (5.49) was recorded in 7.5% honey level of inclusion and 2% Omega-3 fatty acid fortified yoghurt level of inclusion on treatment 6.

Treatment 1 had the lowest score on flavour, colour, taste and overall acceptability (Table 5). Yoghurt texture showed no significant difference across the treatments. T3 had the highest colour (6.50), and T2 had the highest flavour, taste, and overall acceptability, with values of 6.80, 6.82 and 7.10, respectively.

According to Mousavi *et al*. (2019), sensory evaluation aids in defining important product characteristics in terms of consumer acceptability. The scores for colour and texture showed that an increase in honey content improved the texture and colour of the products.

A study by Bakr *et al*. (2015) indicated that the content of honey up to 5% preserves the physicochemical properties and improves the organoleptic characteristics of the resultant functional product from yoghurt.

Greek yoghurt fortified with honey at a 5% level of inclusion (treatment 2) was considered to have the best taste, while Greek yoghurt fortified with honey at a 7.5% level of inclusion (treatment 3) had the best colour. Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 fatty acid at a 2% level of inclusion (treatment 6) had the best texture among differently fortified yoghurts.

CONCLUSIONS

The fortification of Greek yoghurt with honey at a 7.5% level of inclusion (treatment 3) proved effective as a natural antioxidant in producing highly nutritious and good-quality Greek yoghurt. From the studies conducted on the physicochemical and organoleptic

Table 5: Organoleptic properties of differently Fortified Greek–type yoghurt

Treatment/ Parameters	T1	T2	T3	T4	T5	T6	T7
Taste	4.30 ± 1.70^{c}	6.82 ± 1.14^{a}	6.40 ± 0.97^{ab}	6.20 ± 1.14^{ab}	4.70 ± 2.00^{bc}	6.30 ± 1.83^{ab}	5.00 ± 2.67^{bc}
Texture	6.30±1.49	5.20±2.20	6.00±1.05	6.10±1.60	6.40±0.97	6.70±1.16	5.30±2.79
Colour	3.60 ± 0.70^{c}	6.30 ± 1.08^{a}	6.50 ± 1.25^{a}	5.80 ± 1.69^{ab}	4.60 ± 1.71^{bc}	5.60 ± 2.12^{ab}	4.50 ± 2.37^{bc}
Flavour	3.20 ± 1.93^{c}	6.80 ± 0.79^{a}	5.70 ± 1.49^{ab}	4.80 ± 2.04^{bc}	4.50 ± 2.68^{bc}	5.40 ± 1.71^{ab}	5.40 ± 2.22^{ab}
Overall Acceptability	3.90 ± 1.60^{c}	7.10 ± 1.60^{a}	6.70 ± 1.34^{ab}	6.60 ± 1.17^{ab}	5.30 ± 2.31^{abc}	6.30 ± 1.95^{ab}	5.10 ± 2.47^{bc}

Means along the same rows with different superscripts are significantly different ($P<0.05$)

T1: Control, T2: Greek yoghurt fortified with honey at a 5% level of inclusion, T3: Greek yoghurt fortified with honey at a 7.5% level of inclusion, T4: Greek yoghurt fortified with honey at a 10% level of inclusion, T5: Greek yoghurt fortified with honey at a 5% level of inclusion and Omega-3 at a 2% level of inclusion, T6: Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 at a 2% level of inclusion, T7: Greek yoghurt fortified with honey at a 10% level of inclusion and Omega-3 at a 2% level of inclusion

properties of Greek yoghurt fortified with honey and Omega-3 fatty acid, it can be concluded that the varying inclusion levels improved the product's sensory and physicochemical properties. The antimicrobial properties of honey reduced the microbial population of the sample, as the control yoghurt had a higher microbial count than honey-fortified yoghurt. Honey contains glucose oxidase, an enzyme that converts glucose into hydrogen peroxide, a potent antimicrobial agent.

The best combination for Greek yoghurt fortified with honey and Omega-3 fatty acid was Greek yoghurt fortified with honey at a 7.5% level of inclusion and Omega-3 fatty acid at 2% (treatment 6), based on the antioxidant effect of added honey at that level to stabilise yoghurt quality.

AUTHOR CONTRIBUTION

ABO and OOO conceptualised the study. IOY and ABO carried out the experiments. IOY, OAA, and ROA wrote the paper with input from all authors. All authors discussed the results and commented on the manuscript.

REFERENCES

AOAC. (2005) "Official method of Analysis," 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.

Al-Rowaily, M. A. (2008) "Effect of heating treatments, processing methods and refrigerated storage of milk and some dairy products in lipids oxidation," *Pakistan Journal of Nutrition*, 7(1), pp. 118–125.

Amegovu, A. K., Ogwok, P., Owor, S., Yiga, P., Musalima, J. H., and Mandha, J. (2014) "Sensory Acceptability of Sorghum Peanut Blend (SPB) and Corn Soy Blend Plus (CSB +) By Young Children with Moderate Acute Malnutrition in Karamoja," *Uganda*, 3 (2).

Aportela Palacios, A., Aosa-Morales, M. and Velez-Ruiz, E. (2005) "Rheological and physicochemical behaviour of fortified yoghurt, with fiber and calcium," *Journal of Texture Studies*, 36(3), pp. 333–349.

Bakr, I., Mohamed, A. Tammam and Gazzar, F. (2015) "Characteristics of bio-yoghurt fortified with fennel honey," *International Journal of Current Microbiology and Applied Sciences*, 4(3), pp. 959–970.

Behare, P., Lule, V. K. and Patil, P. (2016) "Yogurt: Dietary Importance," in *Encyclopedia of Food and Health*. Elsevier, pp. 612–616.

Boran, G., Karaçam, H. and Boran, M. (2006) "Changes in the quality of fish oils due to storage temperature and time," *Food chemistry*, 98(4), pp. 693–698. doi: 10.1016/j.foodchem.2005.06.041.

Chick, H., Shin, H. S. and Ustunol, Z. (2001) "Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey." *Journal of Food Science*, 66(3), 478-481. http:// dx.doi.org/10.1111/j.1365-2621.2001.tb16134.x

Choi, Y. J., Jin, H. Y. and Yang, H. S. (2016) "Quality and Storage of Yoghurt containing *Lacobacillus sakei* AL1033 and cinnamon ethanol extract." *Journal of Animal Science and Technology*. https://doi.org/10.1186/ s40781

Estrada Andino, J. (2022) *Production and processing of a functional yogurt fortified with microencapsulated Omega-3 and vitamin E*. Louisiana State University.

Gassem, M. A. and Frank, J. F. (1991) "Physical properties of yogurt made from milk treated with proteolytic enzymes," *Journal of dairy science*, 74 (5), pp. 1503–1511. doi: 10.3168/ jds.s0022-0302(91)78310-0.

Granato, D., Barba, F.J., Bursa´c Kova˘cevi´c, D., Lorenzo, J.M., Cruz, A.G., Putnik, P. (2020) "Functional foods: Product development, technological trends, efficacy testing, and safety," *Annual review of food science and technology*, 11(1), pp. 93–118. doi: 10.1146/ annurev-food-032519-051708.

Gullón, B., Gagaoua, M., Barba, F.J., Gullón, P., Zhang, W., Lorenzo, J.M. (2020)

"Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products," *Trends in food science & technology*, 100, pp. 1–18. doi: 10.1016/j.tifs.2020.03.039.

Huber, G., Rupasinghe, H., and Shahidi, F. (2009) "Inhibition of oxidation of Omega-3 polyunsaturated fatty acids and fish oil by quercetin glycosides," *Food chemistry*, 117(2), pp. 290–295. doi: 10.1016/j.foodchem.2009.04.007.

Ivarez, S.J., Tulipani, S., Romandini, S., Bertoli, E. and Battino, M. (2010) "Contribution of honey in nutrition and human health: a review," *Mediterranean Journal of Nutrition and Metabolism*, 3, pp. 15–23.

Karr, J. E., Alexander, J. E. and Winningham, R. G. (2011) "Omega-3 polyunsaturated fatty acids and cognition throughout the lifespan: A review." Nutritional Neuroscience, 14: 216–225.

Kibui, A. N., Owaga, E., and Mburu, M. (2018) "Proximate composition and nutritional characterization of chia enriched yoghurt," *African Journal of Food Agriculture Nutrition and Development*, 18(01), pp. 13239–13253. doi: 10.18697/ajfand.81.17635.

Kwasi Kpodo, F. M., Afoakwa, E. O., Amoa, B. B., Budu, A. S., and Saalia, F. K. (2014) "Effect of Ingredient Variation on Microbial Acidification, Susceptibility to Syneresis, Water Holding Capacity and Viscosity of Soy-Peanut-Cow Milk Yoghurt," *J Nutritional Health Food Engg*, 1(2).

Lamye, G. M., Lunga, P. K., Pamo, T. E. and Kuiate, J. R. (2017) "Seasonal microbial conditions of locally made yoghurt (shalom) marketed in some regions of Cameroon," *International Journal of Food Science*, 2017, pp. 1–16.

Latimer, G. W. (Ed.). (2023) "Official methods of analysis of AOAC INTERNATIONAL." New York: Oxford University Press. doi: 10.1093/9780197610145.001.0001.

Let, M.B., Jacobsen, C., Meyer, A.S., (2007) "Lipid oxidation in milk, yoghurt, and salad dressing enriched with neat fish oil or pre-emulsified fish oil," *Journal of agricultural and food chemistry*, 55 (19), pp. 7802–7809. doi: 10.1021/jf070830x.

López-Pedrouso, M., Lorenzo, J.M., Cantalapiedra, J., Zapata, C., Franco, J.M., Franco, D. (2020) "Aquaculture and by-products: Challenges and opportunities in the use of alternative protein sources and bioactive compounds," *Advances in food and nutrition research*, 92, pp. 127–185. doi: 10.1016/bs.afnr.2019.11.001.

Mckinley, M. C. (2005) "The nutrition and health benefits of yoghurt," *International journal of dairy technology*, 58(1), pp. 1–12. doi: 10.1111/j.1471-0307.2005.00180.x.

Metcalf, R. G., James, M. J., Mantzioris, E., and Cleland, L. G. (2003) "A practical approach to increasing intakes of n-3 polyunsaturated fatty acids: use of novel foods enriched with n-3 fats," *European journal of clinical nutrition*, 57(12), pp. 1605–1612. doi: 10.1038/sj.ejcn.1601731.

Mohammed, M., Ibrahim, M., Siti, A. and Siew H. (2013) "Physiochemical and antioxidant properties of Malaysian honeys produced by Apis Cerena, Apis dorsata and Apis mellifera," *BMC Complementary and Alternative Medicine*, 13.

Mousavi M., Heshmati A., Garmakhany, A. D., Vahidinia A. and Taheri, M. (2019) "Texture and sensory characterization of functional yoghurt supplemented with flax seed during cold storage," *Journal of Food Science and Nutrition*, 7(3), pp. 907–917.

Pereira G, G. Rafael L, M. Gajo, A, A. Ramos, T, M. Pinto, S, M. Abreu, L, R. Resende, J, V. (2012) "Influencia do pH nas caracteristicas fisico-quimicas e sensoriais de frozenyoghurtde morango," *Semina: Ciencias Agrarias*, 33(2), pp. 675–686.

Rajunaik. B, Shilpa. V, Ramachandra. B, Praveen. A. R, Mahesh Kumar. G. (2017).. "Enhancement of Shelf Life and Therapeutic Value Of Dairy Product (Yoghurt) By Incorporation Of Omega-3 Fatty Acids From Marine Source." ISBN: 978-93-86291-88-2.

Rashid, A. and Thakur, E. (2012) "Studies on quality parameters of set yoghurt prepared by the addition of honey," *International Journal of Science and Research. Publ*, 2, pp. 1–10.

Roumyan, N., Zapryanov, P., Kondareva, S. and Georgiev, G. (1996) "On some aspects of a new fermented milk product Medina," *Biotechnology, biotechnological equipment*, 10(2–3), pp. 86–89. doi: 10.1080/13102818.1996.10818904.

Sadeghi, A. (2016) "In vitro Assessment of Some Probiotic Properties of Lactobacillus fermentum Isolated from Pickled Garlic," *Journal of Food Quality and Hazards Control*, 3(2), pp. 67–72.

Sarkar, M. M., Nahar, T. N., Alam, M. K., Rahman, M. M., Rashid, M. H. and Islam, M. A. (2012) "Chemical and bacteriological quality of popular Dahi available in some selected areas of Bangladesh," *Animal science journal of Pakistan*, 41(1), pp. 47–51. doi: 10.3329/bjas.v41i1.11977.

Sarkar, S. (2019) "Potentiality of probiotic yoghurt as a functional food-A review," *Nutr. Food Sci*, 49, pp. 182–202.

SAS (2012) "Statistical Analysis System, User's Guide." Statistical Version 9.1, ed. SAS Institute Inc., Cary, NC, USA. [1]

Seema, P. and Simon, C. (2013) "Manuka honey: an emerging natural food with medicinal use" *Review Nat. Prod. Bioprospect*, 3, 121–128 http://dx.doi.org/10.1007/s13659-013-0018-7

Seydim, A. C., and Sarikus, G. (2006) "Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils," *Food research international (Ottawa, Ont.)*, 39(5), pp. 639–644. doi: 10.1016/j.foodres.2006.01.013.

Siegel, G. and Ermilov, E. (2012). "Omega-3 fatty acids: benefits for cardio-cerebrovascular diseases." Atherosclerosis. 225, 291–295.

Sodini, I., Remeuf, F., Haddad, S. and Corrieu, G. 2014) "The relative effect of milk base, starter, and process on yoghurt texture: A review," *Critical Reviews in Food Science and Nutrition*, 44, pp. 113–137.

Solowiej, B., and Szafrańska, J. (2018) "Characteristics of traditional yoghurt microflora and its metabolites," *Pol. Dairy J*, 3, pp. 10–15.

Stip, T. and Bels, A. (2009) "Antioxidant properties and phenolic content of different floral origin honeys," *Journal of Apiproduct and Apimedical Science*, 1(2), pp. 43–50. http://dx.doi.org/10.3896/ibra

Vareltzis, P., Adamopoulos, K., Stavrakakis, E., Stefariakis, A. and Goula, A. M. (2016) "Approaches to minimize yoghurt syneresis in simulated tzatziki sauce preparation," *International Journal of Dairy Technology*, 69(2), pp. 191–199.

Vernon, W., Gary, C., Krause, F., and Bailey, M. E. (1970) "A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage," *Jour. Food. Sci. Technol*, 35, pp. 582–585.