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PRODUCTION, MICROBIOLOGICAL AND QUALITY EVALUATION OF LOW-FAT SPICED YOGHURTS WITH LOW GLYCEMIC LOADS

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ABSTRACT

Spiced yoghurt improves health in various ways such as ability to improve metabolism and burn extra calories. Consumption of full fat yoghurt has declined due to the awareness of the probable harmful effects of fat on consumers' health, thus dietary habits of consumers have changed and market interest has also tended to change in favour of low or non-fat yoghurt. This study was aimed at highlighting the physico-chemical, microbiological analysis and nutritional importance of spiced yoghurts. Standard methods were used for the production of plain and spice -treated yoghurts and the spices used were turmeric, *Aframomum danielli* and clove (1% w/v), respectively. All the yoghurt samples were stored at 4°C.Control sample (plain yoghurt) had no spice. Physicochemical profile, proximate composition and microbiological analysis were determined for all the yoghurt samples. The glycemic load of each sample was calculated by multiplying the glycemic indices (GIs) of non-fat yoghurt (International Glycemic Index Table) by the available carbohydrate and dividing the product by 100. While the carbohydrate content of plain yoghurt was 24.15 ± 0.69%, turmeric and *Aframomum danielli*- spiced yoghurts had 14.75 ± 0.69 %and 14.37 ± 1.29%, respectively. The fat contents of plain, turmeric, *Aframomum danielli* and clove spiced - yoghurts were 0.10±0.05%, 0.03±0.01%, 0.20±0.13% and 0.25±0.63%, respectively. The spiced yoghurts had decreased pH and increased titratable acidity values during the storage period. Total bacterial counts of turmeric (1.4×10^4 cfu/ml), *Aframomum danielli (*1.0×10^4 cfu/ml) and clove (1.6×10^4 cfu/ml) spiced yoghurts decreased when compared to plain yoghurt with 2.4×10^4 cfu/ml. Some fungi were detected in spiced yoghurts toward the end of storage period. The results of GIs obtained were found to be under low glycemic load. Glycemic load of plain yoghurt (7.86± 0.24) was significantly ($P \leq 0.05$) higher than those of clove (4.56 ± 0.45) and *Aframomum danielli* (4.64 ±0.43) spiced yoghurts, respectively. In conclusion, spiced yoghurt is a nutritionally beneficial product which is considered to be safe. The findings of the present work can be useful from the standpoint of health of yoghurt consumers.

Key words: Spice, yoghurt, glycemic load, quality evaluation, *Aframomum danielli,* turmeric, clove

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INTRODUCTION

Fermented dairy products are products that can be produced via fermentation of lactose by microorganisms especially by lactic acid bacteria. Fermented dairy products in question are usually produced by using lactic acid bacteria and yeasts [1]. Yoghurt is a dairy product obtained by lactic acid fermentation as an action of starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. There are many varieties of yoghurt such as non-fat or low-fat and this classification is due to health considerations as high level consumption of fat has been attributed to many health problems [2]. The addition of specific lactic acid bacteria to milk used in the production of yoghurt enhances the digestibility and nutrient value of milk. First, the active cultures used in the fermentation process metabolize the milk sugar lactose, improving tolerance to this disaccharide that is difficult to digest. Secondly, yoghurt manufacturing induces the denaturation of proteins, and thus improving overall bioavailability. Thirdly, lactic acid bacteria improve the absorption of minerals such as calcium, phosphorous and magnesium. Milk fermentation to produce yoghurt also extends the shelf life of the product and enhances the taste. The decrease in pH inhibits the growth of pathogenic bacteria. The lactic acid produced is also responsible for the characteristic flavour and aroma of yoghurt and helps to maintain the quality of the yoghurt during storage.

According to the Code of Federal Regulations of Food and Drug Administration FDA (United States Department of Health and Human Services Washington DC) [3], low fat yoghurt and non-fat yoghurt are similar in description to yoghurt but contain 0.5% to 2% and less than 0.5% milk fat, respectively [2]. Milk fat has an important role in the texture, flavour and colour development of dairy products. The reduction of fat and subsequently reduction of total solids content in low-fat and non-fat yoghurts exhibit weak body, poor texture, and whey separation unless various stabilizer blends are used.

Spices impart aroma and colour to food preparations and sometimes mask undesirable odours. Volatile oils give the aroma, and oleoresins impart the taste [4]. Due to their antimicrobial nature, spices are used to improve taste and enhance shelf life. Some spices are also known to contribute to the self defence of plants against infectious organisms [5]. Spices are derived from different parts of specific plants such as the barks, flowers, roots, seeds and fruits.

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae*. Curcumin is the main active ingredient in turmeric and is responsible its anti-oxidative, anti-microbial and anti-inflammatory properties. Curcumin is also a food preservative, coloring agent and chemo preventive agent [6]. Turmeric contains very high amounts of minerals and vitamins. Turmeric is sometimes used as an agent to impart a rich, custard-like yellow colour. Turmeric is used in canned beverages, baked products, dairy products, ice cream, yoghurt, yellow cakes, orange juice, biscuits, popcorn colour, cereals, sauces, and gelatins.

Aframomum danielli seeds are smooth, shining olive brown with a turpentine taste. *Aframomum danielli* had been reported to stabilize colour, flavour and pH of cashew

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juice and with a good health promoting profile, bio preservative properties and antioxidant properties [7].

Cloves (*Syzygium aromaticum L.*) are the unopened pink flower buds of the evergreen clove tree. The buds are picked by hand when they are pink and dried until they turn brown. In fact, their English name is actually derived from the Latin word *clavus*, which means nail. Although cloves have a very hard exterior, their flesh features contain an oily compound that is essential to their nutritional and flavor profile. The main properties include antioxidant, antifungal and antibacterial properties. By tradition, it has been used in food preservation as flavoring and antimicrobial substance [8]. Cloves are an excellent source of minerals, vitamins, proteins, and carbohydrates.

Spices have been used in the production of yoghurt in order to improve its nutritional qualities. Studies have been done on production and quality evaluation of flavoured yoghurts using carrot, pineapple, ginger and pepper fruit [9] and production of probiotic yoghurt flavored with the spice, *Aframomum danielli*, strawberry and vanilla [10].

This present study was carried out to highlight the physico-chemical, microbiological properties and nutritional importance of yoghurts spiced with turmeric, cloves and African cardamom (*Aframomum danielli*).

MATERIALS AND METHODS

Collection of samples

Skimmed powdered milk, fresh milk (Lactel Company, France), gelatin and granulated sugar (Dangote Sugar Company) were obtained at FoodCo Bodija, Ibadan Oyo State, Nigeria. *Aframomum danielli* seeds, turmeric, and cloves were purchased in Ibadan, Nigeria.

The *Aframomum danieli* seeds were removed from the pods and cleaned and extraneous materials were removed. The seeds were winnowed, milled into powder and sieved through 250µm mesh size to obtain a fine powder. It was then stored at room temperature for use.

Turmeric and cloves were dried and milled separately into powder. Spice powders were kept in glass bottles with screw cap at ambient temperature.

Starter culture used was a freeze-dried lactic culture (YC 381 Hansen, Denmark) purchased from a chemical store in Lagos. Solvents used were all of analytical grade.
Plain and spiced yoghurts were produced in the Food Processing Laboratory of the Department of Food Technology University of Ibadan, Oyo State. The laboratory analysis of the yoghurt samples was carried out at Jaagee Food Laboratory Ibadan and laboratories of the Department of Food Technology University of Ibadan.

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Preparation of Aqueous Extract

Turmeric extract was prepared by soaking 1 g of ground turmeric in 100 ml water, followed by filtration, pasteurization and storage at 4°C prior to use [11]. *Aframomum danielli* aqueous solution was prepared by dissolving 1g of ground *Aframomum danielli* powder in 100 ml of distilled water followed with storage in the refrigerator at 4°C for 5 days [12]. The mixture was centrifuged at 2000 rpm for 15mins and filtered. Clove aqueous extract was prepared based on the method specified by Wilson [13] after undergoing slight modifications, 100 ml of distilled water was added into 1g of ground clove, and the mixture was centrifuged at 2000 rpm for 15mins and filtered. The extract obtained at the end of this process was used for further analysis. The extract was kept in the refrigerator (4°C) for further analysis.

Preparation of Spiced yoghurt

Fresh milk (skimmed) was processed into yoghurt according to the method described by Muhammed *et al*. [14] with slight modifications. Skim milk powder and sugar were mixed and added to previously warmed (60°C) fresh milk followed by heating to 90°C for 30 minutes. The pasteurized milk was cooled down to 45°C. Yoghurt culture (0.5g of freeze-dried lactic culture) was added to each of the 1000ml sugar-milk mixture according to manufacturer's instruction and incubated at 45°C for 6 hours, during which the milk coagulated. The product was stored in a refrigerator at 4°C for subsequent analysis. The yoghurt was then treated with extract solutions of turmeric, *A*. *danielli* and cloves, respectively. Thereafter, 1ml of 1% (w/v) spice extract solution was added to the milk-sugar-culture mixture to enhance the flavour, colour and preservation. Four yoghurt samples were obtained as follows: plain yoghurt, turmeric spiced yoghurt, *Aframomum danielli* spiced yoghurt and clove spiced yoghurt. All the yoghurt samples were stored at 4°C until used for analysis.

Physicochemical Analysis of Spiced yoghurt

Physico-chemical analysis was carried out over 20 days of storage period. pH was measured using a pH meter [15]. Titratable acidity value of the yoghurts was determined according to Association of Official Analytical Chemist (AOAC [16] method 942.15). All samples were analyzed in triplicates.

Proximate Analyses of Spiced Yoghurt

Moisture, ash, protein, fat, and fibre content of all the yoghurts were determined according to (AOAC [16] methods 990.20, 945.46, 991.20, 905.02 and 991.43, respectively). Carbohydrates content was determined by difference calculation as described by AOAC [16].

Microbiological analysis of Spiced Yoghurt

Yoghurt samples maintained at 4°C were analyzed on 0, 5, 10, 15 and 20 days after which pour plate technique [17] was used for viable counts.

Glycemic Load of Spiced Yoghurt

Glycemic index (GI) is a method of classifying foods based on the blood glucose response after food consumption. Published GI ranking is as follows: GI of 70+ (high), GI of 56-69 (moderate) and GI of 55 and less (low) [18]. Glycemic load (GL) is the

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weight of glucose which raises blood glucose by the same amount [19]. Glycemic loads of the yoghurts samples were determined [34] and available carbohydrate (AC) less fibre in serving of samples was known from the proximate analysis of the each sample. Then the AC value was multiplied by standard glycemic index value (GI Estimates, International Glycemic Index Table) for that particular food and divided by 100.

Available carbohydrate = Total carbohydrates–Fibre

$$\text{Glycemic Load} = \frac{\text{Glycemic Index} \times \text{Available Carbohydrate}}{100}$$

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using SPSS 17.0 version and the means were separated using the Duncan's multiple range tests. Significant differences were determined at $P \leq 0.05$.

RESULTS

Physicochemical Characteristics of Spiced Yoghurt

The results of the physico-chemical analyses of plain yoghurt and spiced yoghurts are shown in Table 1. The pH of the yoghurts decreased while titratable acidity content increased during the storage period.

Change in pH and titratable acidity (%) of spiced yoghurt.

At the end of the 20thday of storage, turmeric-spiced yoghurt had the highest mean pH of 4.68±0.08 while *Aframomum danielli*-spiced yoghurt had the lowest mean pH with 4.50±0.07.

The titratable acidity (%) of all yoghurt samples showed opposite trends to pH values. Plain yoghurt (control) had the highest mean titratable acidity of 0.95±0.44% while *Aframomum danielli*-spiced yoghurt had the lowest mean titratable acidity of 0.80±0.25%. The pH decreased and titratable acidity increased in all yoghurt samples during the 20thday of storage (Figures 1 and 2).

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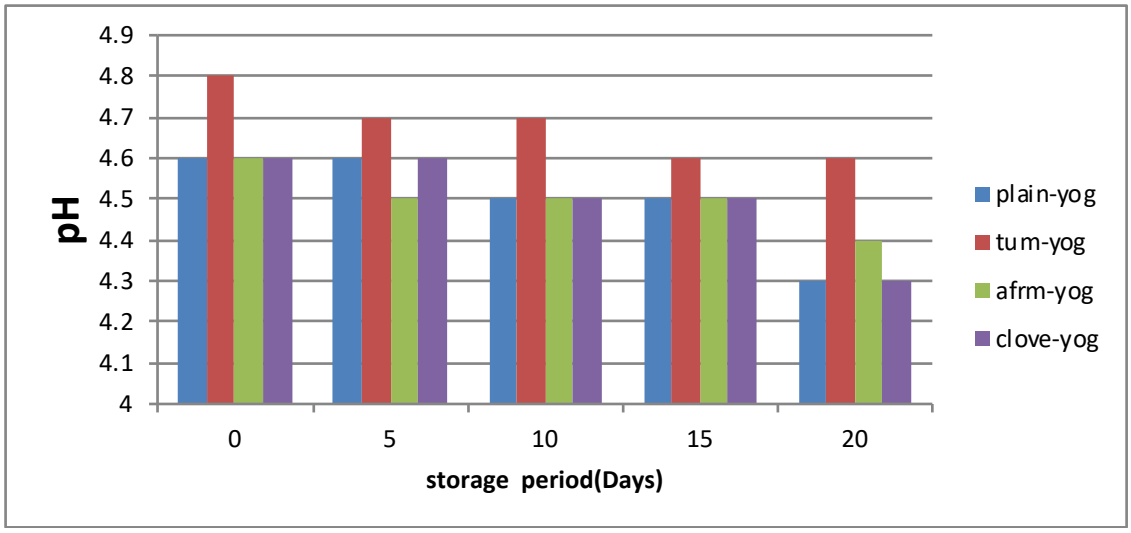
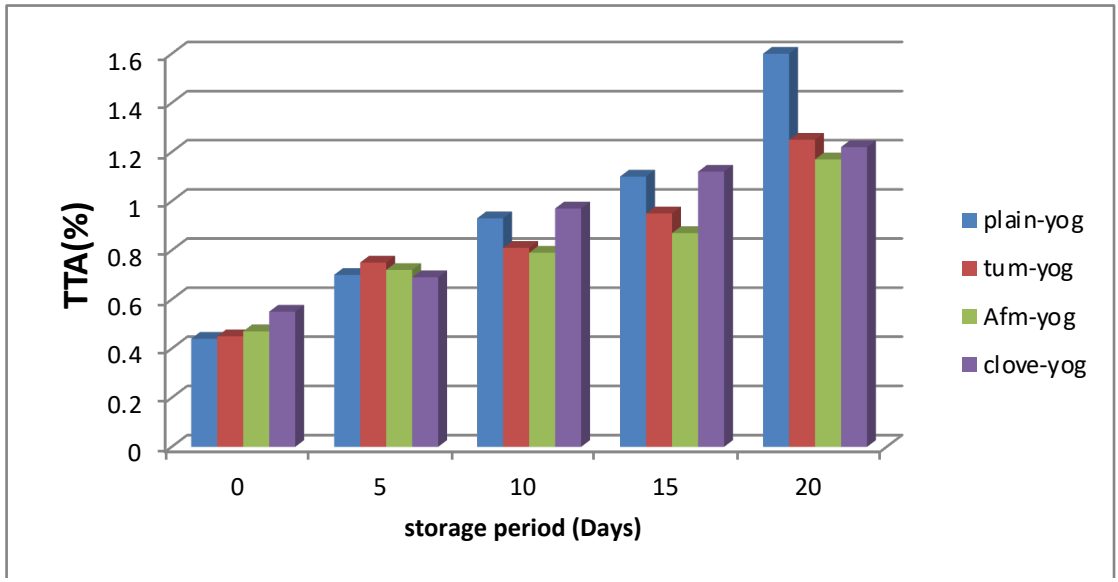


plainyog: plain yoghurt ; tum-yog: turmeric spiced yoghurt;
afrm-yog: *Aframomum danielli* spiced yoghurt; clove-yog: clove spiced yoghurt

Figure 1: Changes in pH values of spiced yoghurts during 20 days of storage



plain-yog : plain yoghurt ; tum -yog : turmeric spiced yoghurt;
afrm-yog : *Aframomum danielli* spiced yoghurt; clove-yog: clove spiced yoghurt

Figure 2: Changes in titratable acidity (%) of spiced yoghurts during 20 days of storage

Proximate Composition of Spiced Yoghurt

The moisture contents of plain yoghurt, turmeric yoghurt, *Aframomum danielli* yoghurt and clove yoghurt were 73.90±0.38%, 79.10±0.30%, 80.60±0.36% and 80.80±0.63%, respectively. The moisture contents of plain yoghurt and spice-treated yoghurts decreased during storage at refrigeration temperature. The protein contents of the yoghurt samples were low. There were no significant differences in the protein levels between turmeric yoghurt and A*framomum danielli* yoghurt. Fibre and ash contents of plain yoghurt differed significantly from clove yoghurt, which were 1.62±0.07% and 1.49±0.42%, respectively. The ash and fibre contents of all yoghurt samples were low.

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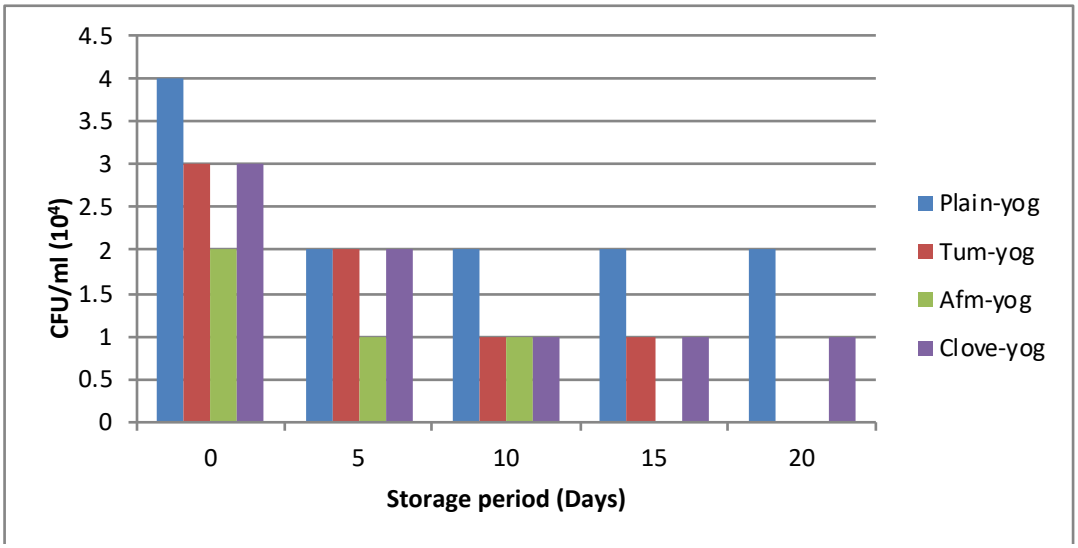
There were significant reductions in the fat contents of the yoghurt samples. The fat content of turmeric-spiced yoghurt was 0.03±0.01% thus curcumin decreased the fat level of treated yoghurt. The carbohydrate content of the plain yoghurt 24.15±0.69% was significantly ($P \leq 0.05$) higher than those of spiced yoghurts. There were no appreciable differences in energy values among the spiced yoghurts except for plain yoghurt with 99.82±1.14 kcal (Table 2).

Microbiological Analysis of Spiced yoghurt

The mean total bacterial counts of plain yoghurt, turmeric yoghurt, *Aframomum danielli* yoghurt and clove yoghurt were 2.4×10^4 cfu/ml, 1.4×10^4 cfu/ml, 1.0×10^4 cfu/ml and 1.6×10^4 cfu/ml, respectively (Table3). Fungal counts in the plain yoghurt were within stipulated limits.

Changes in the total bacterial and fungal counts of spiced yoghurt

At the end of the 20th day of storage, bacterial counts decreased in all the samples (fig 3). The bacterial counts of turmeric yoghurt and *Aframomum danielli* yoghurt were 3×10^4 cfu/ml and 2×10^4 cfu/ml, respectively on the first day of storage and towards the end of 20 days of storage there were no bacteria detected in the samples. Fungi were not detected in the spiced yoghurts on the first day and towards the end of 20 days of storage (Fig 4).



plain-yog : plain yoghurt ; tum- yog : turmeric spiced yoghurt;
afrm-yog : *Aframomum danielli* spiced yoghurt; clove-yog: clove spiced yoghurt

Figure 3: Changes in total bacterial counts of spiced yoghurts during 20 days of storage

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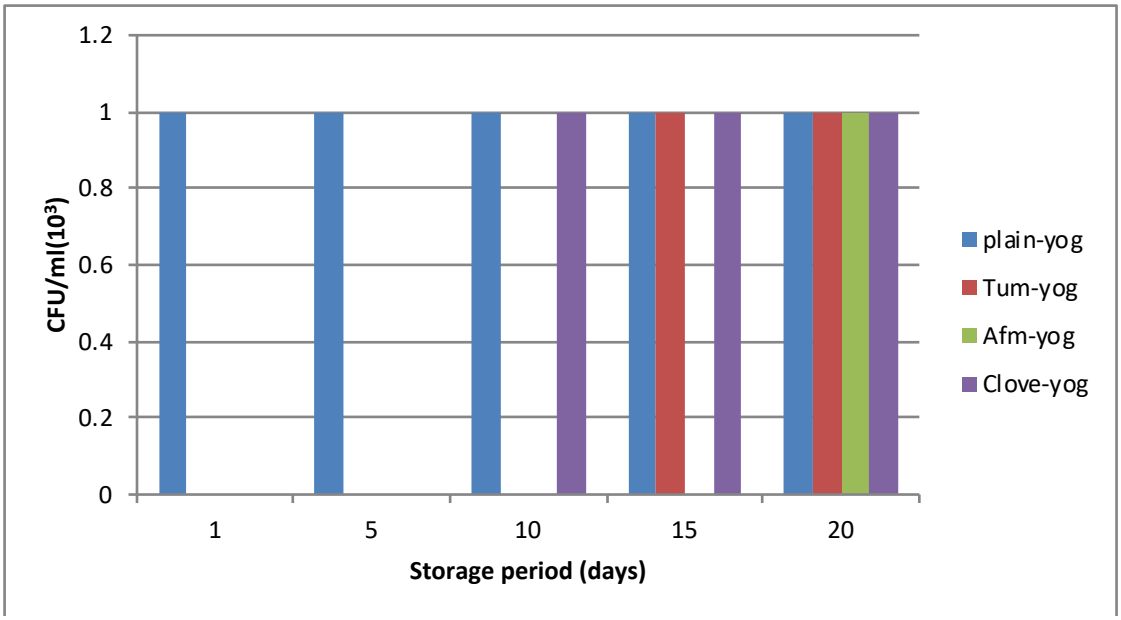

plain -yog : plain yoghurt; tum -yog : turmeric spiced yoghurt;
afrm-yog : *Aframomum danielli* spiced yoghurt; clove-yog: clove spiced yoghurt

Figure 4: Changes in total fungal counts of spiced yoghurts during 20 days of storage

Glycemic Load of Spiced yoghurt

The glycemic load of food puts together its carbohydrate content and its glycemic index to give a more accurate estimate of how much it will affect blood glucose level. In general, food with glycemic load of 1—10 is considered to have a low glycemic load, 11—19 is a medium glycemic load, and 20 or higher is a high glycemic load. The glycemic load of plain yoghurt 7.86±0.24 was significantly ($P \leq 0.05$) higher than those of spiced yoghurts (Table 4).

DISCUSSION

Physicochemical Characteristics of Spiced Yoghurt

The reduction in pH of the spiced yoghurt could be attributed to the breakdown of lactose into lactic acid. Generally, acidity and pH values of concentrated yoghurt vary depending on the starter culture and draining conditions [20]. The decreases in pH values of spiced yoghurt samples could have been responsible for the absence of growth of bacteria as storage period progressed. The mean pH of plain yoghurt 4.50±0.12 was significantly different from that of turmeric yoghurt but did not differ from that of *Aframomum danielli* yoghurt and clove yoghurt. The lactic acid produced during fermentation period is known to be responsible for the characteristic flavour and aroma of yoghurt and this helps to maintain the quality of yoghurt during storage and packaging [21].The titratable acidity of plain yoghurt and spiced yoghurts showed no significant differences at ($P \leq 0.05$).Aroma, colour and taste were imparted by the spices added to the yoghurt [4].The addition of turmeric in turmeric spiced yoghurt gave rise to yoghurt with a custard-like yellow colour and the addition of clove gave intense flavor to clove yoghurt.

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Proximate composition of Spiced Yoghurt

The moisture contents of plain yoghurt and spiced yoghurts examined in this study were in agreement with the findings of other researchers [22] who noted that the maximum moisture content of yoghurt should not be more than 84%, because excess water in yoghurt makes it less viscous thereby affecting its texture and mouth feel. The moisture contents of plain yoghurt and spiced yoghurts which decreased with storage period is not unusual as it has been reported [23], that the decrease in the moisture content of yoghurt during storage period is due to the evaporation rate of moisture during storage at refrigerated condition. In this study it was found that the moisture content of plain yoghurt 73.9±0.38% was significantly ($P \leq 0.05$) lower than those of spiced yoghurts. The increase in the moisture content of spiced yoghurts could be due to the mechanism of antibacterial activities of spices involving the hydrogen bonding resulting in formation of water in the electron transport system [6].

The protein contents of both plain and spiced yoghurts examined in this study were lower when compared with the 11-18% recommended for commercial yoghurts by the National Yoghurt Association [24]. The values obtained were however, comparable to the 3.5% protein content of yoghurt reported elsewhere [25].

The ash and fibre contents of yoghurt samples examined in this study were low, this observation was in agreement with results obtained by other workers [26] that generally, yoghurt has poor fibre level because it is milk-and water- based product.

When it is realized that nonfat (zero%) yoghurt can be produced, however, the fat level of every yoghurt depends on the fat content of the milk, whether it is skimmed or full cream milk [27]. The low fat contents of yoghurt samples examined in this present study could be attributed to the low oil content of the milk (skimmed milk) which was the major substrate of the yoghurt produced. Furthermore, it has been reported that yoghurt manufactured from skimmed milk will likely have very low fat content while those produced from full cream milk will have fat content of about 4% or slightly above [27] and with curcumin being able to decrease cholesterol and fat levels [28], therefore, the 0.03±0.01% fat content of turmeric yoghurt could be of beneficial health effects. The fat contents of plain yoghurt did not differ significantly from turmeric yoghurt 0.03±0.01%, clove yoghurt 0.25±0.63% and *Aframomun danielli* yoghurt 0.20±0.13%.

Carbohydrates are the major constituents of milk that are converted to lactic acid during yoghurt production. The carbohydrate content of plain yoghurt was significantly higher than those of spiced yoghurts. The differences in energy values found in the yoghurt samples were probably due to the fact that there had been appreciable reduction in protein, carbohydrate and fat levels of the samples due to the activities of microorganisms and also to reactions of enzymes that might have degraded the nutrients

Microbiological Analysis of Spiced yoghurt

Microorganisms used as starter culture contributed to the total bacterial counts of yoghurt samples examined in this study. The total bacterial count (TBC) of spiced yoghurts decreased when compared with plain yoghurt and this could be due to the antibacterial effects of the spices used in this study [6, 7, 8]. The bacterial counts of the spiced yoghurt

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samples were below the acceptable range of 10^6 to 10^7 [29, 30] and the total fungal counts were within stipulated values [29].

Glycemic Load of Spiced yoghurt

Lowering of glycemic load has been shown to make a difference in weight control, and prevention of heart disease [31]. The glycemic load of plain yoghurt which was 7.86±0.24 was significantly ($P \leq 0.05$) higher than those of spiced yoghurts. Spice-treated yoghurts had low glycemic loads and this could be attributed to the effectiveness of the spices in regulating the rate of utilization of blood sugars by inhibiting the hydrolysis of carbohydrate. The low glycemic load of turmeric yoghurt might have been due to the effectiveness of curcumin the active component of turmeric which is responsible for inhibitory activities of α- glucosidase [32]. Furthermore, the active components of *A. danielli* were able to inhibit the α-amylase and α-glucosidase responsible for the breakdown of carbohydrate into oligosaccharides and disaccharides before being converted to monosaccharides thus regulating and stabilizing the release and absorption of sugars into the blood stream [33,34].

CONCLUSION

This study has shown that the nutritional quality of plain yoghurt could be improved by the addition of turmeric, *Aframomum danielli* and clove. The total bacterial and fungal counts of spiced yoghurts obtained are within stipulated limits. The glycemic loads of the spiced yoghurts are lower than that of the plain yoghurt. Spiced yoghurt can be consumed for its health and nutritional benefits and this study showed that spiced yoghurts have low glycemic load and low fat content, which can be considered when planning food diets. Consumption of spice-rich foods with low glycemic load could be a more effective way of preventing various ailments.

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Table 1: Physico-chemical changes of spiced yoghurt during storage

PARAMETERS	Plain-yog	Tum-yog	Afm-yog	Clove-yog
pH	4.50±0.12^{b}	4.68±0.08^{a}	4.50±0.07^{b}	4.52±0.08^{b}
Titratable Acidity (%)	0.95±0.44^{a}	0.84±0.29^{a}	0.80±0.25^{a}	0.91±0.28^{a}

*Mean with the same superscript letter found within the same row are not significantly different (P<0.05).
Plain-yog: Plain yoghurt;
Tum-yog: Turmeric spiced yoghurt;
Afm-yog: *Aframomum danielli* spiced yoghurt;
Clove-yog: Clove spiced yoghurt
Values are represented as mean ± standard deviation (n=3)

Table 2: Proximate composition of spiced yoghurt (Mean ±S.D)

Composition	Plain-yog(%)	Tum-yog(%)	Afm-yog(%)	Clove-yog(%)
Moisture	73.90±0.38^{c}	79.10±0.30^{b}	80.60±0.36^{a}	80.80±0.63^{a}
Crude protein	0.58 ±0.31^{b}	4.80 ±2.77^{a}	3.77 ±0.66^{a}	0.40±0.20^{b}
Crude Fat	0.10±0.05^{ab}	0.03 ±0.01^{a}	0.20±0.13^{b}	0.25±0.63^{b}
Crude Fibre	0.32±0.15^{c}	0.54 ±0.11^{b}	0.30 ±0.06^{c}	1.62±0.07^{a}
Total Ash	0.92±0.05^{b}	0.78 ±0.26^{b}	0.76±0.12^{b}	1.49±042^{a}
Carbohydrates	24.15±0.69^{a}	14.75 ±3.41^{b}	14.37 ±1.29^{b}	15.44±1.29^{b}
Total Energy (Kcal)	99.82±1.14^{a}	78.47±2.58^{b}	74.36±1.43^{b}	65.61±4.22^{c}

*Mean with the same superscript letter found within the same row are not significantly different (P<0.05).
(Plain-yog): Plain yoghurt; (Tum-yog): Turmeric spiced yoghurt;
(Afm-yog): *Aframomum danielli* spiced yoghurt; (Clove-yog): Clove spiced yoghurt

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Table 3: Microbial Analysis of Spiced yoghurts (cfu/ml)

		Storage time (Days)				
Count	Yoghurt sample	1	5	10	15	20
Total bacteria count	Plain	4×10^4	2×10^4	2×10^4	2×10^4	2×10^4
	Turmeric	3×10^4	2×10^4	1×10^4	1×10^4	ND
	Aframomum danielli	2×10^4	1×10^4	1×10^4	ND	ND
	Clove	3×10^4	2×10^4	1×10^4	1×10^4	1×10^4
Total fungi count	Plain	1×10^3	1×10^3	1×10^3	1×10^3	1×10^3
	Turmeric	ND	ND	ND	1×10^3	1×10^3
	Aframomum danielli	ND	ND	ND	ND	1×10^3
	Clove	ND	ND	1×10^3	1×10^3	1×10^3

ND = Not Detected

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Table 4: Glycemic Loads of Spiced Yoghurt (Mean ±S.D)

SAMPLE	GLYCEMIC LOAD
Plain-yog	7.86±0.24[a]
Tum-yog	4.69±1.16[b]
Afm-yog	4.64±0.43[b]
Clove-yog	4.56±0.45[b]

*Mean with the same superscript letter found within the same column are not significantly different (P<0.05)
(Plain-yog): Plain yoghurt; (Tum-yog): Turmeric spiced yoghurt;
(Afm-yog): *Aframomum danielli* spiced yoghurt; (Clove-yog): Clove spiced yoghurt

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