World Sustainable Development Outlook 2013

391

POLYPHENOL, VITAMIN C, CAFFEINE, PROTEIN, ASH, FREE FATTY ACID AND PEROXIDE PROFILES OF GREEN TEA-BASED YOGHURTS

Abiodun A. Olapade[1]
University of Ibadan, Nigeria

Shamsideen Olusegun Aroyeun[2]
Cocoa Research Institute of Nigeria, Nigeria

G. O. Ajibola[1]
University of Ibadan, Nigeria

Abstract
Purpose: To develop a new functional food incorporating green tea infusions into yoghurt.
Design/methodology/approach: The concentrations of green tea infusions used were 0.5%, 1.0%, 1.5%, 2.0% and 2.5% (v/v). Yoghurt was produced using standard methods. The effects of incorporating green tea in the formulation were studied by measuring the chemical parameters such as free radical scavenging activity, polyphenols, minerals, vitamin C, caffeine, pH, total soluble solids, titratable acidity and other proximate chemical compositions. The effects of adding green tea to yoghurt were also determined by measuring the free fatty acids and the peroxide values of the yoghurts stored for fourteen days under ambient storage. All measurements were done in triplicate. Sensory evaluation of the yoghurts was carried out by a panel of tasters comprising students of the food technology department of the University of Ibadan who are accustomed to consuming yoghurts.

World Sustainable Development Outlook 2013

[1] Faculty of Technology, University of Ibadan, Ibadan, NIGERIA
[2] Crop Processing and Utilization Department, Cocoa Research Institute of Nigeria, PMB, 5244, Ibadan, Oyo State, NIGERIA, Email: aroyeun2000@yahoo.co.uk

Findings: The yoghurts contained 0.5-16.67 gGAE/100g of polyphenols which were significantly different with respect to concentrations ($P<0.05$). Significant differences also occurred in their radical scavenging activity, which fell between 8–22.2%. The peroxide values as well as the free fatty acid concentration of the yoghurts reduced as a result of the inclusion of green tea in the formulation after fourteen days of storage, which confirmed its bio-preservative properties. The mean caffeine content of the yogurts increased with the increase in concentration of green tea infusion. The pH value of the control yoghurt was 4.50± 0.12, while green tea incorporated samples ranged between 4.30 and 4.63. The taste became bitter, the colour became duller and overall quality became lower.
Practical implications: This paper shows that incorporation of tea in yoghurt can make a functional food capable of enhancing the health status of its consumers.
Originality/value: The development of this product can create good markets for tea farmers because it gives added value to green tea.

Keywords: Green tea, Yoghurts, Polyphenols, Health

Paper type: Research paper

INTRODUCTION

Naturally occurring dietary components and their influence on the human body are interesting to scientists. Numerous epidemiological studies have indicated that food and beverages rich in polyphenols are important factors in preventing diseases, slowing down the ageing processes, and decreasing mortality from cardiovascular and meoplastic diseases (Di Carlo *et al.*, 1999). Polyphenols, biologically active compounds of plant origin, are classified as non-nutrients. An average daily intake of polyphenols can be important in fighting free radicals activity in the human body (Scalbert and Williamson, 2000). Free radicals are constantly generated due to environmental pollution, radiation, chemicals, toxins, physical stress and the oxidation process of drugs and food. Many plant phenolic compounds have been reputed to have antioxidant properties that are even stronger than vitamins E and C. In addition, currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and gallic acid esters have been suspected to cause or prompt negative health effects and hence the need to substitute them with naturally occurring antioxidants (Amie *et al.*, 2003; Aqil *et al.*, 2006; Pourmorad *et al.*, 2006).Tea (*Camellia sinensis*) is the most consumed drink in the world after water (Cabrera *et al.*, 2006). Green tea leaves (GTL), a non-fermented tea, contain10–30% polyphenols (dry leaf weight), including catechins, flavonols, flavonones, phenolic acids, glycosides and the aglycones of plants pigments (Pan *et al.*, 2003). Polyphenols are natural antioxidants (Tanizawa *et al.*, 1984) present in green tea as well as black tea. They are considered to be responsible for the anticarcinogenic, antimutagenic properties of teas as well as protective action against cardiovascular diseases (Shahidi and Wanasundara, 1992; Tijburd *et al.*, 1997; Wiseman *et al.*, 1997). Tea also contains alkaloids, namely caffeine, theophylline and theobromine, which are also important factors in determining the quality of green tea (Sharma *et al.*, 2008). Considering the health benefits derived from the use of green tea presently and its future potential as a natural antioxidant, it has been incorporated into some foods such as cereals, cakes, biscuits, dairy products, instant noodles, confectionery, ice cream and fried snacks thus giving a healthier appeal to the consumer (Wang and Zhou, 2004). Its incorporation has been extended to yoghurt (Jaziri *et al.*, 2009).

Yoghurts are gaining in popularity due to their acceptability for consumers as well as their nutritional properties and potentially beneficial effects on human health. In the food market today, both yoghurt and green tea are popular choices for health-conscious consumers because of their health benefits and also their reasonable price compared to other dietary supplements such as Ginseng and Ginko leaves. Yoghurts and green tea both contain antioxidants and phenolic compounds related to anti-ageing properties, and may be related to cancer prevention. There is currently considerable growth in industrialised countries in the economical importance of yoghurts. In the last few years, this tendency has driven the manufacturers to develop and produce a wide variety of these products with different characteristics. Yoghurt can be a good source of essential nutrients for minerals in the human diet. They could contribute significantly to the recommended daily requirements for calcium and magnesium to maintain physiological processes. Yoghurts are also a good dietary source of phosphorus (besides calcium, considered the most important nutrient for bone health) and its contribution to total phosphorus intake has been reported as 30–45% in western countries (Cashman and Flynn, 1999).

Tea antioxidants have drawn increased attention in recent years because of their potential health benefits, not only as antioxidant agents but also as anti-arteriosclerotic, anti-carcinogenic, and antimicrobial agents. They may contribute to reducing risks of chronic diseases and cancer, promoting oral health, and prolonging the shelf life of food products without damaging their organoleptic or nutritional qualities (Wang and Zhou, 2004). The possible health benefits that the consumption of local tea varieties may bring include the reported possession of antimicrobial activity against a large spectrum of pathogenic bacteria (Jaziri *et al.*, 2005). The use of green tea extracts in foods such as bread, cereals, cakes, biscuits, dairy products, noodles, instant noodles, confectionery, ice cream and fried snacks gives 'a healthier appeal to the consumer'. Therefore, the marketing potential for these foods can be improved by the presence of catechins. A successful example has been their application in mooncake, a traditional cake eaten during the Chinese Middle Autumn Festival. The incorporation of green tea extract both increased the shelf life and improved the flavour of mooncake. A more recent example is the addition of green tea extract to bread by Wang and Zhou (2004). Commercial utilization of tea or tea products in yoghurts is not available in the literature and no scientific information is available on the function of tea products on biscuit quality. The objective of this study is to evaluate the effect of incorporating local green tea in yoghurt on its antioxidant, caffeine, protein, ash and sensory quality of the made yoghurts.

MATERIALS AND METHODS

Procurement of materials

The dried green tea leaves were obtained from the Cocoa Research Institute of Nigeria (Kusuku station, Mambilla, Taraba State). The full cream powdered milk (Dano, by AmbaDenmark), stabiliser (corn starch produced in Nigeria), granulated sugar (Dangote, Nigeria) and freeze dried starter culture (yogourmet) was procured from the food processing laboratory of the department.

PREPARATION OF GREEN TEA INFUSION

The dried green tea leaves were ground into powder using a porcelain mortar and pestle. This was weighed and used for the formulation of the infusion (Table 1). The green tea infusion of various concentrations was prepared as described by Staszewski *et al.* (2011).About 500 ml distilled water at 95 ^{o}C was added to a known weight of ground green tea leaf and vigorously stirred. The mixture was maintained at 95 ^{o}C for 20 min in a water bath and the infusion obtained was later passed through sterile cotton wool to remove all other particles.

DEVELOPMENT OF GREEN TEA BASED YOGHURT

Yoghurt was produced as described by Balami *et al.* (2004) with slight modifications. The various concentrations of green tea infusion prepared were used separately to replace water in green tea yoghurt production. A control sample was also prepared with distilled water. Twenty-five grams of powdered milk was reconstituted with 200 ml of each of green tea infusion. About 15g corn starch and 12.5g granulated sugar were added and the mixture was blended with a warring blender to obtain a uniform mixture. The mixture was pasteurised at 85 ^{o}C for 30 min and rapidly cooled to about 42 ^{o}C in a water bath. The samples prepared in duplicate were inoculated with 3g starter culture. The sample containers were covered to prevent contamination and incubated at 42±2 ^{o}C for 6 h.

DETERMINATION OF TOTAL POLYPHENOLS AND RADICAL SCAVENGING ABILITY

The radical scavenging activity of the samples was determined using the DPPH model system as described by Sharma *et al.* (2008). The 1, 1-diphenyl-2-picrylhydrayl (DPPH) was freshly prepared, stored in a flask covered with aluminium foil and kept in the dark at 4 ^{o}C between measurements.

DETERMINATION OF ACIDITY

The pH change was monitored by a pH meter (Fisher Scientific, Pittsburgh, PA). Titratable acidity (TA) was determined as described by Pyo and Song (2009). A 5 g sample and 45 ml of distilled water was titrated with 0.1 N NaOH to an end point of pH 7.0. TA was calculated on the basis of lactic acid as the predominant acid and was expressed as percentage lactic acid. The sample temperature was maintained at 25 ^{o}C for each analysis. The chemical compositions of the control and green tea based yoghurt samples were determined: Total polyphenols content by spectrophotometry using Gallic acid as standard according to the method described by ISO (2005). (b) Radical scavenger's activity by DPPH model (c) Caffeine content as described by Smith (2005) and Rogers and Dernoncourt (1998). The physico-chemical analysis of the samples was evaluated by determining (a) pH using Inolab 740 pH meter with combined electrode of Hanna instruments (Deutschland, Germany). Titratable acidity (AOAC, 2005), Total solids, fat, protein and ash contents were determined using (AOAC, 2000) methods while the free fatty acid and peroxide values were determined in accordance with AOAC (2000).

Calcium was determined with Jenway Digital Flame Photometer (PFP7 Model) and phosphorus by spectrophotometric method. Vitamin C content was also analysed as described by Onwuka(2005). Microbiological analysis was determined by evaluating: (a) total viable count as described by Adegoke (2000), fungal count as described by Olutola *et al.* (1991) while coliform counts were based on the methods described by Adegoke (2004).

SENSORY EVALUATION

For the sensory properties, the samples were evaluated by a panel of 25 assessors who are regular consumers of yoghurt. The samples were served at 10 ^{o}C in glass cups and were coded with three letters. The assessors were selected among the students and staff of the Faculty. The order of presentation of samples was randomised. The panellists cleansed their mouth between samples with water during the evaluation. A test form comprising taste, flavour, colour, consistency and overall acceptance was given to each assessor. The samples were scored using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

STATISTICAL ANALYSIS

In this present study, all experiments were performed at least in triplicate and results were expressed as mean values ± standard deviation (SD). Statistical analysis was performed by one- way analysis of variance using SPSS 7.5 (SPSS Institute, Cary, NC, USA) and individual comparisons were obtained by Duncan's multiple range test, which was used to determine the difference of means.

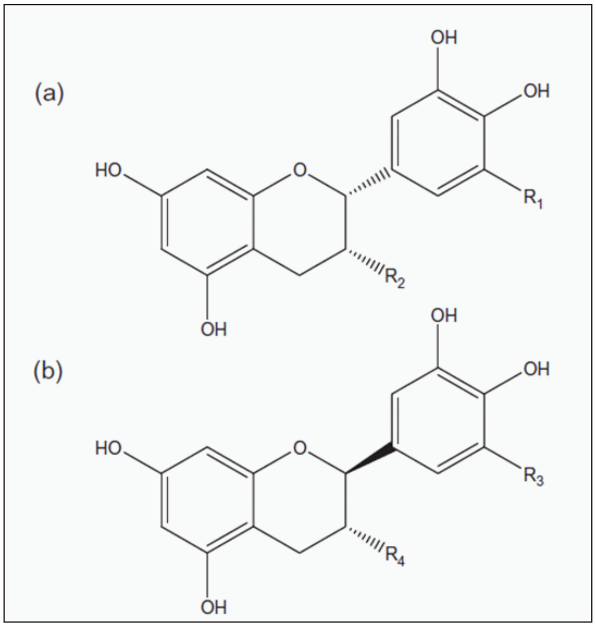
RESULTS AND DISCUSSION

Total polyphenols increased with increase in concentration of green tea infusion (Table 1). The polyphenols content ranged between 0.5 and 16.67 gGAE/100g and it was observed that the samples were significantly ($P<0.05$) different from one another based on total polyphenols content. This result was lower than the value reported by Anesini *et al.* (2008). Similarly, the results of radical scavenging activity of the samples increased with increase in the concentration of green tea infusion. There were also significant ($P<0.05$) differences among all the samples based on their radical scavenger activity. The radical scavenging activity values were 16.8– 22.2%.

The result of caffeine content in the green tea leaf was 578±12 ppm. The values for green tea infusion samples ranged from 140 to 411 ppm, while corresponding values for yoghurt were 148-338 ppm. The samples were significantly ($P < 0.05$) different from one another and the mean caffeine content of the samples increased with increase in concentration of green tea infusion (Fig. 1). It was also observed that the concentration of caffeine in the samples decreased after processing into yoghurt (Fig. 2). The values observed in this study were comparable to previous observations reported by Kaplan *et al.* (1974), which stated that growing conditions, processing conditions and other variables affect caffeine content. The calibration curves in Figures 1 and 2 were obtained using a computer algorithm and these showed a positive linear relationship between absorbance and concentration of caffeine of the samples. This linear relationship obeyed the Beer-Lambert Law, which stated the linear relationship exists between absorbance and concentration of an absorbing species.

Table 1. Green tea infusion formulation

Dried ground Tea leaves (g)	Distilled water (ml)	Concentration of tea extract (%)
2.5	500	0.5
5.0	500	1.0
7.5	500	1.5
10.0	500	2.0
12.5	500	2.5



A is Epicatechin where R1 is H, R2 is OH B is (+)- Catechin where R3 is H and R4 is OH

Figure 1. General chemical structures of green tea

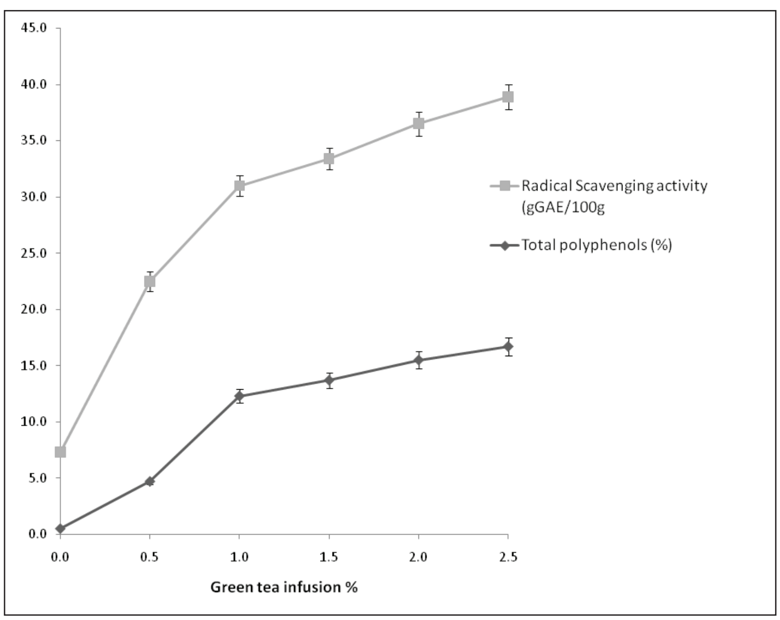


Figure 2. Effects of green tea on radical scavenging activity and polyphenol contents of yogurts

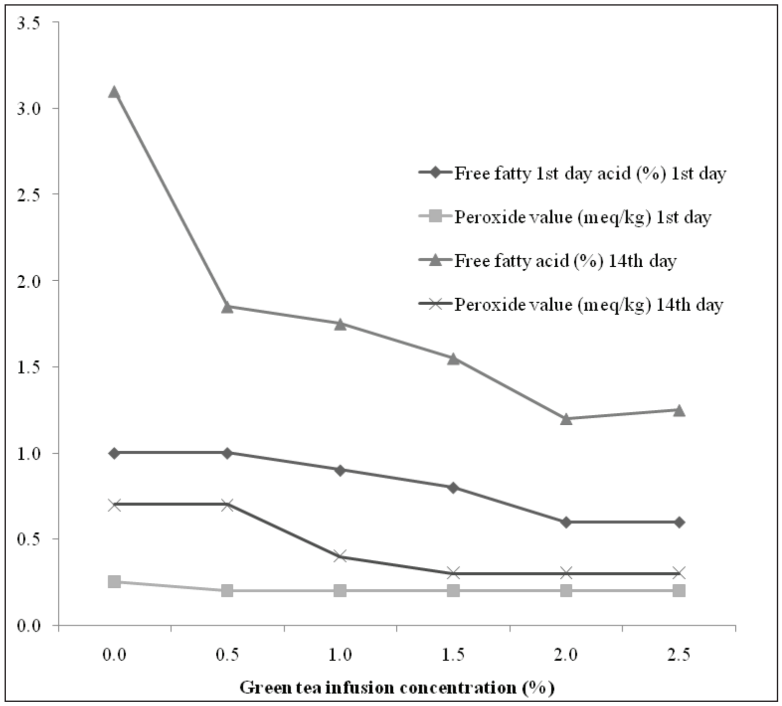


Figure 3. Effects of Green Tea infusion on the free fatty acids and peroxide values of green tea yogurts stored for fourteen days

PHYSICO-CHEMICAL ANALYSIS

Table 2 shows the physico-chemical properties of control yoghurt and green tea yoghurt samples. The mean pH value of control yoghurt samples was 4.50± 0.12, while the values for green tea yoghurt samples ranged from 4.30 to 4.63. There were no significant (*P*>0.05) differences among the pH value of control yoghurt and those of green tea yoghurt samples. The pH values obtained in this study were comparable to values previously reported by Dublin-Green and Ibe (2005), Hassan and Amjad, (2010) and Okoye and Animalu (2009). The pH values of these samples were also in accordance with FDA specifications for yoghurt (≤4.6) (Olugbuyiro and Oseh, 2011).

The mean values of titratable acidity for green tea yoghurt samples were 0.85-0.95%, compared with 0.95% obtained for the control sample. Statistically, there no were significant (*P* > 0.05) differences in the titratable acidity values of control and other samples. The titratable acidity values obtained in this study fell within the range reported for some yoghurt samples, with values between 0.78 and 0.95 according to Kirk and Sawyer (1991) which were above the standard recommended value of 0.7% by (FDA, 2009). There was no direct relationship observed between pH values and titratable acidity as has been previously reported (Dublin-Green and Ibe, 2005). There were no significant ($P > 0.05$) differences among all the samples based on their total solids content. The results were comparable to those obtained by Mahdian and Tehrani (2007), who reported that concentrated yoghurt has total solids of 23–27%. These results also fell within the range obtained by (Gogo *et al.*, 2012; Okoye and Animalu, 2009). There was no significant ($P < 0.05$) difference among control yoghurt, 0.5%, 1.0% and 1.5% green tea infusion samples based on their fat content. The results obtained in this study fell within the recommended values and was supported by Codex Alimentarius Commission (2000) which recommended that the minimum fat content of whole milk yoghurt should be 3.0%. Meanwhile, samples were not significantly($P > 0.05$) different from one another in terms of their protein content. Statistically, there was no significant ($P < 0.05$) difference among all the samples in terms of their ash content. The results obtained were higher than those reported by Ashraf *et al.* (2010). Table 3 shows the results of free fatty acid and peroxide values on the first day and after fourteen days of storage There was no significant ($P < 0.05$) difference in free fatty acid content of all the samples except samples containing 1.5% to 2.5% green tea infusion, which were different

Samples	pH	Total titratable acidity (%)	Total solids (%)	Crude fat (%)	Crude protein (%)	Ash (%)
Control yoghurt	$4.50±0.12^{ab}$	$0.95±0.07^{a}$	$23.40±0.53^{a}$	$3.13±0.32^{a}$	$3.30±0.23^{a}$	$1.65±0.71^{a}$
0.5% GTI	$4.40±0.10^{bc}$	$0.95±0.14^{a}$	$23.87±0.81^{a}$	$3.20±0.35^{a}$	$3.57±0.40^{a}$	$1.65±0.71^{a}$
1.0% GTI	$4.30±0.10^{c}$	$0.87±0.21^{a}$	$23.17±1.26^{a}$	$3.37±0.64^{a}$	$3.55±0.31^{a}$	$1.70±0.26^{a}$
1.5% GTI	$4.30±0.15^{abc}$	$0.90±0.02^{a}$	$23.30±0.14^{a}$	$3.43±0.81^{a}$	$3.55±0.32^{a}$	$1.70±0.35^{a}$
2.0% GTI	$4.43±0.06^{abc}$	$0.85±0.03^{a}$	$24.17±0.07^{a}$	$3.49±0.39^{a}$	$3.70±0.44^{a}$	$1.70±0.40^{a}$
2.5% GTI	$4.63±0.15^{a}$	$0.86±0.07^{a}$	$23.33±0.58^{a}$	$2.90±0.17^{a}$	$3.45±0.44^{a}$	$1.70±0.15^{a}$

Means in the same column with the same superscript are not significantly different at (P<0.05). Values are means± standard deviation of duplicate determinations.
GTI - green tea infusion

Table 2. Score for physico-chemical analysis of yoghurt samples

Table 3. Mean free fatty acid and peroxide value of yoghurt at 1st and 14th day of storage

\Samples	1st Day free fatty acid (%)	1st Day peroxide value (meq/kg)	14th Day free fatty acid (%)	14th Day peroxide value (meq/kg)
Control Yoghurt	1.00±0.10a	0.25±0.07a	3.10±0.13a	0.7±0.04a
0.5% GTI	1.00±0.11a	0.20±0.05a	1.85±0.07b	0.7±0.07a
1.0% GTI	0.90±0.14ab	0.20±0.06a	1.75±0.07b	0.4±0.01b
1.5% GTI	0.8±0.13b	0.20±0.08a	1.55±0.07c	0.3±0.05b
2.0% GTI	0.6±0.10c	0.20±0.02a	1.20±0.07d	0.3±0.08b
2.5% GTI	0.6±0.08c	0.20±0.05a	1.25±0.10d	0.3±0.05b

Means in the same column with the same superscript are not significantly different at ($P<0.05$). Values are means± standard deviation of duplicate determinations.
GTI means green tea infusion

from other samples. Meanwhile, the highest increase in free fatty acid was observed in the control sample. The reduction in the free fatty acid values of the other samples was as a result of green tea infusion incorporated into those samples. There was no significant ($P<0.05$) difference among all the samples based on their peroxide values on first day of production. Neither was there any significant difference in peroxide values of all the samples except the control and the sample containing 0.5% green tea infusion, which were different from the others after fourteen days of storage, although, a more pronounced increase occurred in the control sample. This result was comparable to that obtained by Ersöz *et al.* (2011).

MINERALS AND VITAMIN C EVALUATION

The results of minerals and vitamin C determination are summarised in Table 4. These results show that the vitamin C, calcium and phosphorus content of the samples increased with an increase in concentration of green tea infusion. The results obtained in this study were higher than those obtained by the Food Standards Agency (2002).

MICROBIOLOGICAL ANALYSIS

The results of microbiological analysis, as shown in Table 5, revealed that the total viable count of the samples fell within the range of 0.5×10^3

Samples	Calcium (%)	Phosphorus (%)	Vitamin C (mg/100g)
Control yoghurt	285±24b	165±20b	2.50±0.34d
0.5% GTI	290±34ab	167±35ab	2.80±0.14c
1.0% GTI	287±35ab	170±30ab	2.90±0.14bc
1.5% GTI	290±30ab	170±23ab	3.10±0.24ab
2.0% GTI	290±31ab	175±28a	3.24±0.70a
2.5% GTI	292±35a	175±14a	3.35±0.73a

Means in the same column with the same superscript are not significantly different at (P<0.05). Values are means± standard deviation of duplicate determinations.
GTI means green tea infusion

Table 4. Minerals and vitamin C composition of yoghurt samples

Samples	TVC (cfu/ml)	TCC (cfu/ml)	TFC (cfu/ml)
Control	0.9×103	NG	NG
0.5% GTI	1.8×103	NG	NG
1.0% GTI	0.5×103	NG	NG
1.5% GTI	1.3×103	NG	NG
2.0% GTI	3.6×103	NG	NG
2.5% GTI	17.4×103	NG	NG
Green tea powder	NG	NG	NG

NG means no growth

Table 5. Total viable count, coliform count and fungi count of yoghurt samples

to 17.4×10^3. These current findings were lower compared to the report of Khan *et al.* (2008). The disparity in the mean value of the total viable count of bacteria might be a reflection of variations in manufacturing practices (Nwamaka and Chike, 2010).

The absence of coliform and fungi followed by a reduction in total viable count recorded by the yoghurt samples indicated that the products are fit for human consumption and will also have good keeping quality (Okoye and Animalu, 2009). The colony counts were in conformity with food regulations and remained so throughout the experiment.

These results are in accordance with the literature emphasising the antimicrobial activities of tea against many pathogenic bacteria (Chou *et al.*, 1999; Hamilton-Miller, 1995; Jaziri and Hamidi, 2005; Yam and Hamilton-Miller, 1997).

SENSORY EVALUATION

The results of the sensory evaluation of twelve yoghurt samples obtained from a 9-point hedonic scale are summarised in Table 5. It was observed that samples incubated at room temperature were preferred in terms of all attributes tested than those incubated at 42–43 °C.

CONCLUSION

This study thus revealed that the samples containing 0.5% to 2.0% concentration incubated at room temperature and 0.5% to 1.5% incubated at 42–43°C were of good quality and acceptable for human consumption. However, samples containing 2.0% green tea infusion and 2.5% green tea infusion both incubated at 42–43°C were not

Table 6. Mean hedonic ratings (n= 64) for sensory evaluation of yoghurt samples

Incubation temp.	Attributes	Aroma	Colour	Consis-tency	Taste	Overall acceptability
28 °C	Control	7.4 ± 1.1^{a}	7.6 ± 1.0^{a}	6.9 ± 1.3^{a}	7.5 ± 1.0^{a}	7.5 ± 0.9^{a}
	0.5% GTI	6.7 ± 1.1^{bc}	6.2 ± 1.2^{b}	6.7 ± 1.4^{ab}	6.4 ± 1.6^{b}	6.4 ± 1.3^{b}
	1.0% GTI	6.3 ± 1.2^{bcd}	6.0 ± 1.3^{b}	7.2 ± 1.4^{a}	6.2 ± 1.5^{bc}	6.3 ± 1.4^{bc}
	1.5% GTI	6.4 ± 1.3^{bcd}	5.9 ± 1.5^{b}	6.6 ± 1.5^{abc}	5.8 ± 1.6^{bcde}	6.0 ± 1.3^{bcd}
	2.0% GTI	6.3 ± 1.6^{bcd}	5.7 ± 1.8^{bc}	6.9 ± 1.5^{a}	6.0 ± 1.8^{bcd}	6.3 ± 1.3^{bc}
	2.5% GTI	5.8 ± 1.6^{de}	5.0 ± 1.9^{d}	5.9 ± 1.9^{bcd}	3.8 ± 1.7^{f}	4.9 ± 1.5^{e}
43 °C	Control	6.8 ± 1.4^{ab}	7.3 ± 1.7^{a}	5.9 ± 1.7^{bcd}	6.3 ± 1.9^{b}	6.6 ± 1.4^{b}
	0.5% GTI	6.3 ± 1.2^{bcd}	6.4 ± 1.2^{b}	6.1 ± 1.5^{bcd}	5.4 ± 1.8^{cde}	6.0 ± 1.4^{bcd}
	1.0% GTI	6.2 ± 1.1^{bcd}	6.07 ± 1.4^{b}	5.76 ± 1.6^{d}	5.3 ± 1.6^{de}	5.7 ± 1.5^{cd}
	1.5% GTI	6.1 ± 1.1^{cde}	5.78 ± 1.4^{bc}	5.87 ± 1.7^{cd}	5.0 ± 1.6^{e}	5.6 ± 1.5^{d}
	2.0% GTI	5.5 ± 1.6^{e}	5.18 ± 1.6^{d}	5.00 ± 1.8^{e}	4.3 ± 1.8^{f}	4.7 ± 1.3^{e}
	2.5% GTI	4.3 ± 1.9^{f}	4.20 ± 1.9^{e}	3.56 ± 1.9^{f}	3.0 ± 1.7^{g}	3.5 ± 1.6^{f}

Mean in the same column followed by the same letter are not significantly different from one another at $P<0.05$. Values are means± standard deviation.

acceptable. Also, the sample containing 2.5% green tea infusion incubated at room temperature was not acceptable to the respondents. A higher concentration of green tea infusion adversely affects the sensory acceptability of green tea yoghurt.

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ABOUT THE AUTHOR

Dr Shamsideen Olusegun Aroyeun assumed duty at the Cocoa Research Institute of Nigeria in June, 1999 and attained the rank of Chief Research Officer in the crop processing Unit in 2009 under the Product Development Programme. He completed his PhD programme in the department of Food Technology at the University of Ibadan, Nigeria. Dr Aroyeun has won two international awards on tea in 2004 and 2007 in Shizouka Japan. He is an international scientist who has presented papers in Hungary, Italy, China, South Africa, Japan and most currently the United States of America. He is a prolific writer with many international journal papers to his credit. He is currently the Head of Station of Cocoa Research Institute of Nigeria, Kusuku, Mambilla.