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Effect of Local Stabilizers on the Physicochemical, Sensory and Microbial Qualities of Thermized Set Yoghurt

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Abstract

The effect of achi (Brachystegia eurycoma), ofor (Detarium microcarpum) and Carboxyl methylcellulose (CMC) on the physicochemical, sensory and microbial qualities of thermized set yoghurt was investigated. The concentrations used were 0.2, 0.4 and 0.6 % each of achi, ofor and CMC, respectively. Thermized set yoghurt was produced by balancing the yoghurt mix, homogenizing, pasteurizing, cooling to inoculation temperature, incubation, and heating. The physicochemical, sensory, and microbial qualities of thermized set yoghurt were determined using standard methods. Data analysis was by one-way analysis of variance (ANOVA) in a completely randomized design. The results showed that thermized set yoghurt stabilized with local stabilizers had a pronounced effect on the pH and titrable acidity when compared to sample with no stabilizer. Achi raised significantly (p < 0.05) the level of ash, specific gravity, viscosity and total solids in thermized set yoghurt. Achi stabilized the set thermized yoghurt than ofor. Samples stabilized with CMC had a significantly (p < 0.05) lower moisture content, higher crude fat content, protein content, pH, total solids and phosphorous than with samples stabilized with the local stabilizers. Scores for the overall acceptability showed that the sample containing no stabilizer had the highest score for all the parameters evaluated but which did not differ significantly (p > 0.05) from the score assigned to sample containing achi at 0.2 %. Microbial count revealed that no growth was obtained with lactic acid bacteria and mould enumeration but low growth (< 30 cfu/ml) was obtained for the total viable growth. Thus, thermized set yoghurt could be produced from local stabilizers such as achi and ofor..

Practical application

It was observed that local stabilizers particularly achi (optimal concentrations of 0.2 to 0.6 %) has a comparable stabilizing activity in thermized set yoghurt relative to CMC. It has been found from the results of this work that achi was a better stabilizer than ofor in stabilizing thermized yoghurt samples and which had a comparable effect with samples stabilized with CMC. Thus, it suffices to say that achi could be employed on a commercial scale in the production of thermized set yoghurt. It is recommended that yoghurt manufacturers should adopt a usage of this stabilizer. Manufacturers could shift to production of thermized yoghurt containing these local stabilizers. This would overcome the constraints imposed by unusual high cost of purchasing exotic stabilizers which invariably would impact on the cost of the finished product.

Keywords: *Brachystegia enrycoma*, Carboxylmethylcellulose, *Detarium microcarpum*, Thermized set yoghurt.

1. Introduction

Yoghurt is a fermented food produced through the lactic acid fermentation of milk or milk products by the symbiotic activities of *Lactobacillius bulgaricus and Streptococcus* thermophilius while containing one or more of the optional ingredients of sweeteners, stabilizers, colourants among others (Athar *et al.*, 2000). The fundamental basis of yoghurt manufacture is the acidic destabilization of milk protein by yoghurt starter culture together with optimum heat induced protein denaturation, producing a three dimensional protein network capable of holding a serum phase (Loveday *et*

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al., 2013). A wide range of classification methods has been adopted for yoghurt in literatures. However, depending on the method of production and physical structure of the coagulum, yoghurt has been classified into set, stirred, drinking, frozen, concentrated, and flavored. (Tamime & Robinson, 1999). Set and stirred yoghurt which are the two most commercially produced variants of yoghurt differs one from another in that set voghurt are produced in a retail pot and the final yoghurt consistency is not sheared prior to cooling whereas stirred yoghurt is sheared after fermentation prior to cooling of the yoghurt variant (Loveday et al., 2013). On a separate classification system, yoghurt may be typified based on their shelf life into fresh and thermized yoghurt. Thermized yoghurt is a yoghurt variant which has been heat-treated after incubation in order to reduce lactic acid bacteria load and by so doing, extend the shelf life of the product outside refrigeration (Alakali et al., 2008).

The microstructure of yoghurt has a major impact on the texture and other physical properties of acid milk gels. Textural properties of yoghurt represent all the rheological and structural attributes perceptible by means of mechanical, tactile and visual receptors. Yoghurt texture can be influenced by the composition of milk, dry matter content, heating, homogenization, the type of starter culture, incubation temperature, cooling, storage time, among others (Karsheva et al., 2013). The shelf life of yogurt is relatively short falling in the range of 3-4 weeks under refrigerated conditions. Yoghurt stability during storage is influenced by the standard of hygiene observed during their manufacture, the microbiological quality of the ingredients and the packaging material. Manufacturers of yoghurt are faced with the

challenge of producing a product with minimal textural defect, the most significant of which is 'spontaneous whey-separation' that is able to keep for as long as the product label says so. Problems of whey-separation is attributable to high incubation temperature, strain of the starter culture used, time and temperature at which the milk base is preheated, packaging material, mechanical stress during transportation, cooling temperature and duration of storage. Wheyseparation could also result when yoghurt is thermized in a bid to control microbiological/enzymatic activities as well as physicochemical interactions. More commonly manufacturers have sought to control or eliminate this problem by increasing the level of milk solids to approximately 15 % (Tamine & Robinson, 1999; Shah, 2003). Other alternatives include the use of stabilizers (including modified food starch, gelatine and vegetable gum) or exopolysaccharide (EPS) producing starter culture (Amatayakul et al., 2006). Polysaccharide derived from Streptococcus thermophillus and Lactobacillus bulgaricus show large variation in composition, charge, spatial arrangement, rigidity and ability to interact with proteins, no defining correlation between exopolysaccharide (EPS) concentration and viscosity has been established in real studies (Amatayakul et al., 2006).

Stabilizers are food-grade additives or hydrocolloids that smoothen the texture of a food product including yoghurt, by allowing for the homogenous dispersion of two or more immiscible materials. The system function by binding water as well as they interact with the milk protein thus increasing their level of hydration and stabilizing protein molecules into a network that prevents the movement of water. The food product acquires a definite framework

with well-maintained physicochemical attributes after their incorporation. In addition to their stabilization ability, stabilizers are able to behave as nano-structures to control, retain, intensify an existing colour and flavor of the food product. Their ability to bind water also guarantees a better shelf life of the product in question. In yoghurt production, manufacturers employ the use of stabilizers in preventing wheyseparation by improving the body/ texture and increasing the viscosity of the product (Tasneem et al., 2014). Stabilizers are greatly used in production yoghurt such as gelatin, carboxylmethyl ccellulose, and starch. The choice of the stabilizer is based on the functional properties of the stabilizer, intended use, outcome, interactions with other ingredients and legal aspects. The problem of stabilizers' usage in yoghurt formulation bothers on overstabilization and under-stabilization, as such, the rule of thumb in determining their concentration depends on trial and error (Tamime & Robinson, 1999; Melesa, 2015). The conventional/exotic stabilizers are expensive and may not be affordable by the small and medium-scale yoghurt manufacturers. So, there is a need to seek for cheap alternative, affordable and available stabilizers such as 'achi', 'ofor' among others.

However, some local stabilizers which are underutilized could be used in place of the exotic ones (Mbaeyi-Nwaoha & Odo. 2019). Indigenous seed crops such as Detarium microcarpum 'ofor' and Brachystegia eurycoma 'achi' could confer comparable stabilization effect on yoghurt as the exotic ones. Flour from the seeds of these wild plants has traditionally been employed in the thickening of soup. Detarium microcarpum and Brachystegia nutritionally eurycoma flour are dense

leguminous plant containing 27. 01 % and 9.98 crude protein, 2.76 and 3.26 % crude fibre, 14.45 and 2.12 % fat, 5.17 and 14.36 % moisture content, 1.4 and 1.98 % ash and 49.21 and 68.3 % carbohydrate, respectively Igwenyi & Azoro (2015). The index of the gelation capacity of both seed flours are found at 10 % (w/v) for the undefatted seed flour and 15 % for the defatted seed flour. The gelation concentration obtained may be regarded as interplay of both the starch, non-starch carbohydrate and the protein components of these seed flours. Both Detarium microcarpum and Brachystegia eurycoma have good pasting properties as well as they have good thermal stabilities. Detarium microcarpum has higher peak viscosity than Brachystegia eurycoma and is appropriate for food where high gelling strength and elasticity is needed. Brachystegia eurycoma has a good potential for use in frozen foods owing to the fact that it has a lower retrogradation profile than Detarium microcarpum when subjected to cooling (Aviara et al., 2015; Uzomah & Odusanya, 2011). The seeds of Detarium microcarpum and Brachystegia eurycoma have been used to formulate stirred yoghurt at concentrations of 0.1 - 0.4 %, respectively while comparing the results to a control sample without the addition of the local stabilizers (Mbaeyi-Nwaoha et al., 2017). It was reported that yoghurt formulation containing 0.4 % 'achi' had the highest fat, ash, while sample phosphorous, and calcium containing 0.4 % 'ofor' had the highest protein, carbohydrate, and pH content. The yoghurt formulation containing 0.1-0.2 % 'achi' was most preferred by the sensory panelist while sample containing 0.3 % 'ofor' was the least preferred. The previous work by Mbaeyi-Nwaoha et al. (2017) with the same local stabilizers was on plain yoghurt that was stored in the refrigerator (without thermization) and

microbiologically, the stabilizers were able to interfere with the activities of the starter culture, the highest total viable count was got for samples containing 0.1% 'achi' and the least lactic acid bacteria count was got for samples containing 0.4 % 'achi'. Also, the researchers reported that no mould growth was seen in yoghurt with the highest lactic acid bacteria count. However, this current research was on thermized set-type voghurt stored at ambient condition (room temperature without refrigeration). The main objective of this study was to evaluate the performance of local stabilizers 'achi', 'ofor') in comparison to 'carboxylmethylcellulose the on physicochemical, sensory and microbial qualities of thermized set yoghurt.

2. Materials and Methods

2.1 Materials

Peak brand of milk powders (whole and skim milk types in different ratios), yoghurt culture (Hasen), local stabilizers, 'ofor' (*Detarium microcarpum*) and 'achi' (*Brachystegia eurycoma*) were purchased from Ogige market, Nsukka Local Government Area, Enugu State, Nigeria.

2.2 Methods

2.2.1. Processing of Detarium microcarpum (ofor) and Brachystegia eurycoma (achi)

Seeds of *Detarium microcarpum* (ofor) and *Brachystegia eurycoma* (achi) were processed into flour (Figure 1) following a modification of the methods described by Nwosu (2012). *Detarium microcarpum* (ofor) and *Brachystegia eurycoma* (achi) seeds received preliminary cleaning by winnowing to remove visible loose dirt. The seeds were boiled (100±2°C for 45-60 mins) for approximately 60 minutes; thereafter it

was drained of the cook water and was dehulled by rasping between the hands. The dehulled seeds (Plates 1 and 2) were sun- dried for 4 hours and was milled using a commercial milling machine and sieved by passing through a 1 mm pore-sized sieve. Figure 1 shows a modified method of processing achi and ofor into flour (Nwosu, 2012).

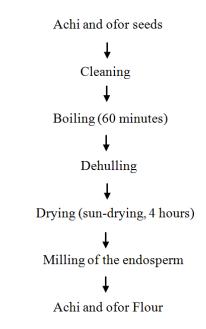


Figure 1: Flow diagram for processing 'achi' and 'ofor' Nwosu (2012)



Plate 1: Processed seeds of *Brachystegia* eurycoma (achi)



Plate 2: Processed seeds of *Detarium microcarpum* (ofor)

2.2.2. Preparation of yoghurt mix

Peak milk powder of 100g weight composed of full cream and skim milk variant in a 50:50 ratio was employed in the production of thermized set

yoghurt. Sugar weight of 2 % was added, rest ratio of the yoghurt system was balanced with water and was made up to a volume of 1000 ml. The Control sample (Y+C) was formulated addition of without the any stabilizer. Additionally, nine yoghurt formulations were produced in the same way as sample Y+C, but 'achi'. 'ofor' they contained 'carboxylmethylcellulose (CMC)' at different concentrations (0.2, 0.4 and 0.6 %). Table 1 shows the formulation of thermized set yoghurt samples.

Table 1: Ingredients formulations for the production of thermized set yoghurt samples

Sample code	Achi (%)	Ofor (%)	CMC (%)	Liquid (ml)	Starter culture (g)
A1	0	-	-	1000	0.5
A2	0.2	-	-	1000	0.5
A3	0.4	-	-	1000	0.5
A4	0.6	-	-	1000	0.5
O1	-	0	-	1000	0.5
O2	-	0.2	-	1000	0.5
О3	-	0.4	-	1000	0.5
O4	-	0.6	-	1000	0.5
C1	-	-	0	1000	0.5
C2	-	-	0.2	1000	0.5
C3	-	-	0.4	1000	0.5
C4	-	-	0.6	1000	0.5

Keys: A1 = No Achi; A2 = 0.2% Achi; A3 = 0.4% Achi; A4 = 0.6% Achi; O1 = No Ofor; O2 = 0.2% Ofor; O3 = 0.4% Ofor; O4 = 0.4% Ofor; C1 = No Carboxylmethylcellulose; C2 = 0.2% Carboxylmethylcellulose; C3= 0.4% Carboxylmethylcellulose; C4 = 0.6% Carboxylmethylcellulose

2.2.3 Production of thermized set yoghurt

Thermized set yoghurt (Figure 2) was produced in accordance with the procedure Alakali *et al.*, (2008). The distinct levels of stabilizers ('achi', 'ofor' and 'CMC'), was stirred into a portion of

the balance water that has been warmed to about 60 °C. Thereafter, the dissolved stabilizer at each of the concentration was blended with the rest of the ingredient mix (milk and sugar) using a to achieve homogenization. Each blender yoghurt formulation containing the distinct stabilizer concentration was pasteurized at 85 °C for approximately 20 minutes to inactivate the pathogens in a water bath. The pasteurized yoghurt formulations were filled into plastic containers, cooled down to temperature of 43 ± 2 °C and was inoculated with 0.5 % levels of freeze-dried yoghurt culture (Hansen) consisting of Lactobacillus bulgaricus and Streptococcus thermophillus. The yoghurt formulations were fermented for 8 hours at inoculation temperature using a water bath. They were cooled down to 10 °C to stop fermentation. Following cooling, thermization of the yoghurt formulations was achieved at 75 °C for 60 seconds. Figure 2 below shows the methods of producing thermized set yoghurt using achi, ofor and CMC.

2.2.4 Physicochemical analysis of stabilized samples of thermized set yoghurt using 'achi', 'ofor' and 'CMC'

Samples of thermized set yoghurt containing various concentrations of 'achi', 'ofor' and 'CMC' were subjected to physico-chemical analysis. The Viscosity, pH, total titratable acidity, the specific gravity and total solids were determined following the AOAC standard method (AOAC, 2010).

2.2.5 Proximate analysis of stabilized samples of thermized set yoghurt using 'achi', 'ofor' and 'CMC'

The fat, ash and protein contents of thermized set yoghurt containing various concentrations of 'achi', 'ofor' and 'CMC' was determined using standard analytical methods described by AOAC (2010) procedures.

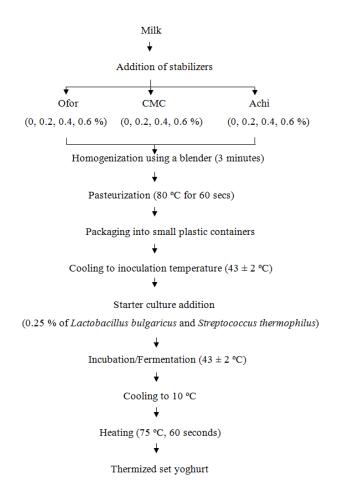


Figure 2: Flow chart for the processing of thermized set yoghurt Alakali *et al.* (2008)

Ash content was determined by incineration of the dried pulps at 550 °C according to the AOAC procedures 942.05. Nitrogen (N) content was micro-Kjeldahl determined using method. according to AOAC procedures 984.13, the protein content was calculated as N x 6.25. Lipid content was determined using Soxhlet apparatus hexane, following **AOAC** 963.15 with methodology. The total percentage carbohydrate content was determined by the difference method as reported by Onyeike et al. (2015). This method involved adding the total values of crude protein, crude fat and ash constituents of the sample and subtracting it from 100. All samples were analyzed in triplicate.

2.2.6Micronutrient analysis of stabilized samples of thermized set yoghurt using 'achi', 'ofor' and 'CMC'

2.2.6.1 Determination of calcium

The calcium content of the formulated yoghurt samples was determined following the methods described in AOAC (2010). Three (3) g of the sample was first digested with 30 ml of aqua regia which is a mixture of concentrated HNO₃ and HCL in the ratio of 1:3. The digested sample was filtered and made up to 50 ml with deionized water. The aliquot of the digested filtrate was used for AAS using filters that match the element. Calibration curve was prepared for the element using a standard solution. A hollow cathode lamp that directs the particular wavelength is chosen the concentration of the mineral in the sample is determined with its corresponding value.

2.2.6.2 Determination of Phosphorus

Phosphorus was determined using UV-Absorption spectrophotometer as described by AOAC (2010). Three (3) g of the sample was weighed into a crucible. The sample was ashed in a muffle furnace at a temperature of 550-600 °C after which 5 ml of distilled water and hydrochloric acid were added to the ash. The solution was heated, filtered, and cooled to room temperature before being neutralized with 50% potassium hydroxide solution. Hydrochloric acid (0.5N) was added and the mixture was diluted to volume with distilled water. Ten (10) ml of this solution was transferred into a 50 ml volumetric flask, 8.0 ml of hydrazine sulphate solution and 2.0 ml of sodium molybdate solution were added. The solution was heated, cooled and transferred into a clean, dry, cuvette. The transmittance was measured at 650 nm using the UV-Spectrophotometer. A reagent blank was also prepared and the phosphorus content of the sample and blank was read from the transmittance graph. The phosphorus content was calculated following the formula:

Phosphorous =
$$\frac{10 (A - B)}{Wv}$$

Where: A= phosphorus content of the sample aliquot in mg, B =phosphorus content of blank aliquot in mg W= weight of sample after addition of hydrazine sulphate and sodium molybdate solution and V= volume of aliquot taken before addition of hydrazine sulphate and sodium molybdate solution

2.2.6.3 Determination of Vitamin B_2 (Riboflavin)

Riboflavin content was determined according to the method of Onwuka (2005). Riboflavin is extracted with dilute acids and then quantified after removing the interfering substance by treatment with KMnO₄. Five grams of the sample was weighed and 50 ml of 0.2N HCl was added and boiled for one hour. This was followed by addition of 1N HCl to lower the pH to 4.5. The solution was filtered into 100 ml volumetric flask and made up to mark with distilled water. 10 ml aliquot was taken from 100 ml volume and had 1 ml of acetic acid added to it, it was mixed and 0.5 ml of 3 % KMnO₄ solution was added. After 2 minutes 0.5 ml of 3 % H₂O₂ was added and the absorbance reading taken at 470 nm. The riboflavin content was calculated as follow:

Ribloflavin =
$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$$
 x $\frac{\text{Concentration of standard}}{\text{Weight used}}$

2.2.7 Microbial analysis of stabilized samples of thermized set yoghurt using 'achi', 'ofo' and 'CMC'

Samples of thermized set yoghurt containing various concentrations of 'achi', 'ofor' and 'CMC' were subjected after 24 hours to microbial analysis.

2.2.7.1 Determination of the total viable count (TVC)

The total viable count test was carried out using the method described by Prescott *et al.* (2005). Using sample and sterilized quarter strength ringer solution as diluents, one milliliter (1 ml) of the water sample was pipetted into a sterile test tube and 9 ml ringer solution pipetted into it and other test tubes arranged for serial dilutions (10⁻³). The diluted sample was pipetted into a marked petri dish and sterile nutrient agar of 20 ml was poured in the same petri dish and swirled to mix and incubated at a temperature of 37 °C for 24 hours. After incubation, the number of colonies was counted and represented as colony forming unit per millilitre (cfu/ml).

 $Cfu/ml = average count \times dilution factor (D. F.)$

2.2.7.2 Determination of the mould count

The mould count test was carried out using the method described by Prescott et al. (2005). Using sample and sterilized quarter strength ringer solution as diluents, one milliliter of the water sample was pipetted into a sterile test tube and nine-millilitre ringer solutions was pipetted into it and other test tubes arranged for serial dilutions (10⁻¹). The diluted sample was pipetted into a marked petri dish and Sabouraud dextrose agar of 20 ml was poured in the same petri dish and swirled to mix and incubated at room temperature for 48 hours. After incubation, the number of colonies were counted

represented as colony forming unit per millilitre (cfu/ ml).

 $Cfu/ml = average count \times dilution factor (D. F)$

2.2.7.3 Determination of the lactic acid bacteria (LAB)

The lactic acid bacteria (LAB) in the formulated yoghurt samples were determined using deMan Rogosa Sharpe (MRS) agar (CM 361) as described by Oxoid manual (Oxoid Ltd., Basingstoke England). Samples were serially diluted in duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37 °C for 48 hours (Prescott *et al.*, 2005). After incubation, the number of colonies were counted and represented as colony forming unit per millilitre (cfu/ml).

Cfu/ ml = average count \times dilution factor (D.F)

2.2.8 Sensory evaluation of formulated samples thermized yoghurt stabilized using 'achi', 'ofor' and 'CMC'

The sensory evaluation was carried out according Iwe (2002) using a 20-man semitrained panelist. Samples were analyzed after 24 hours. The panelists were instructed to indicate their preference of the samples. A nine-point Hedonic scale (where 9 was the highest score and 1 the lowest score) for each characteristic such as colour, flavour, mouth feel, and overall acceptability was determined.

2.2.9 Data analysis and experimental design

Data obtained were subjected to one-way analysis of variance (ANOVA) in a completely randomized design according to the methods of Obi (2001). When means were found to be different, they were separated using Duncan's new multiple range test. Correlation studies were

also performed where necessary to determine the extent of interaction between determinations.

3. Results and Discussion

3.1 Physicochemical composition of thermized set yoghurt stabilized with different stabilizers

Values obtained for specific gravity in the samples ranged from 1.033 (for sample 02) to 1.06 (for sample A4). The result showed (Table 2) that between the stabilizer types and the different concentrations, there were significant difference (p < 0.05) in their effect on specific gravity. It was seen that the specific gravity was increasing with increase in concentrations. The value of specific gravity for sample containing zero stabilizer was 1.04, which was found not to be significantly different (p > 0.05) from samples O2, O4 and C4, respectively. Between the local stabilizers, it was found that achi contributed more to the level of specific gravity than those samples containing ofor on an average basis. The result also showed that sample A4 (1.06) had the highest value of specific gravity among samples stabilized with achi, and this differed for samples stabilized with ofor where the highest specific gravity value was obtained in sample O4 (1.05). The highest value of specific gravity among yoghurt samples stabilized with CMC was obtained in sample C4 (1.06 %).

Table 2 shows the pH values of freshly thermized set yoghurt samples. Values obtained ranged from 3.85 (for sample containing zero stabilizer) to 4.40 (for sample C3). Between the stabilizer types and the different concentrations, there was no significant (p > 0.05) difference in their effect on pH.

Table 2: Physicochemical composition of thermized set yoghurt stabilized with achi, ofor and carboxylmethylcellulose

Sample code	Conc.	Specific Gravity	$^{\mathrm{hd}}$	Viscosity	Titratable	Total Solids
	(%)			(cP)	Acidity (%)	(%)
Achi A1	(0.0)	1.04⁰±0.00	3.85ab±0.07	0.84 ≥ ±0.02	0.85 ° ±0.01	13.68 € ±0.01
A2	(0.2)	1.05bc±0.00	3.80 ≥ ±0.00	1.29 ₺±0.01	0.86 ° ±0.00	$13.84^{f} \pm 0.01$
A3	(0.4)	1.05°d±0.00	3.90 b ±0.00	1.58 ° ±0.03	0.85 ° ±0.01	13.02 ° ±0.04
A4	(0.0)	1.06€±0.00	3.90 ₺±0.00	1.71 € ±0.01	0.84 ° ±0.00	$14.58^{h} \pm 0.021$
Ofor O1	(0.0)	1.04≈±0.00	3.85 ab±0.07	0.84 ≥ ±0.02	0.85 ° ±0.01	13.68 € ±0.01
00	(0.2)	1.03 ≥±0.00	3.90 b ±0.00	$1.64^{d} \pm 0.03$	0.83 ° ±0.01	$12.74^{b} \pm 0.01$
03	(0.4)	1.042±0.00	4.00 ° ±0.00	$1.68^{\text{ de}\pm0.028}$	$0.77^{b} \pm 0.02$	$12.67^{b} \pm 0.03$
94	(0.0)	$1.05^{\rm de}{\pm}0.00$	3.90 b ±0.00	1.77 ± 0.01	0.85 ° ±0.01	11.93 ² ±0.03
CMC C1	(0.0)	1.04≥±0.00	3.85 ab±0.07	0.84 ≥ ±0.02	0.85 ° ±0.01	13.68 € ±0.01
23	(0.2)	1.04≥±0.00	$4.10^{d} \pm 0.00$	1.69 € ±0.01	$0.74^{b} \pm 0.02$	14.34 € ±0.035
ස	(0.4)	1.04⁵±0.00	4.40 € ±0.00	1.72 ° ±0.00	0.69 a ±0.01	$14.53^{\rm h}{\pm}0.02$
2	(0.0)	1.06ef±0.00	4.05 cd±0.07	1.805f±0.01	0.74 b ±0.023	13.39 d ±0.08
Values are me	ean = standard deviat	Values are mean = standard deviation of duplicate readings. Means on the same column with different superscripts are significantly (p < 0.05) different. Key: Conc. = Concentration; CMC=	he same column with different supe	uperscripts are significantly (p	: 0.05) different. Key: Conc. = (Concentration; CMC=

For achi stabilized yoghurt samples, there is no significant (p > 0.05) difference between and samples containing 0.4 0.6 concentrations of achi, while a significant difference existed between samplescontaining 0.2 % of achi and the other concentrations at which achi was utilized. A similar pattern of interaction was seen for CMC and ofor types of stabilizer. A comparison between the local stabilizers showed that achi reduced the level of pH than ofor. This affirms the earlier established

claim that achi stabilized yoghurt samples had a lower moisture content on an average basis than did samples containing ofor owing to its low retrogradation profile (Uzomah & Odusanya, 2011). The lower the level of moisture, the more the pH. This may be attributed to the fact that at a lower moisture content, the lactic acid bacteria had little moisture for their metabolism thus the pH was high than in samples where a higher moisture content was observed. On the other hand, a direct correlation (r = 0.581) could be found between the pH and the level of protein, increasing with increase in pH. Firmer gels were formed at pH > 4.0, a further drop in pH below this level leads to a greater increase in whey separation (Tamime & Robinson, 1999).

The viscosity values (Table 2) obtained for the yoghurt samples ranged from 0.84 cP (for sample containing zero concentration of stabilizers to 1.81 cP (for sample C4). There was no significant difference (p > 0.05) between the effect of the different stabilizers types and different stabilizer concentrations. However, the result shows that an increasing level of concentration, there was an increase in the viscosity. A comparison between the local stabilizers showed that samples stabilized with ofor had a higher viscosity on an average basis compared to samples stabilized with achi. However, CMC stabilized yoghurt samples had the highest viscosity than did samples stabilized with any of the local stabilizers. The result also showed that sample A4 (1.71 cP) had the highest value of viscosity among samples stabilized with achi, and this differed from samples stabilized with ofor where the highest viscosity value was obtained in sample O4 (1.77cP). The highest value of viscosity among yoghurt samples stabilized with CMC was obtained in sample C4 (1.81 cP). Since sample with zero stabilizer had

the least viscosity, it can be concluded that stabilizers were able to increase viscosity in a yoghurt system either by binding water, hydrating the protein or establishing a bond between itself and the protein structures.

Table 2 shows the titratable acidity of freshly thermized set yoghurt samples. Values obtained ranged from 0.69 % (for sample C3) to 0.86 % (for sample A2). Between the stabilizer types there was a significant difference (p < 0.05) in their effect on the level of acidity produced, with CMC stabilized samples having the highest titratable acidity than did samples containing any of the local stabilizers. However, a comparison between the local stabilizers showed achi to contribute significantly (p < 0.05) to an increased level of titratable acidity in the yoghurt system. This may be related to the fact that the least pH was observed for samples stabilized with achi on an average basis, thus showing that a significant negative correlation exits between pH and titratable acidity. Between the concentrations used, result showed that no significant difference (p > 0.05) existed between 0.2 % and 0.6 %, concentrations, but a significant difference (p < 0.05) existed between 0.4 % and the rest of the concentrations. Sample containing zero stabilizer had 0.85 % titratable acidity and which did not differ significantly (p > 0.05) with samples stabilized with achi (A2, A3, and A4 respectively) and ofor (O2 and O4) respectively.

The total solids of freshly thermized set yoghurt samples is represented in Table 2. Values obtained ranged from 11.93 % (for sample O4) to 14. 575 % (for sample A4). Between the stabilizer types there was a significant (p < 0.05) difference in their effect on the level of total solids produced, with CMC stabilized samples having the least total solids than did samples containing any of the local stabilizers. On the

other hand, a comparison between the local stabilizers showed achi to contribute significantly (p < 0.05) to an increased level of total solids in the yoghurt system. The result also showed that sample A4 (14.58 %) had the highest value of total solids among samples stabilized with achi, and this differed from samples stabilized with ofor where the highest total solids value was obtained in sample O2 (12.74 %). The highest value of total solids among yoghurt samples stabilized with CMC was obtained in sample C3 (14.53 %). Interactions between the stabilizers and their concentrations showed that there is a significant difference (p < 0.05) suggesting that the effects caused by concentration were different for different stabilizers. The total solids of sample containing zero stabilizer was 13.60 % and it differed significantly (p < 0.05) from that of all the other samples.

3.2 Proximate composition of thermized set yoghurt stabilized with achi, ofor and carboxylmethylcellulose

Plates 3-6 show thermized set yoghurt samples formulated from different levels of achi (0.2, 0.4 and 0.6 %), ofor (0.2, 0.4 and 0.6 %) and CMC (0.2, 0.4 and 0.6 %). Plate 6 shows themized set voghurt produced without stabilizer. Sample containing no stabilizer had milkish white colouration with no visible separation of whey from the set yoghurt gel. On a similar note, samples containing 0.2 % each of the stabilizer, had a colour apppeal closest to the control. Sample stabilized with ofor at 0.4concentration showed the highest visible whey separation. Addition of either achi and ofor at concentrations beyond 0.2 % gave a milkish white colouration.



Plate 3: Themized set yoghurt samples stabilized with achi



Plate 4: Themized set yoghurt samples stabilized with ofor



Plate 5: Themized set yoghurt samples stabilized with CMC



Plate 6: Themized set yoghurt sample with no stabilizer

Table 3 shows the moisture, ash, fat, fibre, protein and carbohydrate content of thermized set yoghurt. The moisture content was within the range of 84.63 % (sample C3) to 88.10 % (sample O4), and it differed significantly (p < 0.05) between stabilizers and their concentrations (Table 3). Moisture content for sample containing zero concentration of stabilizer was found to be 85.15 %. On an average basis, a significant difference (p < 0.05) exists between the types of stabilizers, in their effect on moisture content. with carboxylymethylcellulose having the lowest mean value (84. 96 %) followed by achi (85.53 %) and ofor (87.01 %). However, no significant (p > 0.05) difference was observed for the different concentration of stabilizers used, with the least effect on moisture recorded at 0.2 % concentration. A comparison in the effect on moisture between the local stabilizer types shows that incorporation of achi at the concentrations used lowered the moisture content better than those containing ofor. This is not in agreement with the report by Ikeagwu et al. (2010), that achi has a lower absorption capacity than ofor.

However, the result affirms the report from Uzomah & Odusanya (2011), that achi has a lower final viscosity (51.25 RVU) than ofor (100.08 RVU), which indicates a lesser retrogradation profile. On the other hand, CMC at the different concentrations used had the least moisture content relative to samples containing either achi or ofor. This may be attributed to the fact that CMC is a modified food starch and its solubilization is found to be in the pH range at which yoghurt gel was set. For achi stabilized yoghurt samples, the least moisture content was obtained in sample A4 (85.02 %). This was different for samples stabilized with ofor, where the least moisture content was obtained in

sample O2 (85.89%). The least moisture content of samples stabilized with CMC was got for sample C3 (84.63 %) and which does not differ significantly (p > 0.05) from the moisture response of sample C4 (84.69 %).

Carbohydrates 5.95cd ±0.06 5.32ªb±0.05 5,93cd ±0.26 6.23d±0.23 5.37ab±0.06 5.69bc ±0.23 6.23d±0.23 5.04a±0.29 $6.23^{d} \pm 0.23$ 5.00ª±0.16 6.30d ±2.25 6.91 €±0.14 Crude Protein 3.89 € ±0.06 3.67cd ±0.11 3.37b ±0.06 3.02 ±0.06 3.89 ≠0.06 4.69h ±0.06 4.418±0.06 3.81 € ±0.06 4.25f±0.06 3.89 ≠0.06 3.89°±0.06 3.5° ±0.11 Fibre (%) 0.10 ±0.00 0.20 ±0.00 0.20 ±0.00 0.10 ±0.00 0.20 ±0.00 0.30 ±0.00 0.20 ±0.00 0.10 ± 0.00 0.10 ±0.00 0.10 ± 0.00 0.10 ±0.00 0.10 ± 0.00 Crude Fat 3.55 ±0.07 3.96d±0.01 4.728±0.03 4.37f±0.28 3.83°±0.04 3.60 ±0.07 3.25 ± ±0.00 4.37f±0.28 4.08° ±0.04 4.88h±0.04 4.758±0.00 $4.37^{f} \pm 0.28$ (%) Ash Content (%) 90.0± ₹2.06 0.43 € ±0.00 0.08 ±0.00 0.34d ±0.00 0.112 ±0.00 0.16 ±0.00 0.51f±0.01 0.49f±0.00 0.49f±0.01 0.27 € ±0.1 0.27 0 ±0.1 Moisture Content (%) 85.15 b ±0.20 84.63 a ±0.04 86.09 d ±0.11 85.02 b ±0.09 85.89 d ±0.11 87.03 ° ±0.09 88.10° ±0.09 85.15 b ±0.20 85.15 b ±0.20 85.48°±0.13 85.56° ±2.09 84.69 a ±0.01 (0.0) (0.0) (0.0) (0.2) 0.4) (0.4) (0.0) CMC C1 Sample Ofor O1 9 3 C3 5 Achi Al 02 03

Table 3: Proximate composition (%) of thermized set yoghurt stabilized with achi, ofor and carboxylmethylcellulose (Wet basis)

superscripts are significantly different (p < 0.05). Keys: Conc. = Concentration; CMC = Carboxylmethylcellul

Values for the ash in produced yoghurt samples shows it to be in the range of 0.08 % (sample C2) to 0.67 % (sample O6) and ash level differed significantly (p < 0.05) between stabilizers and between the concentrations used (Table 3). The ash content for sample containing concentration of stabilizer was found to be 0.27 %. Between the types of stabilizers, there was a significant difference (p < 0.05) in their effect on the level of ash, with achi having the lowest mean value (0.38 %) followed by CMC (0.42 %) and ofor (0.53 %). A comparison between samples stabilized with the local stabilizers shows that ofor contributed more to raising the level of ash in yoghurt than achi does on an average basis. This may be attributed to a significant level of ash in ofor (5.12 %) than in achi (2.58%) as reported by Amah et al. (2017). CMC was intermediate in raising the level of ash on an average basis. For yoghurt samples stabilized with achi, the highest ash content was obtained in sample A4 (0.51 %). This was different for samples stabilized with ofor, where the highest ash content was obtained in sample O3 (0.67 %). The highest ash content of samples stabilized with CMC was obtained in sample C3 (0.34%).

The level of fat in formulated yoghurt samples ranged from 3.25 % (sample O4) to 4.88 % (sample C3). A significant difference (p < 0.05) was seen in the effect of the different types of stabilizers on the level of fat in the formulated yoghurt samples (Table 4). However, no significant difference (p > 0.05) exists between the different concentrations of the stabilizers. The sample with zero concentration of stabilizer was seen to differ significantly from all concentrations of stabilizers. A significant negative correlation (r = -0.739) exists between moisture content and the level of fat, showing

that fat was increasing with decreasing level of moisture and vice versa. The highest level of fat recorded for sample **C**3 which was coincidentally had the lowest moisture content. A comparison between the local stabilizer types showed that samples containing achi had a higher mean value of fat than are those containing ofor, but with samples containing CMC having the highest level of fat. The result indicates that sample A4 (4.72 %) had the highest fat content among samples stabilized with achi, and this differed for samples stabilized with ofor where the highest fat was obtained in sample O2 (3.83 %). The highest fat content among yoghurt samples stabilized with CMC was obtained in sample C3 (4.88 %).

From the result obtained, the level of fibre did not differ significantly between stabilizers and their concentrations. Mean values obtained from each determination were similar (Table 3).

Protein content of thermized set yoghurt samples (Table 3) ranged from 3.02 % (for sample O4) to 4.685 % (for sample C3). The result showed that the protein content differed significantly (p < stabilizer 0.05) between the types. significantly inverse correlation (r = -0.779)exists between the crude protein content and the level of moisture, suggesting that at higher protein level, a firmer gel is obtained and the level of expelled moisture (whey) is reduced. Samples containing achi had a higher mean value of protein on an average basis than those containing ofor, when a comparison between the local stabilizers were made. However, samples containing CMC had the highest protein value on an average basis. The result indicates that sample A4 (4.25 %) had the highest protein content among samples stabilized with achi, and this differed for samples stabilized with ofor where the highest protein was obtained in sample O2 (3.67 %). The highest protein content among yoghurt samples stabilized with CMC was obtained in sample C3 (4.69 %). The level of protein in sample containing no stabilizers was 4.89 % and which does not differ significantly from sample A2. (3.81 %).

Carbohydrate content of formulated yoghurt samples ranged from 5.00 % (for sample O4) % to 6.910 % (for sample A2). The result (Table 3) showed that there was significant difference (p < 0.05) between achi and ofor stabilized samples and between ofor and CMC stabilized samples, but no significant (p > 0.05) difference was found between achi and CMC stabilized samples on an average basis. On the other hand, between the concentrations used, a significant (p < 0.05) difference found between was samples containing 0. 2 % and 0.4 % concentration of stabilizers and between 0.2 % and 0.6 % level of stabilizer, however, no significant (p > 0.05)difference existed between 0.4 % and 0.6 % concentrations. A comparison between the local stabilizers showed that achi stabilized samples had a significantly (p < 0.05) higher level of carbohydrate than those containing ofor, with CMC stabilized samples being the intermediate. Sample containing no stabilizer carbohydrate value of 6.23 % which did not differ significantly (p > 0.05) from samples O2 (5.93 %), C2 (6.30 %) and C4 (5.95 %).

3.3 Micronutrient composition of thermized set yoghurt stabilized with different stabilizers.

Table 4 shows the phosphorous content of freshly thermized set yoghurt samples. Values obtained ranged from 0.09 mg/100g (for sample O4) to 0.13 mg/100g (for sample C3). Between the stabilizer types and the different concentrations, there was no significant difference (p > 0.05) in their effect on

phosphorous. Sample with no stabilizer had a higher phosphorous content than when either of the local stabilizers at the different concentrations used was added. A comparison between the local stabilizers effect on the other hand, did not show a significant difference (p > 0.05). Samples stabilized with CMC contributed more to the level of phosphorous in the yoghurt system than did either of achi or ofor.

The calcium content of freshly thermized set yoghurt samples is represented in Table 4. Values obtained ranged from 23.00 mg/100g (for sample O3) to 42.67 mg/100g (for sample containing zero stabilizer). Between stabilizer types, there was a significant difference (p < 0.05) in their effects on calcium content of the yoghurt samples. However, no significant difference (p > 0.05) existed between the different concentrations and their effect on the level of calcium in the yoghurt system. The highest calcium content was obtained for the sample with zero stabilizer, and which differed significantly from the effect of any of the stabilizer type.

A comparison between the local stabilizers showed that achi significantly (p < 0.05) raised the level of calcium in yoghurt system on an average basis than ofor, and this was found also to be significantly (p < 0.05) higher than the effect of CMC in yoghurt system. The result also showed that sample A2 (37.33 mg/100g) had the highest value of calcium among samples stabilized with achi, and this differed from samples stabilized with ofor where the highest calcium value was obtained in sample O4 (24.00 mg / 100g) but did not differ (p < 0.05) significantly within the stabilizer treatment. The highest value of calcium among yoghurt samples stabilized with CMC was obtained in sample C3 (31.00 mg / 100g).

Table 4: Micronutrient composition of thermized set yoghurt stabilized with achi, ofor and carboxylmethylcellulose (Wet basis)

Sample code	Conc	Phosphorus	Calcium	Vitamin B2
	(%)	(mg/100g)	(mg/100g)	(mg/100g)
Achi A1	(0.0)	$0.12^{d}\pm0.00$	42.67 [£] ±2.83	$0.02^{ab} \pm 0.00$
A2	(0.2)	$0.11^{bc}\pm0.00$	37.33e±0.94	$0.02^{a}\pm0.01$
A3	(0.4)	$0.11^{b}\pm0.00$	$28.00^{\rm cd} {\pm} 0.94$	$0.02^{\rm ab}\!\!\pm\!0.00$
A4	(0.6)	0.12℃±0.00	35.33e±0.00	$0.02^{\mathrm{ab}}\!\!\pm\!0.00$
Ofor O1	(0.0)	$0.12^{d}\pm0.00$	42.67 [£] ±2.83	$0.02^{ab} \pm 0.00$
O2	(0.2)	$0.11^{\rm b}\!\!\pm\!0.00$	$23.00^{a}\pm1.89$	$0.02^{a}\pm0.01$
O3	(0.4)	$0.11^{bc} \pm 0.00$	23.33°±1.89	$0.03^{abc} \pm 0.01$
O4	(0.6)	$0.09^{a}\pm0.00$	$24.00^{ab} {\pm} 0.94$	$0.02^{ab}\!\!\pm\!0.00$
CMC C1	(0.0)	$0.12^{d}\pm0.00$	42.67f±2.83	$0.02^{ab} \pm 0.00$
C2	(0.2)	$0.11^{b}\pm0.00$	$26.00^{abc} \pm 2.83$	0.03abc±0.01
C3	(0.4)	0.12e±0.00	31.00d±0.47	0.04°±0.01
C4	(0.6)	$0.12^{d} \pm 0.00$	27.33 ^{bcd} ±0.00	$0.03^{bc} \pm 0.00$

Values are mean \pm standard deviation of duplicate readings. Means on the same column with different superscripts are significantly different (p < 0.05). **Keys:** Conc. = Concentration; CMC= Carboxylmethylcellulose

Table 4 shows the vitamin B_2 values of freshly thermized set yoghurt samples. Values obtained ranged from 0.02 mg/100g to 0.04 mg/100g. Interaction between the stabilizer and their concentrations shows that there was no significant difference (p > 0.05) in their effect on vitamin B_2 content. Such could be said also for the effect of different concentrations on the level of vitamin B_2 content of formulated yoghurt samples. The vitamin B_2 content of sample with no stabilizer was found not to be significantly different (p > 0.05) from samples containing achi (0.2, 0.4 and 0.6 % concentration), ofor (0.2, 0.4, and 0.6 % concentration) and CMC (0.2 % concentration).

3.4 Microbial count of thermized set yoghurt stabilized with different stabilizers

Table 5 shows that no growth was obtained when the yoghurt samples were tested for lactic acid bacteria survival as well as for mould. Negligible counts (below 30 cfu ml⁻¹) were obtained for the

total viable organisms. This shows that the temperature of thermization was effective in addressing the activity and load of the lactic acid bacteria. This is in agreement with the results of Alakali *et al.* (2009), where thermization temperature of 75°C was effective in addressing the microbial load of the yoghurt system than did temperature of 65°C.

3.5 Sensory scores of thermized set yoghurt stabilized with different stabilizers

The sensory scores (Table 6), showed that the sample without stabilizer was ranked the highest for its colour appeal and which differed significantly (p < 0.05) from the colour score allotted to any of the sample containing stabilizers. Sample stabilized with achi showed that the highest colour appeal was obtained at

Table 5: Performance of stabilizers and its effect on the microbial quality of formulated samples of thermized set yoghurt

Sampl	e code	Conc. (%)	Total viable count	Lactic acid bacteria	Mould count
Achi	A1	(0.0)	LG	NG	NG
	A2	(0.2)	LG	NG	NG
	A3	(0.4)	LG	NG	NG
	A4	(0.6)	LG	NG	NG
Ofor	O1	(0.0)	LG	NG	NG
	02	(0.2)	LG	NG	NG
	O3	(0.4)	LG	NG	NG
	04	(0.6)	LG	NG	NG
CMC	C1	(0.0)	LG	NG	NG
	C2	(0.2)	LG	NG	NG
	С3	(0.4)	LG	NG	NG
	C4	(0.6)	LG	NG	NG

Values are means of duplicate determinations. **Keys:** Conc. = Concentration; CMC = Carboxylmethylcellulose, NG = No growth, LG = low growth (< 30 cfu)

0.2 % concentration. Similarly, such was found to be the case with samples stabilized with ofor. A deviation was seen in sample stabilized with CMC, with 0.6 % concentration of the stabilizer having the highest scoring in terms of colour.

Scoring for aroma of stabilized yoghurt samples (Table 6) showed that sample without any stabilizer was scored highest by the panelist and which differed significantly from all the other samples except for sample containing achi at 0.2 % concentration. The result showed that no significant (p > 0.05) difference exists between the different types of stabilizers and between the different concentrations in their score for the aroma of the yoghurt samples. Sample containing no stabilizer was scored the highest for its taste appeal and which differed from the score allotted to the other samples with the exception of sample A2 containing achi at 0.2 % concentration. Samples stabilized with achi and CMC had no significant difference (p > 0.05) on an average basis, but a significant (p < 0.05) difference exists between them and samples stabilized with ofor.

Table 7 showed that the sample with no stabilizer was scored the highest for its consistency, and which differed significantly (p < 0.05) with all other samples but not with containing sample A2 achi 0.2 at concentration. Samples stabilized with achi and CMC had no significant (p > 0.05) difference in their scores for overall acceptability on an average basis, but a significant difference (p < 0.05) existed in the scores allotted to them and samples stabilized with ofor. Samples stabilized with ofor, showed a significant difference (p < 0.05) between sample O2 and either of sample O3 or O4, but no significant difference exists between samples O3 and O4.

Table 6: Sensory attributes of thermized set yoghurt stabilized with achi, ofor and carboxylmethylcellulose

Sample	Conc.	Colour	Aroma	Taste	Consistency	Mouthfeel	Overall
							acceptability
Achi A1	(0.0)	8.00d±0.65	7.50 ⁴ ±0.76	7.20社1.11	7.05 ^d ±1.23	7.40e±1.10	7.45d±0.95
A2	(0.2)	6.90€±0.97	6.950 ₹1.00	6.50°d±1.76	6.30cd±1.34	6.60de±1.31	6.85°d±1.18
A3	(0.4)	4.50a±1.70	5.35ab±1.98	4.55ab±1.90	3.55≈±1.82	3.70 €±2.03	4.25⁴±1.89
A4	(0.0)	5.65⁵±1.76	5.65ab±1.73	5.45bc±1.76	5.50bc±1.47	5.00bc±1.84	5.45b±1.36
Ofor O1	(0.0)	8.004±0.65	7.50d±0.76	7.20 ⁴ ±1.11	7.054±1.23	7.40€±1.10	7.45 ^d ±0.945
05	(0.2)	5.95bc±1.54	6.20b ±1.51	5.50bc±1.73	5.00b±1.89	5.60억±1.67	5.60b±1.54
03	(0.4)	4.15a±2.18	5.05≈±1.82	3.55a±1.64	2.80 ±1.79	3.304±2.08	3.30a±1.66
04	(0.0)	4.40*±1.93	5.35ab±1.76	4.50ab±1.47	3.45°±1.47	4.05ab±1.88	4.056±1.50
CMC C1	(0.0)	8.00d±0.65	7.5040.76	7.20⁴±1.11	7.05年1.23	7.40€±1.10	7.45 ⁴ ±0.95
C	(0.2)	5.75b±1.83	5.95abc±1.39	5.35bc±2.01	4.90⁵±1.86	5.15bc±2.06	5.40b±1.73
ຣ	(0.4)	6.00bc±1.38	5.95abc±1.73	5.45bc±2.01	5.70bc±2.05	5.3542.06	5.60b±2.04
2	(0.0)	6.55bc±1.54	6.30bc±1.53	5.55bc±2.04	6.15cd±1.63	5.90≈41.86	6.05bc±1.70

Sensory scores for the overall acceptability (Table 6) of the formulated yoghurt sample shows that, sample containing no stabilizer was scored the highest for overall acceptability, and which differed significantly from the scores allotted to other samples with the exception of

the sample stabilized with 0.2 % concentration of achi. Samples stabilized with achi and CMC had no significant (p > 0.05) difference in their scores for overall acceptability on an average basis, but a significant (p < 0.05) difference existed in the scores allotted to them and samples stabilized with ofor.

4. Conclusion

This study showed that thermized set yoghurt containing different stabilizers at different concentrations were produced. Thermized set yoghurt stabilized with local stabilizers had a pronounced effect on the physicochemical parameters when compared to sample with no stabilizer. The local stabilizers raised the level of ash, specific gravity, viscosity, and total solids in thermized set yoghurt. Between these local stabilizers, however, achi conferred a better stabilization effect in thermized set yoghurt than ofor. Samples stabilized with CMC had lower moisture content, higher crude fat content, protein content, pH, total solids and phosphorous than with samples stabilized with the local stabilizers. Sensory scores of thermized set yoghurt samples showed that the sample containing no stabilizer had the highest score for all the parameters evaluated but which did not differ significantly (p > 0.05) from the score assigned to sample A2. From the microbial count of the yoghurt samples revealed that no growth was obtained for the lactic acid bacteria and mould enumeration while negligible counts (< 30 cfu/ml) were obtained for all the total viable organisms.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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