



SHELF LIFE STUDY CHANGES ON THE PHYSICOCHEMICAL, IN VITRO ANTIOXIDANT AND NUTRITIONAL CHARACTERISTICS OF YOGHURT WITH DIFFERENT INCLUSION OF DIETARY GINGER (ZINIGIBER OFFICINALE) FIBER STORED AT COLD TEMPERATURE.

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Abstract

This study evaluated the shelf life changes on the physicochemical, antioxidant, proximate compositions and sensory characteristic of yoghurt during cold storage for four weeks. Ginger dietary fiber was added to the yoghurt in different proportions (10 %, 20 %, 30 %, 40 % and 50 %) using standard technology adapted to laboratory conditions. The moisture content of the yoghurt decreases as the storage period increases while the protein content of the yoghurt increases as the storage period increases while the protein content of the yoghurt increases as the storage period. Ginger fiber has a decreasing effect on the pH while the total soluble solids and total titratable acidity increases as the concentration of the dietary ginger fiber increases over a period of four weeks. Inclusion of dietary ginger fiber improves the flavonoid content as the storage period increases. It can therefore be concluded that enrichment of ginger fiber at 20 % level is considered as the best in terms of the nutrient, physicochemical and antioxidant characteristic of the developed yoghurt.

Key words: Shelf life, yoghurt, cold, antioxidant, fiber.

Introduction

Yogurt is one of the most consumed healthy and nutritious foodstuffs worldwide (Shi et al., 2017; Zhi et al., 2018). Yogurt has a better digestibility of proteins than milk and many latent positive effects on health by providing the human body prebiotic and probiotic bacteria. By incorporating fibers in yogurt, researchers have achieved a mean of increasing fibers consumption in all sectors of the populace and they have developed a functional food with an increasing array of beneficial effects. Many studies reported prebiotic fortification by adding dietary fibers in yogurt. Consumption of high fiber yogurt may prevent or reduce obesity, diabetes, cancer, hypercholesterolemia, gastrointestinal disorders, colonic diverticulosis and constipation, ulcerative colitis, hyperlipidemia, hypertension, coronary artery disease, but also promote intestinal microflora and gastrointestinal immunity (Dello et al., 2017; Hoppert et al., 2013; Sah et al., 2016; Tomic et al., 2017). Fibers are found in the cell wall of vegetables, fruits or cereals and these include polysaccharides (pectins, cellulose and hemicelluloses) and lignin. Although both soluble and insoluble fibers are available, however, the insoluble fibers are used with food fortifying intents (Dönmez and Gökmen, 2017; Bertolino et al., 2015; Tejada-Ortigoza et al., 2016). Researchers has enriched the nutrient, antioxidant and rheological properties of voghurt using various plants dietary fibers. For instance, Oat and wheat fibers are the most frequently used auxiliary materials in the dairy industry, leading to fortified finished products. Oat fibers (containing β -glucan, an indigestible polysaccharide) were proven to increase immunity, to improve anticancer activity and lower blood cholesterol, lipids and blood glucose. Adding oat fibers in yogurt fostered the creation of a good fermented product, with insignificant drop in flavour quality and a minor decline in texture quality (Sanz et al., 2008). "Wheat fibers help to accelerate intestinal transit." Wheat bran is extremely rich in fibers, as well as in minerals such as potassium, phosphorus and magnesium. Wheat dextrin, extracted from wheat starch, is widely used to add fibers in processed foods especially for its contribution in lowering cholesterol (lowdensity lipoprotein and total cholesterol) and in reducing the risk of type 2 diabetes and coronary heart disease. Wheat fibers improve the products quality characteristics in dietetic foods, meat products, pasta, bread, baked goods, cheese and other dairy products. However, little information exists on the enrichment of yoghurt using indigenous made dietary fiber from ginger, hence the aim of this study was to evaluate the effect of addition of ginger fiber on the antioxidant, physicochemical, storage stability and sensory properties of yoghurt.





Materials and methods Sources of raw materials

Giner rhizomes, Dano powder milk and starter culture were obtained from a local market in Saki Oyo State, Nigeria. All chemicals used are of analytical grade and obtained from the Food Science and Technology Laboratory, The Oke-Ogun Polytechnic, Saki Oyo State Nigeria

Sample preparation

Production of ginger dietary fiber sample

For fiber extraction, 5 g of dried ginger rhizome was soaked in 50 ml of deionised water for 12 h. The ginger rhizome was blanched at 95 °C for 5 min and filtered. Fibers were obtained by treating the bleached residue in 85 % ethanol with a defined ethanol/residue ratio at fixed time and temperature on a magnetic stirrer (IKAC MAC HS7). The mixture was filtered. and the residue was recovered to undergo several other treatment cycles. The final residue was then sequentially rinsed with 95 % ethanol and acetone before drying in an oven at 50 °C for 24 h. The multiple leaching processes using ethanol allowed gradual removal of nonfibrous compounds from the ginger and thus enriching the residue in fibers and then decreasing of the yield.

Production of yoghurt sample

For the yogurt production, the milk was first pasteurized at 90 °C for 5 min and cooled to 45 °C. After cooling, the milk was inoculated with the starter culture. The milk was directly inoculated with 0.02 % (w/v) starter culture of Lactobacillus bulgaricus and Streptococcus thermophilus. After a strong stirring for the evenly distribution of the culture, the inoculated samples were transferred over containers having 0, 10, 20, 30, 40 and 50 % containing the dietary ginger fibers. The fermentation process was carried out at 43 °C, until a pH of 4.6 was reached. Subsequently, the finished products were stored at 4-6 °C for four weeks for further analysis.

Methods

Determination of the physicochemical properties of the yoghurt

Determination of the pH and total titratable acidity and total soluble solid

The pH of the samples was measured according to Association of Official Analytical Chemists (AOAC) method No. 943.02 [AOAC, 2020] with some modifications. The yoghurt sample (5 g) was weighed into an Erlenmeyer flask and 50 ml of distilled H₂O was added. The suspension was prepared by mixing for 10 min at 25° C. Then, the pH was measured using a Jenway-3505 pH-meter (Barloworld Scientific Ltd., Dunmow, Essex, UK) with a glass electrode standardized by buffer solutions of pH 4 and pH 7, both at 25°C. Each batch was analysed in triplicate.

The measurement of titratable acidity was done according to method described by Virtanen et al., (2007). Erlenmeyer flask used with a transfer pipette 20 ml milk or yogurt and 1 ml of 2 % w/v solution of phenolphthalein. Content is titrated with 0.1 M NaOH solution to appearance the faint pink color that will not get lost for over 2 min. Acidification of milk (yogurt) is calculated by the formula: $K = V \cdot 2$, where V- volume is consumed base neutralization. The total solid of the yoghurt samples were measured using an Abbe Mark II digital refractometer by placing 0.5 g syrup on the lens and reading the sample for temperature corrected brix.

Determination of the antioxidant activity of the yoghurt

Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging abilities of the yogurts were measured according to the method of Shimada et al., (1992) with some modifications. In brief, 50 µm DPPH radical solution (Sigma Aldrich, St. Louis, MO, USA) prepared in 95 % ethanol was added to an equal volume of the yogurt samples. The reaction mixtures were shaken vigorously and kept in the dark at room temperature for 30 min. After centrifugation (80009g, 10 min), the DPPH radical scavenging activity was measured at 517 nm with a microplate reader (BioTeck Inc., Winooski, VT, USA). Scavenging effect of DPPH radicals was calculated according to the following equation

Inhibition (%) = $\frac{(Acontrol - Asample)}{A(control)x}$ 100

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Ascorbic acid and butylated hydroxylanisol (BHA) at a concentration of 0.1 mg/mL were used as positive control





Determination of Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) assay: The reducing power of the extracts was determined according to the method described by Ammar *et al.*, (2015) with some modifications. Briefly, 250 μ L of each sample at different concentration were mixed with a phosphate buffer (500 μ L, 0.2 M, pH 6.6) and potassium ferricyanide (500 μ L, 1%). The mixtures were then incubated at 50°C for 20 min. An amount of 500 μ L of trichloroacetic acid (10%) was added to each sample, and the mixtures were centrifuged at 1.006 × g for 10 min. After that, 750 μ L of the upper layer were mixed with 750 μ L distilled water and ferric chloride (50 μ L, 0.1%). The mixtures were incubated for 10 min in the dark. Absorbance was measured at 700 nm against a control that consisted of all the reagents without the test sample. All tests were performed in duplicate.

Determination of flavonoid content

The method of Zhishen *et al.*, (1999) was adopted to determine the total flavonoid content of the sample whereby 0.1 ml of extract was mixed with 4.9 ml distilled water and 0.3 ml of NaNO₂ was added. Approximately 0.3 ml of AlCl₃ and 2 ml 1 M NaOH were added at 5 min and 6 min, respectively. The volume was made up to 10 ml with distilled water. The mixture was thoroughly mixed using the vortex equipment and the absorbance was read at 510 nm. A calibration curve was prepared using a standard of catechin hydrate (R2 ¹/₄ 0.9994) was used to prepare the calibration curve and the result was expressed as mg catechin equivalents per g of the sample.

Determination of the proximate composition of the yoghurt

Determination of the Moisture content

Moisture content: About 2 ml of the yoghurt sample was weighed into previously dried and weighed glass crucibles. The crucibles with the samples were then placed in a thermostatically controlled oven at 105° C till a constant weight of solid material was obtained after 5 h. The crucibles were then removed and cooled in a dessicator and then weighed. The moisture content of the samples was calculated by difference in weights and expressed as a percentage.

Determination of Crude ash content

About 2 ml of homogenized yoghurt sample was weighed into each of three previously dried and weighed porcelain crucibles and heated for about 20 min over a boiling water bath till they were visibly dry. The crucibles with their content were then transferred into a muffle furnace at 600° C and incinerated for 2 h. The crucibles were removed, placed in a dessicator to cool then weighed and the ash content was calculated and expressed as a percentage.

Determination of Crude protein content

About 2 ml of the sample was placed in a kjeldahl digestion flask containing a selenium-based catalyst and 25 ml of concentrated H_2SO_4 added in a fume chamber until digestion was completed after 5 h. The digest was cooled and transferred into a 100 ml volumetric flask and made up to the mark with distilled water. 10 ml of the diluted digest was put in the steam distillation unit, which was previously flushed with distilled water. 18 mL of 40 % of NaOH was then added to the solution in the steam distiller. 25 ml of 2% boric acid pipette into a conical

flask and two drops of bromocresol green methyl red mixed indicator added. This mixture was placed under the condenser outlet of the distillation system, with the tip of the condenser completely immersed in it. The distillation was carried out until all the boric acid solution turned from pink to yellowish green. The solution in the conical flask was titrated against 0.1N HCl solutions and the end point recorded, the distillation processes were done with triplicate samples of the diluted digest, a blank was taken through the same procedure using distilled water in place of the sample.

Determination of Crude fat content

About 10 ml of each yoghurt samples was poured into a previously weighed Petri dish and dried over a water bath till most of the water had evaporated, the samples were then transferred to an oven and further dried at 105° C till a constant weighed was obtained. The weight of water loss and dried solids obtained were determined by subtraction and later used to calculate the total amount of fat on wet weight basis. 2 g of the dried sample was weighed into each of two paper thimbles, the thimbles were sealed and placed in the soxhlet extractors. About 150 ml of petroleum ether was poured into each of two previously dried and weighed round bottomed





flasks attached to the extractors; extraction was carried out for 16 h. After this, the petroleum ether was recovered from the soxhlet with only small amounts left in the flask. The flasks were then removed and placed in an oven (with the door partially closed) for the ether to completely evaporate. The flasks were cooled in a desssicator, weighed and the fat content was calculated on a wet per weight basis using the water content determined after drying the wet sample.

Determination of carbohydrate content

Total carbohydrate was determined by differences between 100 and total sum of the percentage of fat, moisture, ash, crude fiber and protein content and to calculate the result of the sample. The carbohydrate content of the samples was expressed as a percentage of the differences between the sum of the other chemical composition of the samples and 100 as calculated below.

Carbohydrate % = 100 - (moisture + fat + protein + fibre + ash)

Statistical analysis

All the data obtained from three replications were analyzed as a completely randomized design procedure using the general linear model procedure of the SPSS 23 statistical package program (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to measure the significant difference between means (p < 0.05).

Result and Discussion

Changes in the pH, total titrable acidity and total soluble solid of yoghurt enriched with dietary ginger fiber during cold storage.

The pH values of the yoghurt samples during cold storage are shown in Figure 1. The pH of the yoghurt samples in this study decreased as the storage period increased. For instance, the pH values for (YGH100) decreased from 4.80 to 3.7 and similar trend of decrease was observed for other samples. The total titrable acidity of the yoghurts as shown in Figure 2 increases as the storage period increases. The decrease in pH and concurrent increase in acidity observed in the yoghurt samples may be due to higher availability of carbohydrates sources from the ginger fiber to the metabolic activity of both yoghurt-starter culture resulting into higher level of organic acids (Sah *et al.*, 2016).

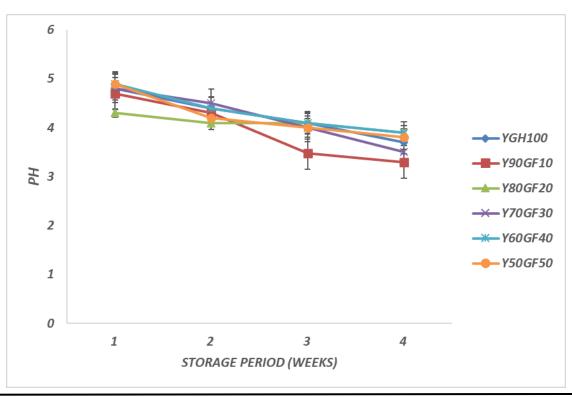






Figure 1. Changes in the pH of yoghurts enriched with ginger dietary fiber during storage

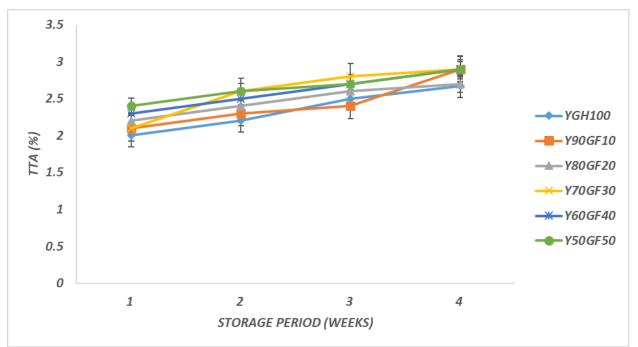


Figure 2. Changes in the TTA of yoghurts enriched with dietary ginger fiber during cold storage

Changes in the Total Soluble Solids of yoghurt enriched with ginger fiber during cold storage.

The effect of storage on the total soluble solids of the yoghurt products is shown in Figure 3. The total solid content of the yoghurt significantly (P<0.05) differed during storage period. The total solid for (YGH100) ranged from 23.00 to 30.00° brix while (Y90GF10) ranged from 24.20 to 29.00° brix. The same trend was observed for other yoghurt samples total solids are an indication of the dry matter content. The relative high values of the total solids of the yoghurt samples especially at the fourth week of the storage period agree with the findings of (Ndife*et al.*, 2014) who also observed a similar trend in yoghurt samples enriched with coconut cakes.

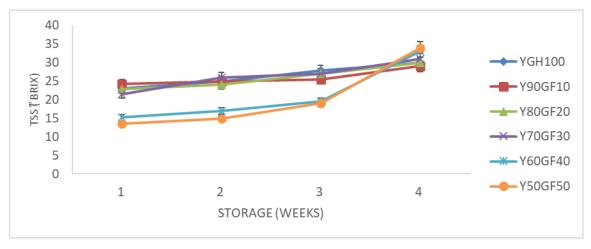


Figure 3. Changes in the Total soluble solid of yoghurts enriched with ginger dietary Fiber during cold storage





Changes in the DPPH radical scavenging activities, ferric reducing activity power (FRAP) and total flavonoids of yoghurt enriched with dietary ginger fiber during cold storage period.

There was a progressive significant (P<0.05) difference in the DPPH radical scavenging activities of the samples as shown in Table 1. The DPPH of the yoghurt samples at the first and second weeks for (YGH100) and (Y90GF10) ranged from 10.73 to 18.45% and 12.12 to 25.45% respectively. It is worth note that the inclusion of dietary fiber from ginger significantly improve the DPPH radical scavenging activities of the yoghurt when compared with the control sample (YGH100). Similarly, addition of sour cheery pulp, grape seed and extracts and grape and callus extracts to yoghurt has been shown to have higher antioxidant than the control yoghurt as reported by (Sengnel et al., 2012; Choachouli et al., 2013). Ferric reducing activity power assay has been reported to be suitable to measure antioxidant activity of substances having half-reaction redox potential below 0.7V. The ferric reducing capacity of the yoghurt is shown in Table 2. Among all the yoghurt samples, the control yoghurt (YGH100) exhibited lowest antioxidant capacity when compared with other yoghurt samples and they also increase as the storage period increases. For instance, for (Y90GF10), the FRAP values increased from 0.13 to 0.76 mgFe²⁺/100g while (Y50GF50) increased from 0.39 to 0.98 mgFe2+/100g. Similar report of yoghurt containing pomegranate fiber was shown to have a higher ferric reducing capacity compared to control yoghurt (Trigueros et al., 2014). The total flavonoid of the yoghurt samples significantly (P<0.05) different as shown in Table 3. An increase in the flavonoid contents was observed as the storage period increased. The (YGH100) showed flavonoid contents of 0.05 to 0.18mg/gQE while the (Y90GF10) ranged from 0.01 to 1.11mg/gQE.

Table 1. Changes in the DPPH radical	scavenging activities of yoghurt	enriched with dietary ginger fiber
during		

cold s	torage.			
Samples	DPPH (%)			
Weeks	1	2	3	4
YGH 100	$10.73^{d} \pm 0.03$	$1212^{c} \pm 0.01$	13.46 ^b ±0.03	14.45 ^a ±0.02
Y90GF10	17.79 ^d ±0.01	19.23°±0.05	$20.98^{b} \pm 0.03$	25.89 ^a ±0.03
Y80GF20	$17.84^{d} \pm 0.02$	20.88°±0.03	$33.76^{b} \pm 0.02$	35.78 ^a ±0.04
Y70GF30	17.90 ^d ±0.04	21.34°±0.02	$34.41^{b} \pm 0.01$	39.67 ^a ±0.05
Y60GF40	18.01 ^d ±0.03	24.44°±0.03	$34.76^{b} \pm 0.03$	40.23 ^a ±0.03
Y50GF50	$18.45^{d} \pm 0.03$	25.45°±0.01	$34.98^{b} \pm 0.03$	43.89 ^a ±0.01
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Values are mean \pm standard deviation of triplicate determinations. Values followed by the same superscript(s) within the same column are not significantly different at (p < 0.05)

Table 2. Changes in the ferric reducing activity power of yoghurt enriched with dietary ginger fiber duri	ing
cold storage.	

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Samples	$FRAP (MgFe^{2+}/100g)$				
Weeks	1	2	3	4	
YGH100	$0.04^{d} \pm 0.03$	$0.12^{c} \pm 0.02$	$0.27^{b} \pm 0.01$	0.30 ^a ±0.03	
Y90GF10	$0.13^{d} \pm 0.02$	0.35°±0.01	$0.53^{b} \pm 0.03$	$0.76^{a} \pm 0.02$	
Y80GF20	$0.18^{d} \pm 0.03$	0.45°±0.03	$0.54^{b} \pm 0.01$	$0.80^{a}\pm0.03$	
Y70GF30	$0.28^{d} \pm 0.01$	0.49°±0.01	$0.57^{b} \pm 0.02$	$0.87^{a} \pm 0.02$	
Y60GF40	$0.33^{d} \pm 0.02$	0.50°±0.03	$0.60^{b} \pm 0.02$	$0.93^{a} \pm 0.03$	
Y50GF50	0.39 ^d ±0.03	0.59°±0.03	$0.68^{b} \pm 0.02$	$0.98^{a} \pm 0.02$	

Values are mean \pm standard deviation of triplicate determinations. Values followed by the same superscript(s) within the same column are not significantly different at (p < 0.05)

 Table 3. Changes in the total flavonoid content of yoghurt enriched with dietary ginger fiber during cold storage.

Samples	TOTAL FLAVONOID CONTENT			
Weeks	1	2	3	4
YGH100	$0.05^{d} \pm 0.02$	$0.08^{c}\pm0.02$	$0.13^{b}\pm0.01$	$0.18^{a}\pm0.01$
Y90GF10	$0.01^{d} \pm 0.03$	$0.50^{c} \pm 0.01$	$0.58^{b} \pm 0.02$	1.11 ^a ±0.03
Y ₈₀ GF ₂₀	1.09 ^d ±0.02	1.10 ^c ±0.03	$1.84^{b} \pm 0.03$	1.89 ^a ±0.02

Adeoti, O. A, Alabi, A.O., Azeez, L. A., Babalola, J. O & Adedokun, S.O





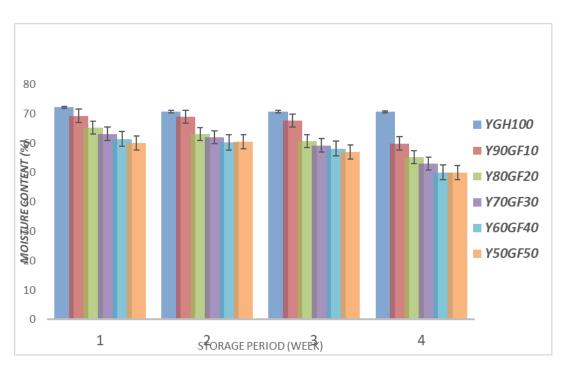
$Y_{60}GF_{40}$ 1.68 ^d ±0.01 1.78 ^c ±0.02 1.98 ^b ±0.01	
	2.69 ^a ±0.02
$Y_{50}GF_{50}$ 2.15 ^d ±0.01 2.67 ^c ±0.03 2.98 ^b ±0.03	$2.98^{a}\pm0.03$

Values are mean \pm standard deviation of triplicate determinations. Values followed by the same superscript(s) within the same column are not significantly different at (p < 0.05)

Changes in the moisture content of yoghurt enriched with ginger fiber during cold storage.

Figure 4.1 shows the changes in the moisture content of yoghurt enriched with ginger fiber and there was significant (P<0.05) difference in the moisture content. The moisture content of the yoghurt decreased as the storage period increases during refrigeration. The moisture content for the first and second weeks ranged from 60.05 to 72.15% for (Y50GF50) and (YGH100); and 60.26 to 70.67% for (Y60GF40) and (YGH100) respectively while the third and fourth weeks showed a moisture content of 57.01 to 70.68% and 50.01 to 70.61% for (Y50GF50) and (YGH100). The moisture content in this study is similar to the values of 73.71 to 84.70% for yoghurts made from tigernut-bambara-coconut milk supplement with baobab pulp emulsion as reported by Adeoti *et al* (2021). The moisture content of yoghurt should be less than 84%, hence the moisture content reported in this study is in agreement with the recommended values.

The fat content of the yoghurt showed a significant difference (P<0.05) as shown in figure 4.2. The fat content of the control sample (YGH100) decrease at second week from 7.12to 6.12 % while it increased at third week to 7.16%. However, the fat content of other yoghurt products decreased as the storage period increased. For instance, (Y90GF10) has fat content of 7.25 and 5.28% at first and second week while there was a decrease in the fat content at third and fourth weeks with values of 4.11 to 2.71% respectively. The fat sample recorded over a storage period in the study was higher than 3.37-3.75% for yoghurt supplemented with papaya juice and gelatin as reported by Tilahun *et al.*, (2019). Fat content plays an important role in yoghurt since it improves the appearance, flavor, taste, and texture of yoghurt. USDA (2001) has reported that yoghurt with more than 3.25% fat should be named as yoghurt while yoghurt with fat content of 0.50-2.008 % should be labelled low-fat yoghurt and yoghurt should have less than 15% fat content, hence the fat contents of yoghurt in this study is in agreement with the standard.







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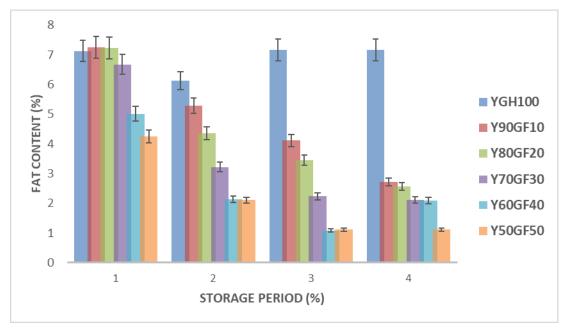
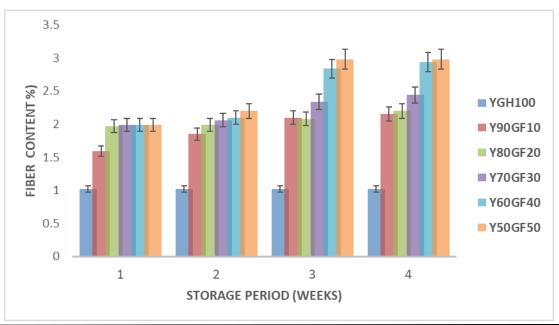


Figure 4.2. Changes in the fat content of yoghurts enriched with ginger dietary fiber during cold storage.

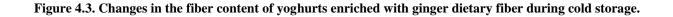
The fiber content of the yoghurt enriched with ginger fiber increases as the storage period increases. The fiber content of (YGH100) was stable throughout the period of storage. However, an increase in fiber was observed for (Y90GF10) and (Y80GF20) with values ranging from 1.59 to 2.15% and 1.97 to 2.20% respectively. The same trend of increase was observed for other samples. The fiber content of the yoghurt samples was higher than 1.26% to 2.87% for yoghurt products from tigernut-bambara-coconut milks supplemented with baobab pulp emulsion (Adeoti *et al.*, 2021). Ginger contains high amount of fiber (soluble and insoluble) which are digestible polysaccharide that could assist in the viscosity and stabilization of yoghurt (Garcia-Perez et al; 2005). It could be therefore deduced from the study that the high fiber in the yoghurt samples may improve the viscosity of the yoghurt.

A similar trend of increase over a storage time was also observed for Ash content of the yoghurts as shown in figure 4.2. The values of ash for (Y90GF10) and (Y80GF20) over a storage period ranged from 1.00 to 2.88% and 1.67 to 2.93%









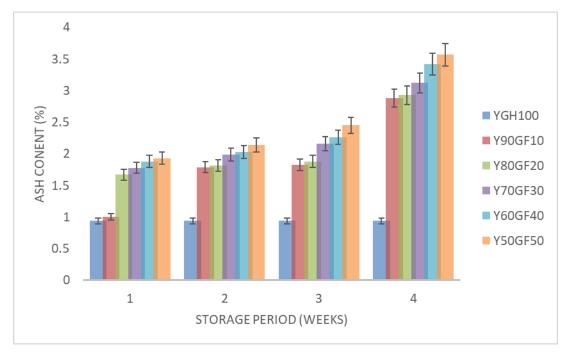
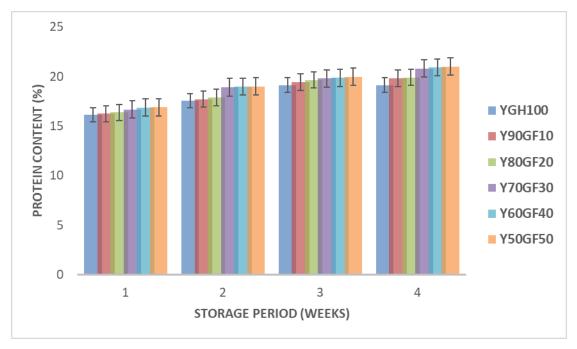


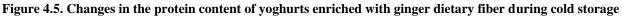
Figure 4.4. Changes in the ash content of yoghurts enriched with ginger dietary fiber during cold storage

The protein content of the yoghurt samples increases over the storage periods as shown in figure 4.5. The protein content for first and second week for the sample ranged from 16.15 to 16.89% for (YGH100) and (Y50GF50); and 17.56 to 18.99% for (YGH100) and (Y50GF50) respectively. The increase in the protein content during storage periods may be due to the fermentation process taking place during refrigeration storage. The protein content of the samples in this study was higher than 3.25-3.80% for yoghurt supplemented with banana, papaya and watermelon pulp (Debashis *et al.*, 2016). Standard codex (2003) stated that yoghurt should contain not less than 2.70% protein, hence the protein content of yoghurts in this study were within the recommended value









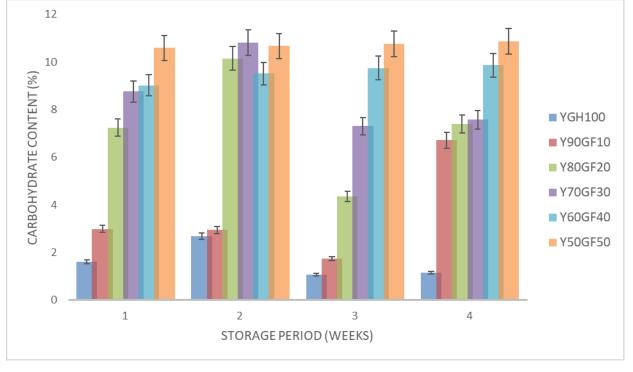


Figure 4.6. Changes in the carbohydrate content of yoghurts enriched with ginger dietary fiber during cold storage

Conclusion

This study evaluated the influence of the yoghurt enrichment with dietary fibers from ginger rhizomes on its physicochemical, antioxidant, nutrient and sensory properties during cold storage. The pH and total titratable acidity values presented that yoghurt samples enriched with ginger fibers could lead to an extension of the shelf life of the yoghurts over a period of four weeks of cold storage. An appreciable increase in the DPPH radical scavenging





activity and total flavonoid content during storage is an indication of bioactive retention in the yoghurt samples. It can therefore be concluded that the developed yoghurt samples might have some bioactive components that can confer health benefits to the consumer upon regular consumption.

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