ENRICHMENT OF MILK WITH FOOD SPICES ENHANCES ANTIOXIDANT, NUTRITIONAL AND PHYSICO-CHEMICAL CHARACTERISTICS OF YOGHURT

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Abstract
Enrichment of milk- based fermentation mix with food spices presents a novel approach for improving the nutritional and food quality of yoghurt. The main aim of this study was to evaluate antioxidant, nutritional, and physicochemical characteristics of yoghurt enhanced with extracts from African black pepper (ABP) (Piper guineense), Turmeric (Curcuma longa), and Cloves (Syzygium aromaticum L.). Yoghurt fermentation mixes containing 4% spice extract was prepared, inoculated with Streptococcus thermophillus and Lactobacillus delbrueckii subsp. Bulgaricus and fermented to produce spiced yoghurt. The results revealed that, addition of clove extracts gave the strongest antioxidant capacity as indicated by significant increase (p < 0.05) in total phenolics, total flavonoids, Total Antioxidant Capacity (TAC), DPPH radical scavenging activity, nitric oxide radical scavenging activity, Ferric ion reducing power (FRAP), and inhibition of lipid peroxidation but also reduced apparent viscosity and organoleptic properties as compared to the plain yoghurt. Similarly, ABP increased the antioxidant characteristics, with increased protein content and gel viscosity. Turmeric extracts significantly increased (p < 0.05) the calcium content and reduced tendency to syneresis. Therefore, enrichment of yoghurt with the food spices undoubtedly constitutes a good way for improving the health utility, nutritional, and techno-functionality. Such recipes may be considered for optimization and development towards producing new functional yoghurt.

Keywords: Functional foods, yoghurt, fermentation, milk, fortification.

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1. INTRODUCTION

Yoghurt, globally regarded as a functional food, not only possess unique organoleptic properties and high nutrient contents but also promotes gut health, heart functions, and natural immune defense in humans (Caleja *et al.*, 2016; Hashemi Gahruie *et al.*, 2015; Tavakoli *et al.*, 2019). During the production of yoghurt, fermentation of milk proceeds in three phases viz: the lag phase characterized by slow pH decrease, logarithmic phase with a rapid pH decrease and eventual decelerated acidification (Corrieu & Béal, 2016). Fermentation helps to induce desirable flavours, texture, nutritional value, and functionality in milk products (Tamang *et al.*, 2016). While the texture components of yoghurt can be maintained and improved by optimizing the production process, the flavour, shelf-life and health benefits can be modified or enhanced by utilization of additives (Brückner-Gühmann *et al.*, 2019; Jaworska *et al.*, 2005).
A current interest in the yoghurt industry is utilization of natural additives towards developing new products that not only cater for consumers' expectations regarding techno-functionality and nutritional value, but also meets the growing need for 'clean label' and value addition with respect to diseases prevention and health promotion in humans. For instance, herbal yoghurts, which are enhanced with *Lycium barbarum* water extract (Baba *et al.*, 2014), tea extracts (Muniandy *et al.*, 2016), and mangosteen rind extract (Wibawanti *et al.*, 2018) have been reported. Thus, the utility of extracts from fruits, food spices and medicinal herbs as natural food additives presents a novel approach for developing functional yoghurts with improved quality, nutritional and

therapeutic properties (Alenisan *et al.*, 2017; El-Said *et al.*, 2014).

Food spices have multifarious roles such as antioxidant, medicinal, food flavouring, and preservative functions (Gyebi *et al.*, 2019; Kapadiya *et al.*, 2016; Olaiya *et al.*, 2013). Our preliminary assessment revealed that, addition of extracts from vegetables, fruits, and spices to milk based fermentation mix improved the antioxidant potentials of yoghurt in a concentration- dependent manner (Ogunyemi *et al*, 2020). The current study focused on further evaluation of the antioxidant, nutritional, and physicochemical characteristics of yoghurt enriched with 4% African black pepper (ABP) (*Piper guineense*), 4% Turmeric (*Curcuma longa*), and 4% Cloves (*Syzygium aromaticum* L.).

2. MATERIALS AND METHODS

1.1 Preparation of spiced yoghurt

Commercial skimmed milk comprising 32% protein, 54% lactose, 0.5% milk fat and 9% minerals was procured from DANO milk, Nigeria. Industrial freeze-dried starter culture (YoFlex CHR Hansen Company, Denmark.) containing *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. bulgaricus (1:1) was purchased. Yoghurt mixes containing 4% spice extracts were prepared, inoculated and fermented as reported in previous studies (Ogunyemi et al, 2020). The fermentation process was monitored for 9 hours via pH value and production of lactic acid. The yoghurt samples were then packed and stored at 4°C until further analysis.

1.2 Antioxidant analysis

Total phenolic content in yoghurt samples was determined using the Folin Ciocalteu Reagent (FCR) method described previously by Singleton *et al.* (1999). The flavonoids were measured by aluminum chloride colorimetric assay as described by Zhishen *et al.* (1999).The DPPH• assay was carried out as described by Shirwaikar *et al.* (2006). Nitric oxide radical scavenging activity was determined according to the method reported by Johnson (1964). The ability of the samples to reduce oxidized iron was carried out as defined by Oyaizu (1986). Total Antioxidant Capacity (TAC) assay was carried out as described by Re *et al.* (1999). Inhibition of lipid peroxidation was carried out in accordance to the methods described by Ruberto *et al.* (2000) with slight modification.

2.3 Chemical and Physicochemical Analysis

Protein, Ash, Moisture content and total solids (TS) of the yoghurts were determined according to Latimer &Association of Official Analytical Chemists (2019). The pH-value and TTA were measured in during fermentation, in finished product, and after storage. pH was measured with a pH-meter (Mettler Toledo, Germany). Titratable acidity in terms of lactic acid was measured according to Hamad *et al.* (2017) The amount of NaOH used up was recorded and TTA was calculated as shown below.

$$\text{Titratable acidity} = \frac{\text{Titre value x 0.009}}{\text{Weight of sample}} \times 100 \quad (1)$$

Apparent viscosity of the homogenized samples was measured using a Brookfield viscometer (Brookfield Programmable Rheometer, Model RVDV-III Ultra; Brookfield Engineering Laboratories, Stoughtm, MA, USA) with a spindle no.4 and 10 rpm rotation speed at 24˚C ± 1˚C. The values of apparent viscosity were recorded in centipoises (cP).

Syneresis of the different yogurt samples was determined according to the methodology proposed by Barkallah *et al.* (2017). 100 mL of each sample was placed in a funnel lined with Whatman filter paper number 1. After 6 h of drainage, the volume of whey was measured and the susceptibility of syneresis was calculated thus:

$$\text{Syneresis} = \frac{\text{volume of whey collected after drainage}}{\text{volume of yogurt sample}} \times 100 \quad (2)$$

The colour of yoghurt samples was determined by the Minolta Colour meter CR-410 model (Minolta Co., Osaka, Japan). The calibration of the meter was achieved using a white standard calibration plate (L*= 92.95, a*=-4.86, b*-6.65). The CIE L*, a*, b* and ΔE values of the yoghurt were obtained directly from the meter.

The hue angle (h*), and chroma (C*) were calculated.

2.4 Sensory evaluation

Twenty (20) panelists evaluated the sensory attributes of the yoghurt samples. A profile test with five pre-defined characteristic properties given as colour, aroma, taste, texture, and overall acceptability was applied. A scaling of the intensity between 0 and 9 with 0 being the weakest and 9 the strongest feature expression.

The samples were equilibrated to room temperature and a heaped teaspoon per person and sample was prepared on a small disposable cup 30 min before the sensory test.

3. RESULTS AND DISCUSSION

3.1 Effects of spice extracts on fermentation process

Figure 1 shows the effects of African black pepper, turmeric and clove extracts on the pH and TTA of yoghurt samples.

All spice extracts had only slight effects on the fermentation process with respect to decreasing pH and accumulation of the total acid in the fermentation vessels as shown in Figures 1a and 1b respectively. The pH of the yoghurt samples ranged between 4.9 and 5.1 as shown in figure 1a, this is in concert with previous reports that lactic acid production results in coagulation of milk beginning at pH 5.4 and completing at pH 4.6 (Güner *et al.*, 2007; Lee & Lucey, 2010). During fermentation of milk, lactic acid bacteria (LAB) utilize lactose and nitrogenous compounds, culminating in production of extracellular lactic acid and various other primary metabolites which impart various sensory characteristics and the nutritional value to the product (Tamang et al., 2016). The biochemical changes accompanying fermentation primarily provoke acidification of the medium, causing chemical and physicochemical changes which include decrease of pH, increased nutrient bioavailability, formation of flavour compounds, coagulation of proteins, and the subsequent gel formation; and thereby influence consumers' acceptability of yoghurt (Brückner-Gühmann *et al.*, 2019). In the acidic medium, the net negative charge on casein micelles decreases, thus reducing electrostatic repulsion between charged groups causing coagulation and destabilization of the casein micelles and the conversion of the fluid milk into a viscoelastic gel (Brückner-Gühmann *et al.*, 2019).

3.2 Effects of spices on antioxidant capacity of yoghurt

Antioxidant capacity assays are useful in measuring the overall antioxidant activity due to the contents of the antioxidant compounds in the food matrix. Figure 2 shows the *in vitro* antioxidant potentials of spiced yoghurt samples.

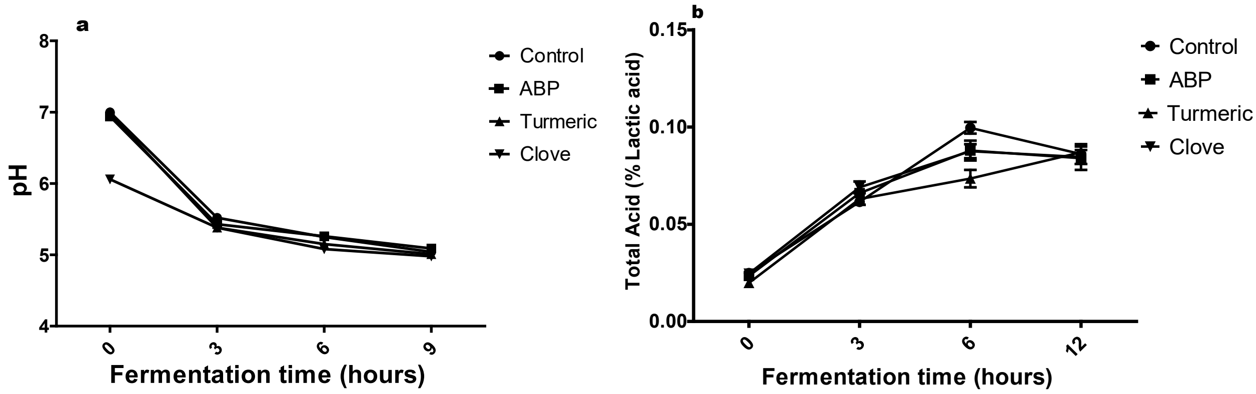

Figure 1: Effects of 4% spices extracts on pH reduction (a), and total acid accumulation (b), of milk during fermentation

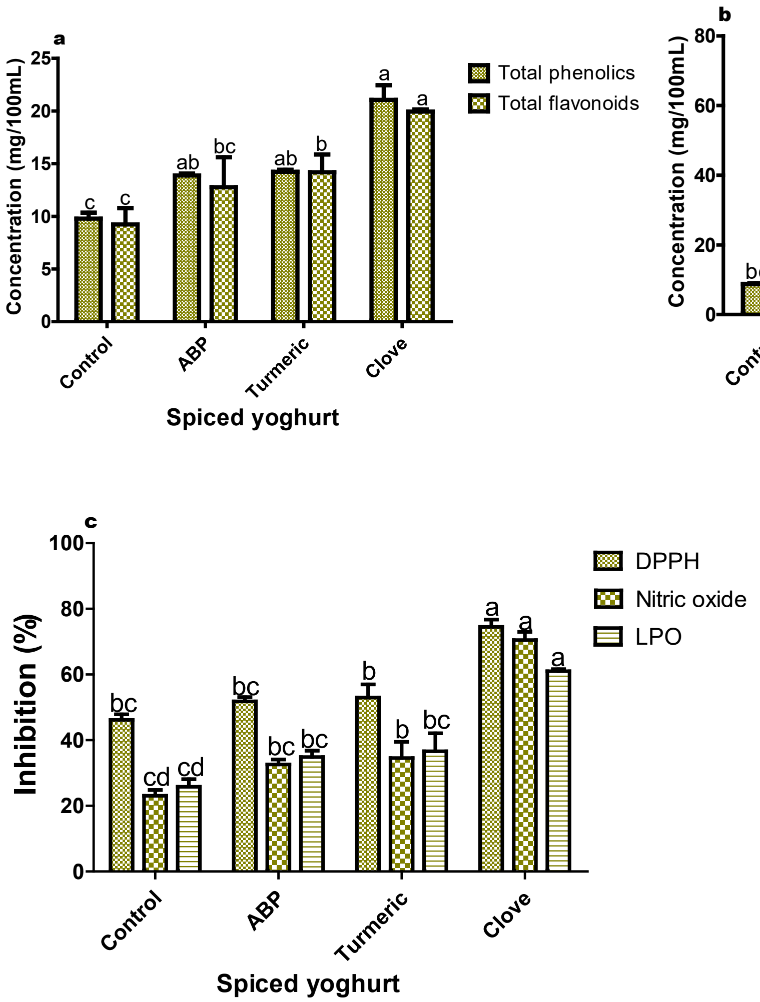

Figure 2: Antioxidant potentials of spiced yoghurt: (a) total phenolic and total flavonoid contents (b)

Ferric reducing antioxidant capacity (FRAP), total antioxidant capacity (TAC). (c) DPPH radical scavenging activity, Nitric oxide scavenging activity and lipid peroxidation. Bars with different alphabets are significantly ($p<0.05$) different from one another. ABP= African Black Pepper.

Both plain and spiced yoghurt samples exhibited antioxidant potentials. Although a small amount of phenolics (9.78 ± 0.57mgGAE/100ml) was detected in the plain yoghurt, this amount significantly increased ($p<0.05$) in all the spiced yoghurt samples. Addition of clove extracts gave the 0.72 fold increase in phenolic content as compared with the plain yoghurt (Figure 2a). Similar effects were also recorded total flavonoid content in the yoghurt samples (Figure 2a). The observed phenolic content recorded in the plain yoghurt may be attributed to the formation of compounds that react with the Folin-Ciocalteu reagent during fermentation process (Raikos *et al.*, 2018). For instance, proteolysis of milk proteins may release amino acids with phenolic side chains, such as tyrosine, which could contribute to the increase in total phenol content. In addition, metabolism of phenolic compounds by the lactic acid bacteria may include flavonoid glycoside hydrolysis or C-ring cleavage and the release of simple phenolics such as phenolic acids (Muniandy *et al.*, 2016). Flavonoids constitute a large group of polyphenolic phytochemicals with antioxidant properties which are overwhelmingly exerted through direct free radical scavenging as reducing agents, hydrogen donors and singlet oxygen quenchers (Beckman, 2000). Flavonoids also exhibit antioxidant properties through chelating with transition metals, primarily Fe (II), Fe (III) and Cu (II), which participate in reactions generating free radicals in biological system. Interestingly, the metal–flavonoid chelates formed are even more potent free radical scavengers than the parent flavonoids and play a prominent role in protection against oxidative stress (Cherrak *et al.*, 2016; Dusan & Vesna, 2007). These antioxidant compounds are of interest in the food industry and human nutrition because of their attractive colour, stability in high acid foods and antioxidant power (Panche *et al.*, 2016). The spices used in this study are known as good sources of phenolic compounds (Kapadiya *et al.*, 2016), thus could be recommended as food additives.

Antioxidant substances donate electrons or hydrogen atoms to free radicals to create a complex. Therefore, the antioxidant potential can be measured based on the electron-donating ability of the substance. This potential can be categorized into hydrogen atom transfer based assays and electron transfer based assays. The plain yoghurt samples exhibited considerable level of antioxidant potentials. For instance FRAP (8.78mg/100ml), DPPH inhibition (46.15%), and nitric oxide inhibition (23.04%) were observed in the plain yoghurt (Figure 2). Clove gave the highest level of TAC and FRAP as observed in Figure 2b and 2c. The FRAP assay is a very important measure of total electron transfer and in combination with other methods can be useful for distinguishing dominant mechanisms with different antioxidants. FRAP assay measures the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion at low pH through the donation of an (Shirwaikar *et al.*, 2006). The results also revealed that, most spice extracts significantly increased ($p<0.05$) the inhibitory activity of yoghurt samples against DPPH radical. Remarkable DPPH radical scavenging activity was detected in the yoghurt fortified with different concentrations of clove extracts. Addition of 4% Clove extract gave the highest inhibitory activity with up to 0.62 fold increases in DPPH scavenging activity as compared with the plain yoghurt. The nitric oxide radical scavenging activity of the yoghurt samples as shown in Figure 2c also shows a similar pattern with the DPPH scavenging assay. Lipid peroxidation refers to a series of chain reactions of unsaturated fatty acids induced by free radicals or other active oxygen species, producing toxic compounds. The results of inhibition of lipid peroxidation evaluation of the plain and spiced yoghurt samples is are presented in figure 2c. Both the plain yoghurt and spiced yoghurt also exhibit inhibition of lipid peroxidation and most of the spiced yoghurt had significant higher inhibition than the plain yoghurt. Similar reports by Srivastava *et al.* (2015) that supplementation of buffalo, and goat milk yogurts with 2% ginger rhizome extract and 2% beet root extracts improved the antioxidant potentials using DPPH and FRAP methods.

While plain yoghurt generally exhibit some levels of antioxidant potential due to substances such as peptides, free amino and fatty acids with antioxidant activities that are generated from the milk during fermentation as reported earlier by Tavakoli *et al.* (2019), enrichment with spice extracts enhanced this endogenous antioxidant properties. Some endogenous peptides may also be able to exert a marked synergistic effects with phenolic antioxidants from plant products (Citta *et al.*, 2017). Besides, such antioxidant compounds may enhance protection against lipid peroxidation, and peroxidability of yoghurt; thereby improving the shelf-life of the fermented product.

3.3 Effects of Spices on the some nutrients in spiced yoghurt

Table 1 shows the effects of incorporation of spice extracts on some nutrients of the yoghurt.

Table 1: Effects of 4% spice extracts on the contents of some nutrients in yoghurt

Yoghurt	Moisture (%)	Protein (%)	Ash (%)	Ca (mg/100mL)	P (mg/100mL)
Control	79.46 ± 1.70[a]	2.54 ± 0.12[b]	11.28 ± 0.53[ab]	610.67 ± 5.21[ab]	2159.83 ± 81.67[a]
ABP	82.09 ± 0.88[a]	3.67 ± 0.19[a]	9.32 ± 0.56[b]	462.67 ± 93.93[b]	1549.67 ± 57.76[b]
Turmeric	82.38 ± 0.19[a]	3.80 ± 0.23[a]	10.34 ± 0.36[ab]	682.50 ± 58.97[a]	1215.10 ± 98.84[c]
Clove	82.58 ± 0.30[a]	3.96 ± 0.16[a]	12. 40 ± 1.50[a]	635.83 ± 32.73[ab]	1120.77 ± 15.12[c]

Mean values with different superscript in each column are significantly ($p<0.05$) different from one another. ABP= African Black Pepper

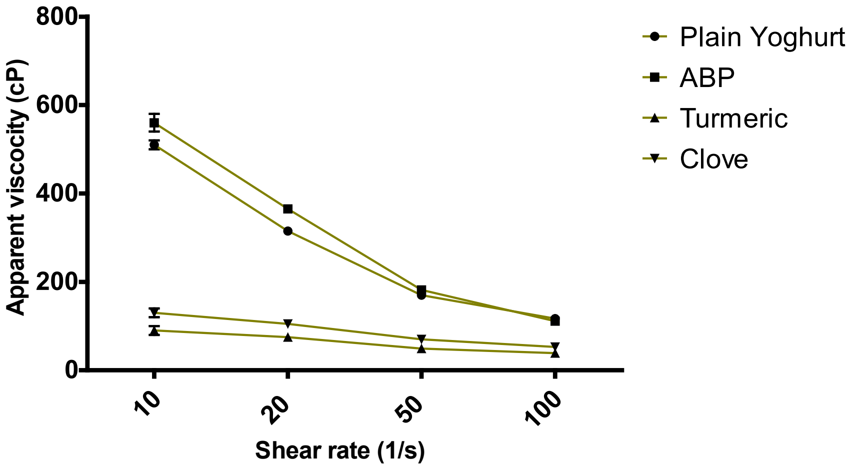

Figure 3: apparent viscosity of yoghurt enriched with 4% spice extracts

Table 2: **Effects of 4% spice extracts on the contents of some nutrients in yoghurt**

Parameters	Control	ABP	Turmeric	Clove
pH	4.82 ± 0.05[a]	4.79 ± 0.02[a]	4.76 ± 0.01[a]	4.66 ± 0.12[a]
Total Acid (%)	7.82 ± 0.05[a]	7.65 ± 0.09[a]	6.48 ± 0.16[b]	7.65 ± 0.18[a]
TSS (Brix)	14.75 ± 0.58[a]	13.50 ± 1.01[ab]	12.83 ± 0.36[ab]	12.17 ± 0.58[b]
Syneresis	64.00 ± 1.15[a]	65.33 ± 0.67[a]	53.33 ± 3.53[b]	59.33 ± 1.76[b]
WHC	80.00 ± 1.15[a]	74.00 ± 1.53[a]	76.33 ± 3.18[a]	73.33 ± 1.76[a]
L*	70.54 ± 0.11[a]	66.62 ± 2.58[a]	66.90 ± 4.71[a]	66.61 ± 6.24[a]
a*	-13.37 ± 0.09[a]	-12.40 ± 0.59[a]	-13.44 ± 0.99[a]	-13.00 ± 2.23[a]
b*	11.25 ± 0.16[a]	11.18 ± 0.56[a]	13.61 ± 1.22[a]	12.76 ± 1.14[a]
H*	-0.69 ± 0.00[a]	-0.73 ± 0.01[a]	0.79 ± 0.03[a]	0.79 ± 0.05[a]
C*	17.46 ± 0.18[a]	16.69 ± 0.80[a]	19.15 ± 1.48[a]	18.27 ± 2.31[a]
ΔE	45.33 ± 1.76[a]	41.03 ± 2.51[a]	31.05 ± 9.64[a]	38.71 ± 6.97[a]

Mean values with different superscript in each column are significantly ($p<0.05$) different from one another. ABP= African Black Pepper

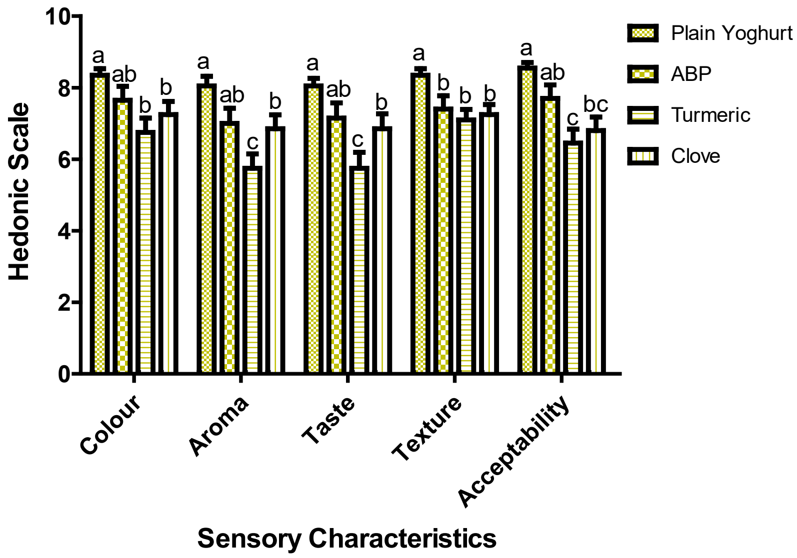

Figure 4: Sensory characteristics of spiced yoghurt samples. Mean values with different superscript in each column are significantly ($p<0.05$) different from one another.

Supplementation of yoghurt with the food spices had little or no effects on the moisture contents. Incorporation of all spice extracts significantly increased ($p < 0.05$) the protein content of the yoghurt samples as compared with the plain yoghurt. Clove extracts gave the highest increase with 0.61 fold significant increases, followed by turmeric and then ABP. On ash content while addition of ABP and turmeric had little or no effect on the yoghurt samples, clove extract significantly increased ash. While addition of clove extracts had little or no effect on the calcium content, turmeric significantly increased ($p< 0.05$) the content of calcium in the yoghurt sample as compared with the control. The moisture content of the spiced yoghurt was maintained within the range of most yoghurts available in the market (80-86%) (Ndife, 2014). The improved protein content of the spiced yoghurt might have resulted from enrichment with plant protein from the spices. Addition of plant proteins lead to the improvement of the quality of yogurt and an increase in its nutritional value (Dabija *et al.*, 2018). Many researchers have achievements in the field of fortification of protein-based yogurt, with plant proteins (Akalin *et al.*, 2012; Morell *et al.*, 2015). Such fortification may have positive imparts the textural and physical properties of yogurts such as yogurt firmness, viscosity and functional properties (Dabija *et al.*, 2018). The firming effect of different proteins addition in the formulation of yogurts could lead to a distinctive structure of the casein, with beneficial influences on the textural characteristics of finished product. On the other hand, for the consumer, the increase in the protein content improves the degree of satiety, especially beneficial for those who consume fermented dairy products for health reasons, certain diets, etc. (Dabija *et al.*, 2018). The ash content is a reflection of the amount of minerals in a food material (Gemede *et al.*, 2015). Calcium, which plays key roles in bone formation and mineralization, is highly required during growth, pregnancy, and lactation (Gemede *et al.*, 2015; Soetan *et al.*, 2009). This, therefore, means that the turmeric enriched yoghurt would be a beneficial drink for the children, pregnant and lactating mothers as well as the elderly whose calcium requirement is high.

3.4 Physico- chemical characteristics of spiced yoghurt samples

The results shown in figure 4 revealed the effects of spice addition on the viscosity of the spiced yoghurt samples.

Supplementation of yoghurt African black pepper gave the highest viscosity as compared with the control and other food spices. In contrast to the most generally accepted reports that, addition of plant extracts decreases the consistency of the products owning to reduced water-binding capacity of proteins (Ramaswamy & Basak, 1992) and as demonstrated with addition of pomegranate peel extracts to yoghurt (El-Said *et al.*, 2014), addition of 4% African black pepper improved the viscosity of the product. These effects may be related to the contribution of protein (as reported in table 4) to the fermentation medium, leading to improvement of the techno-functionality of the product. This can be attributed to the positive impact of protein enrichment on the aggregation of casein network in yoghurts via electrostatic interaction, and on the resistance for the yoghurt matrix to flow. During yogurt making process, the level of lactic acid becomes higher as fermentation goes on, and a series of cascade such as increase in acidity, dissociation of the carboxyl groups, ionization of serine phosphate and increase in the negative charge between casein micelles take place respectively. At this point, whey proteins are denatured and are more susceptible to association with casein and casein micelles. Additionally, during acidification, the denatured whey proteins, associated or not with casein micelles, aggregate. This results from the neutralization between calcium phosphate and the increasing negative charge and due to the presence of these attractive forces, repulsive charge is reduced and the casein micelles aggregate and eventually coagulate into a network of small chains, yoghurt coagulum and a visco-elastic gel (Brückner-Gühmann *et al.*, 2019).

Addition of both turmeric and clove extracts significantly reduced syneresis of the yoghurt samples by 0.45 fold and 0.48 fold respectively. Tendency to syneresis gives information about the stability of the gel samples. The rate of syneresis in acidified milk gels like yoghurt is mainly determined by the microstructure of the protein network. If the water binding is not sufficient, whey will be expelled on the surface of the product during storage (Brückner-Gühmann *et al.*, 2019). Addition of spice extracts to yoghurt did not have significant effect on the colour characteristics of the yoghurt (Table 2).

Results in table 2 revealed that enrichment of the milk-based fermentation mix with the spice extracts did not cause significant change to the colour characteristics of the yoghurt samples in terms of the degree of lightness (L*), red- green range (a*), yellow-blue range (b*), chroma (C*), hue angle (h), and the total colour difference (ΔE) with respect to the standard. Colour is the first contact perceived as a measure of quality and could greatly influence the consumer's acceptability of food products. It is used to measure other quality attributes such as flavour, sensory, nutritional, and pigments, due to its simplicity and good correlation with other physicochemical properties.

3.5 **Sensory Characteristics**

Consumer acceptability is a key factor in product development. Results of sensory profile analysis of the spiced yoghurts are summarised in figure 4.

Although, instrumental viscosity and colour measurements revealed little or no effects of spice extracts on the yoghurt samples, sensory evaluation revealed significant reduction in colour, aroma, taste, texture and overall acceptance of the spices yoghurts as compared to the plain yoghurt.

4 CONCLUSION

Yoghurt containing clove extracts showed the highest antioxidant potential and reduced rate of syneresis but also reduced viscosity and organoleptic properties. In the case of ABP, antioxidant characteristics, protein content and viscosity were enhanced with minimal effects on consumer acceptability. Effects of turmeric extracts include improved calcium content and reduced syneresis but reduced total solids. Overall, African black pepper showed remarkable desirable effects on the product as its addition to the milk-based fermentation mix resulted in yoghurt, which combines improved antioxidant characteristics with increased protein content as well as product quality, with respect to viscosity. Therefore, enrichment of yoghurt with the food spices undoubtedly constitutes a good way for improving the techno-functionality, nutritional and health benefits of yoghurt. Such recipes may be considered for optimization and development towards producing new functional yoghurt.

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