Effects of Local Stabilizers (Achi, Ofor, And Cocoyam) on the Physicochemical, Sensory and Microbiological Properties of Yoghurt

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KEYWORDS

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ABSTRACT

This study was conducted to evaluate the use of local soup thickeners known as plant-based stabilizer to produce yoghurt and determine their effects on the physicochemical and microbiological properties of yoghurt. Reconstituted powdered cow milk was homogenized, pasteurized at 95°C for 5 min, cooled to 45˚C and inoculated with a direct-vat-set (DVS) yoghurt starter culture of Streptococcus thermophilus and Lactobacillus bulgaricus for 12 h and cooled at 5˚C. Achi and Ofor seeds were sorted, cleaned, soaked (6 h), toasted, dehulled, dehydrated, milled and sieved to obtain their respective flours. Cocoyam were sorted, peeled, washed, sliced, dehydrated, milled, and sieved to obtain cocoyam flour. These flours were incorporated at 10% into yoghurt samples as stabilizers. The yoghurt samples were subjected to proximate, functional and microbial, and organoleptic evaluation. Proximate analysis revealed significant differences (p <0.05) in carbohydrate, moisture, protein, fat, and ash content among different yogurt samples. Functional properties assessment indicated that the yogurt containing 100% Cocoyam flour displayed the lowest water release. Microbiological evaluation recorded varying total viable bacterial counts (TVC) across samples, with the lowest count observed in yogurt containing 0.33% Cocoyam and 0.67% Ofor. Notably, coliforms were absent in all samples. Overall, the findings suggested the potential of local plant-based soup thickeners as effective stabilizers in yogurt production, enhancing its physicochemical and functional attributes. The study highlights the feasibility of incorporating these indigenous hydrocolloids into yogurt formulation for improved product quality and possibly broader market appeal.

INTRODUCTION

Yoghurt is one of the most popular fermented dairy products, which is semi-solid and most popular of all fermented milk products in Nigeria (Shiby and Mishra, 2013). Yoghurt is made by the fermentation of milk using a starter culture of a particular strain of *Lactobacillus* or a mixed culture of *Lactobacillus delbreickii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Sansawal *et al.*, 2017). It can be manufactured using fresh animal milk or using reconstituted powdered milk. In recent times, powdered cow milk and vegetable milk such as soymilk are a being used a major base material to produce quality yoghurt (Obiora *et al.*, 2020). Lactic acid and the other compounds formed during the fermentation of milk makes yogurt a food product that is acidic and creamy, appreciated for its taste and nutritional qualities, notably for its calcium content (Widayat *et al.*, 2020). Yoghurt is classified primarily according to its chemical composition (full-fat, reduced-fat, and low-fat), manufacturing method (set and stirred yogurt), flavour type, and post incubation process.

Stabilizers are essential in dairy products to increase the viscosity, and inhibit the formation of large crystals. Stabilizers include agar, cornstarch, gelatin, and pectin. Cellulose compounds such as methylcellulose and CMC (sodium carboxyl methyl cellulose) are also used (Bakirci and Macit, 2017). Achi and Ofor seed flours serve as traditional emulsifier and thickening agent. Their seed kernels are added to all kinds of soup such as *egusi*, *oha, onugbu* soups and local beer in Nigeria. Their leaves and flowers are used as condiments or vegetables for cooking (Kouyate, 2011).

Their utilization can potentially reduce the reliance on imported synthetic hydrocolloids (carboxy methyl cellulose, guar gum, etc.) that has no nutritional value, thereby boosting Nigeria's import dependence and ease the pressure on the Nigerian currency. This study also encourages the use of underutilized locally sourced raw materials for commercial production which are readily available, serve as a novel route to increase the nutritive and functional properties of yoghurt. The use of local stabilizers (*achi*, *ofor*, and cocoyam) may also increase the spectrum of purchase of yoghurt to low income and poor families and reduce the overdependence on imported synthetic hydrocolloids (carboxy methyl cellulose, guar gum, etc.) that has no nutritional value as well as boosting the Nigeria's gross domestic product.

MATERIALS AND METHODS

Sources of Raw material

The raw materials used in this study include powdered cow milk (Full Cream Dano milk), *Achi* seed, *Ofor* seed, and Cocoyam tubers (*xanthosomas agittifolium*). The materials were purchased from Eke-Awka market, Anambra State. The raw materials *(Achi* seeds, *Ofor* seeds, and co

coyam tubers) were transported to the lab where they were processed into flours, incorporated into yoghurt and the samples were analyzed for proximate and sensory properties in the laboratory. Reagents and equipment used for the analysis were obtained from laboratory of the Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Experimental Design

A simple centroid mixture design approach using JMP Pro software version 23 was employed in this study. Eight samples of local stabilizers and their combinations were were added to yoghurt samples as shown in Table 1.

Table 1: Experimental Design

S/N	Sample code	Cocoyam	*Achi*	*Ofor*
1	CO1	0.33	0	0.67
2	CY2	1	0	0
3	CA3	0.67	0.33	0
4	AC4	0	1	0
5	CH5	0.33	0.67	0
6	AO6	0	0.33	0.67
7	CF7	0.67	0	0.33
8	OF8	0	0	1
9	STD	0	0	0

Proximate Analyses

Reconstituted powdered cow milk (400g to 2 liters of distilled water) was homogenized using a homogenizer for 3 minutes, pasteurized at a temperature of 95˚C for 5 minutes, cooled to 45˚C, inoculated with 2% starter culture (yogourmet), fermented at a temperature of 45˚C for 8 hours and cooled to 5˚C (Lee, 2006; Bristone *et al.,* 2016). The processed stabilizers (*Achi, Ofor* and cocoyam) and their combinations were incorporated into the yogurt as shown in Table 1. Each of the samples was analyzed for carbohydrates, crude protein, crude fibre, fat, moisture and ash using standard methods described by AOAC (2023).

Functional properties Evaluation

The viscosity of the sample was determined using the method outlined by Onwuka (2005), involving washing the viscometer tube with acetone and then suspending it to fill with exactly 20 milliliters of the sample. The sample was allowed to run down to the primary/calibration line. Stop watch was used to ascertain the reading.

The water absorption capacity of the yoghurt sample was determined using a method described by Iwe, (2003). A 10 ml of yoghurt sample was centrifuged for 30 minutes at 3000 rpm. The quantity of whey expelled after centrifugation was expressed as millimeters of water bound in yoghurt sample.

The syneresis of yoghurt sample was measured using drainage method described by Tamime *et al.* (1995) with minor modification. The method was based on spontaneous movement of whey out of the gel under the force of gravity. 25 gram of yoghurt was poured into a funnel with filter paper placed on a 50 ml of volumetric flask and allowed to stand for 6 hours. The amount of drained off whey from the yoghurt was measured.

Microbiological Evaluation

Ten millimeter (10 ml) of each sample of the yoghurt samples was put in 9 ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1 ml was inoculated separately on to MacConkey agar, Nutrient agar and Potato Dextrose agar plates and spread evenly using sterile bent glass rod. The inoculated MacConkey agar, Nutrient agar and Potato Dextrose agar plates were incubated at 30 °C and 35 °C for 24 and 48 hours respectively. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per mil, cfu/ml (Cheesebrough, 2006).

Sensory Evaluation

Yoghurt samples were subjected to sensory evaluation using 25 semi-trained panelists. The panelists evaluated the samples using a questionnaire provided and the points based on; colour, taste, aroma, flavour and overall acceptability using a 9-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like or dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like very extremely) as described by Ihekoronye and Ngoddy (1985).

Statistical analysis

The data was analyzed using Statistical Package for Social Sciences version 23.0. All data were represented as mean of three replicates. The mean, range and standard deviation of each parameter was determined. Duncan Multiple Range Test was employed for separation of means.

RESULTS AND DISCUSSION

Proximate analyses of the yoghurt samples

Table 2 showed the proximate compositions of nine yoghurt samples. Sample CY2 (100% cocoyam) had the highest carbohydrate content while sample AC4 (100% *achi*) had the least value for carbohydrate. Wursburg (2015) mentioned that carbohydrates are good sources of energy and its high concentration contributes to the texture, viscosity, and moisture retention of yoghurt. Sample CY2 (100% cocoyam) had the highest carbohydrate content while sample AO6 (0.33% cocoyam and 0.67% *achi*) recorded the lowest value for carbohydrate content. This suggests that cocoyam has higher carbohydrate content than achi, and that increasing the proportion of cocoyam in the yoghurt increases its carbohydrate content. Sample AC4 (*achi* 100%) had the highest moisture content while sample OF8 (100% *ofor*) had the least moisture content among the yoghurt samples. Sample OF8 (100% *Ofor*) had the highest protein content while sample AC4 (100% *achi*) had the least protein content. This suggests that ofor had higher protein content than achi, and that increasing the proportion of ofor in the yoghurt increases its protein content. Sample AO6 (0.33% *achi* and 0.67% *ofor*) had the highest fat content while sample CY2 (100% cocoyam) had the least fat content. This suggests that ofor has a higher fat content than cocoyam, and that increasing the proportion of ofor in the yoghurt increases its fat content. Sample AO6 (0.33% *achi* and 0.67% *ofor*) and AC4 (100% *achi)* had highest ash content while sample CY2 (100% cocoyam) had the least ash content. This suggests that achi and ofor have higher ash content than cocoyam, and that increasing the proportion of achi or ofor in the yoghurt increases its ash content.

Table 2: Proximate composition of Nine Samples of Composite Yogurt

Sample Code	Carbohydrate (%)	Moisture (%)	Crude Protein (%)	Fat (%)	Ash (%)
CO1	8.79^{d}±0.50	82.66^{d}±0.57	5.05^{b}±0.01	2.23^{c}±0.05	1.42^{b}±0.01
CY2	13.34^{a}±0.61	84.19^{cd}±0.57	1.04^{e}±0.04	1.12^{d}±0.06	0.31^{f}±0.00
CA3	12.89^{b}0.59	84.29^{cd}±0.57	0.98^{f}±0.01	1.39^{d}±0.90	0.45^{e}±0.01
AC4	7.06^{e}±0.55	88.02^{a}±0.00	2.05^{d}±0.05	2.39^{c}±0.01	0.48^{e}±0.01
CH5	7.24^{e}±0.63	87.49^{b}±0.57	2.44^{d}±0.02	2.33^{c}±0.05	0.50^{d}±0.02
AO6	5.32^{g}±0.50	85.98^{c}±0.57	5.26^{b}±0.01	2.56^{b}±0.05	0.88^{c}±0.01
CF7	10.99^{c}±0.04	83.23^{cd}±0.57	3.63^{c}±0.03	1.72^{d}±0.06	0.43^{e}±0.00
OF8	6.09^{f}±0.67	82.47^{cd}±0.77	5.98^{b}±0.01	3.70^{a}±0.10	1.76^{a}±0.00
STD	9.06^{c}±0.22	81.30^{cd}±0.09	6.21^{a}±0.15	3.20^{a}±0.04	0.23^{g}±0.01

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$).CO1= Cocoyam 33% and *Ofor* 67%, CY2 = Cocoyam 100%, CA3 = Cocoyam 67% and *Achi* 33%, AC4 = *Achi* 100%, CH5 = Cocoyam 33% and *Achi* 67%, AO6 = *Achi* 33% and *Ofor* 67%, CF7 = Cocoyam 67% and *Ofor* 33%, OF8 = *Ofor* 100%, STD= 100% yoghurt

Sensory quality of the yoghurt samples

Table 3 showed the sensory scores of nine yoghurt samples. According to Fellows (2007), the major quality factors for yogurt acceptability include the sensory properties such as the appearance, texture and mouth feel. The mean value of the colour ranged from 4.04 -8.56 relating to "dislike slightly" to "like very m" on the 9-point hedonic scale. The taste ranged from 5.08-7.48 relating to "neither like nor dislike" to "like moderately" on the 9-point hedonic scale. The aroma ranged from 6.76-7.48 relating to "like slightly" to "like moderately" on the 9-point hedonic scale. The texture ranged from 3.96-8.52 relating to "dislike moderately" to "like very much". The mean value for the overall acceptability ranged from 4.24-7.52 relating to "dislike slightly" to "like moderately" on the 9-point hedonic scale. Overall acceptability refers to the general acceptance of the product with reference to all the discriminating sensory attributes of the sample including colour, taste, texture, moldability and flavour (Ogundele *et al*., 2015); it is also an important parameter in organoleptic estimation as it plays a crucial role in decision making and it influences the panelists acceptance and choices of the different samples. Yoghurt made from 100% cocoyam flour (AC4) had the best sensory properties.

Table 3: Sensory Analysis Results of Nine Samples of Composite Yoghurt.

Sample code	Colour	Taste	Aroma	Texture	Overall Acceptability
CO1	7.90^{a}±1.15	7.32^{a}±1.28	7.32^{ab}±1.43	7.20^{b}±1.32	7.52^{a}±0.65
CY2	7.92^{ab}±0.96	7.48^{a}±1.15	6.96^{c}±1.20	8.52^{a}±1.19	7.28^{a}±0.61
CA3	7.08^{a}±1.41	5.08^{c}±1.44	5.16^{d}±1.21	6.36^{c}±1.35	6.36^{c}±0.90
AC4	4.04^{b}±1.36	7.68^{a}±1.14	6.88^{c}±1.30	3.96^{d}±1.39	5.68^{d}±0.85
CH5	6.36^{ab}±1.22	6.92^{b}±1.49	6.76^{c}±1.20	5.24^{a}±1.16	4.24^{e}±0.92
AO6	5.08^{b}±1.38	4.92^{a}±1.28	6.84^{c}±1.10	7.00^{b}±1.25	7.16^{b}±0.80
CF7	7.36^{ab}±1.18	6.88^{b}±0.97	7.04^{b}±1.09	7.16^{b}±1.31	7.28^{a}±0.61
OF8	7.92^{a}±1.18	7.28^{a}±1.54	7.16^{b}±1.49	6.44^{c}±1.26	6.20^{c}±0.95
STD	8.56^{a} ±1.10	6.80^{b}±1.18	7.42^{a}±0.05	6.30^{c}±1.10	7. 30^{a}±1.15

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$).CO1= Cocoyam 33% and *Ofor* 67%, CY2 = Cocoyam 100%, CA3 = Cocoyam 67% and *Achi* 33%, AC4 = *Achi* 100%, CH5 = Cocoyam 33% and *Achi* 67%, AO6 = *Achi* 33% and *Ofor* 67%, CF7 = Cocoyam 67% and *Ofor* 33%, OF8 = *Ofor* 100%, STD = 100% yoghurt.

Functional properties of the yogurt samples

Table 4 showed the results of functional analysis of five yoghurt samples. Five best samples of yoghurt were selected after sensory analysis and subjected to functional and microbial evaluation. The mean values for syneresis ranged from 26.50-54.16% with sample CO1 (0.33% cocoyam, 0.67% *ofor*) having the highest value while sample CY2 (100% cocoyam) had the least value. The water holding capacity ranged from 525 – 901 g/kg. Sample OF8 (100% *ofor*) recorded the least value while sample CA3 (0.67% cocoyam and 0.33% *achi)* recorded the least value. This means that cocoyam and achi are effective stabilizers for reducing the whey separation and increasing the moisture retention of yoghurt, but they also make it more fluid and less thick. The viscosity of the samples ranged from 33.32 – 62.85 mm^2/S. Sample OF8 (100% *ofor*) had the highest value while sample CA3 (0.67% cocoyam and 0.33% *achi*) recorded the least value. This means that *ofor* is a very effective stabilizer for increasing the thickness and firmness of yoghurt, but it also causes more whey separation and shrinkage than *achi* and cocoyam.

Table 4: Functional properties of Six Samples of Composite Yoghurt

Sample code	% Formulation of Cocoyam, *Achi* and *Ofor*	Syneresis (%)	Water Holding Capacity (g/kg)	Viscosity (mm^2/S)
CO1	0.33:0:67	$54.16^a \pm 0.15$	$579.33^d \pm 1.15$	$50.10^b \pm 0.79$
CY2	1:0:0	$26.50^f \pm 0.10$	$701.00^c \pm 1.00$	$39.07^c \pm 2.16$
CA3	0.67:0.33:0	$52.13^b \pm 0.15$	$525.66^{de} \pm 0.57$	$33.32^d \pm 1.96$
CF7	0.67:0:0.33	$50.00^c \pm 0.00$	$588.33^d \pm 0.57$	$41.82^c \pm 1.97$
OF8	0:0:1	$48.73^d \pm 0.05$	$901.33^a \pm 1.52$	$62.85^a \pm 2.91$
STD	0:0:0	$25^f.02^e \pm 0.27$	$500^d.01 \pm 0.15$	$37.02^c \pm 0.85$

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$).CO1= Cocoyam 33% and *Ofor* 67%, CY2 = Cocoyam 100%, CA3 = Cocoyam 67% and *Achi* 33%, AC4 = *Achi* 100%, CH5 = Cocoyam 33% and *Achi* 67%, AO6 = *Achi* 33% and *Ofor* 67%, CF7 = Cocoyam 67% and *Ofor* 33%, OF8 = *Ofor* 100%, STD = 100% yoghurt.

Microbiological quality of the yoghurt samples

Table 4 showed the results of microbiological analysis of six yoghurt samples selected after sensory evaluation. The total viable bacterial count (TVC) ranged from 1.90 x 10^7- 2.93 x 10^7 CFU/ml with sample CA3 (0.67% and 0.33% achi) having the highest value while sample CO1 (0.33% cocoyam and 0.67% *ofor*) had the least value. The total yeast and mold counts ranged from 0.50×10^7 - 1.80×10^7CFU/ml. The lowest amount was found in sample CO1 (0.33% cocoyam and 0.67% *ofor*) and the highest amount was found in sampleCF7 (0.67% cocoyam and 0.33*ofor*). There were no coliforms identified in the analysed samples. The absence of coliform could be due to the significant level of sanitary measures implored by the various processing procedures.

Table 4: Results of Microbiological Analysis of Six Yoghurt Samples

Sample code	**% Formulation**	**Total Viable Bacteria count (cfu/ml)**	**Yeast and Mold (cfu/ml)**	**Total Coliform Count**
CO1	0.33:0:67	$1.90^d \times 10^7 \pm 0.07$	$0.50^c \times 10^7 \pm 0.10$	Nil
CY2	1:0:0	$2.30^c \times 10^7 \pm 0.0.70$	$1.36^{ab} \times 10^7 \pm 0.98$	Nil
CA3	0.67:0.33:0	$2.93^{ab} \times 10^7 \pm 0.90$	$1.70^a \times 10^7 \pm 0.20$	Nil
CF7	0.67:0:0.33	$2.60^{bc} \times 10^7 \pm 0.57$	$1.80^a \times 10^7 \pm 0.63$	Nil
OF8	0:0:1	$2.33^{bc} \times 10^7 \pm 1.15$	$1.03^{ab} \times 10^7 \pm 0.25$	Nil
STD	0	$2.90^{ab} \times 10^7 \pm 0.01$	$1.35 \times 10^7 \pm 0.03$	Nil

Values are means ± standard deviation. Means with the same superscript in the same column are not significantly different ($p \leq 0.05$).CO1= Cocoyam 33% and *Ofor* 67%, CY2 = Cocoyam 100%, CA3 = Cocoyam 67% and *Achi* 33%, AC4 = *Achi* 100%, CH5 = Cocoyam 33% and *Achi* 67%, AO6 = *Achi* 33% and *Ofor* 67%, CF7 = Cocoyam 67% and *Ofor* 33%, OF8 = *Ofor* 100%, STD = 100% yoghurt.

CONCLUSION

This study has shown that locally derived plant-based hydrocolloids commonly used as native soup thickeners could also be used in the formulation of generally acceptable yoghurt. There were significant increase proximate and functional properties of yoghurt samples when compared with the standard yoghurt. The yoghurt samples were considered safe for consumption since the highest total viable bacterial count were within the acceptable range. Considering the economic situation of Nigeria, adoption of locally-derived plant-based stabilizers will encourage consumption of more nutritious food and healthy lifestyle among the populace. However, further analysis such as the anti-nutritional and phytochemical qualities may be carried out on the yoghurt.

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