Available Online at ESci Journals
Journal of Food Chemistry and Nutrition
ISSN: 2307-4124 (Online), 2308-7943 (Print)
http://www.escijournals.net/JFCN

PRODUCTION AND EVALUATION OF IMITATION YOGURT AND ICE CREAM USING *LAGENARIA SICERARIA* PROTEIN ISOLATES

[a]Eunice M. Ogunbusola, [b]Tayo N. Fagbemi, [b]Oluwatooyin F. Osundahunsi
[a] *Department of Food Science and Technology, Federal University Oye- Ekiti, Nigeria.*
[b] *Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria.*

A B S T R A C T

Protein isolates were prepared from two varieties of *Lagenaria siceraria* (Calabash and Bottle gourd) seeds using alkali solubilization and acid precipitation method. Yogurt and ice cream were prepared using the protein isolates to substitute for the milk solid nonfat. Physicochemical properties and sensory evaluation were carried out on the formulated food samples. The pH, titratable acidity and total solid of the yogurt samples produced ranged from 4.02 to 4.52, 0.68 to 0.72% and 8.77 to 12.02% respectively while the pH overrun and specific gravity of the ice cream mixes produced ranged from 5.81 to 6.20, 42.68 to 48.61% and 0.79 to 0.90 respectively. Yogurt and ice cream substituted with *Lagenaria siceraria* protein isolates up to 25% level of substitution showed no significant difference from the control in all the sensory parameters examined. *Lagenaria siceraria* protein isolates may be found useful in ice cream as stabilizer and protein supplement.

Keywords: Protein isolates, *Lagenaria siceraria*, Yogurt, Ice cream, physicochemical properties, sensory evaluation. LS1 - Calabash gourd seed, LS2 -Bottle gourd seed.

INTRODUCTION

Vegetable proteins are widely used as replacement for animal proteins. Food scientists have investigated the complete or partial replacement of milk solid nonfat with plant proteins due to its nutritional and economic values. Consumer interest in the relationship between diet and health increases the demand for information on functional foods. Yogurt and ice cream have become one of the most acceptable desserts in Nigerian market. Many non-milk materials particularly plant proteins have been researched to partially replace mix ingredient in ice cream production, these include soy flour (Hammad *et al*., 1985), soymilk (Saleem *et al.*, 1989), soybean, flaxseed, sesame and almond concentrates (Salama *et al*., 2007).

The application of plant protein isolates in the food trade as both ingredients and nutritional supplement are limited to proteins from soybeans, whereas other vegetable proteins are less used. Among these are those from the Cucurbitaceae such as *Lagenaria siceraria* which form part of agricultural crops in south western Nigeria. *Lagenaria siceraria* belongs to the gourd family. The seeds are widely used in soups as thickener and the outer calabash as containers or bowls for storing water, palm wine and grains. There is dearth of information on the utilization of its protein isolates, this work is thus aimed at production and evaluation of yogurt and ice cream using *Lagenaria siceraria* protein isolates.

* Corresponding Author:
Email ID: riikebusola@yahoo.com

MATERIALS AND METHODS

Collection and preparation of samples: Two varieties of *Lagenaria siceraria* (calabash gourd seed LS_1 and bottle gourd seed LS_2) were bought from Anibaba farm in Irele Ekiti, Ekiti State, Nigeria. The seeds were manually shelled, washed and later dried in a hot air oven at 50^0C. The seeds were pulverized using a Brabender blender, defatted continuously for 8 hours using n-hexane solvent. Defatted meals were dried, pulverised and sieved to pass through a 300µm sieve.

Preparation of Protein Isolates: Defatted seed flours were added to distilled water at a meal to solvent ratio of 1:20 w/v. The mixture was stirred with a magnetic stirrer for 10min and the pH of the slurry was adjusted to 9.0 using 0.1M HCl or 0.1M NaOH dropwise. The

extraction was allowed to continue for 2hours with constant stirring while the pH is kept constant. The slurry was centrifuged at 4000 x g for 20min. The residues were re-extracted with the same solvent under similar conditions. The supernatants were combined and proteins precipitated by adjusting the pH to 4.0 (pH of minimum solubility) with 0.1M HCl, followed by separation by centrifugation at 4000 x g for 20 min. The residue (proteins) was dispersed in distilled water, poured in dialysis tubing (Brooklyn, N.Y.11311, USA), and dialyzed against distilled water for 18 hours at room temperature. The dialysing water was replaced at intervals of 3hours during the period of dialysis. The dialysate was freeze dehydrated using freeze dryer and later stored in an air-tight container in the deep freezer for future analysis.

Preparation of Yogurt: Dano slim powdered milk (0% fat) Suspension was prepared in hot water at 90^0C. The milk was substituted with *Lagenaria siceraria* protein isolates at the levels of 25, 50 and 75%. The suspension was cooled to 45^0C and old yogurt from Fan Milk Company (Fan Yogo) was added to introduce the starter culture. The mixture was incubated at 45^0C for about 12 hours and later cooled in the refrigerator for about 24 hours.

Analysis of Yogurt: The Total Solid of the yogurt samples was determined using the method of AOAC (2005), pH was measured using Bench top pH meter (model pH-016A) (Ruck, 1969) and titratable acidity was determined using the method of Ruck (1969), sensory evaluation was carried out on the yogurt using panel of judges selected from their consistency in scoring and the samples were evaluated for taste, colour, aroma and overall acceptability using nine point hedonic scale from like extremely to dislike extremely (Larmond, 1985).

Preparation of Ice cream: Ice milk base mixes were prepared to contain 16% fat, 16% sugar, with or without 0.5% gelatin and egg white, and 12% milk solid nonfat (msnf). The milk solid nonfat was substituted with *Lagenaria siceraria* protein isolates at the levels of 25, 50, and 75%. The mixes were homogenized, pasteurized at 90^0C and cooled rapidly to 5^0C, aged for about 12 hours and frozen in vertical batch freezer (Armfield model ATE-6073) and hardened for about 24 hours.

Analysis of Ice cream mixes: The pH, specific gravity (at 20^0C) and overrun of the ice cream mixes were determined (Ruck, 1969). The sensory evaluation of ice cream was carried out and sensory parameters examined were taste, colour, aroma and mouthfeel.

Statistical Analysis: All determinations were carried out in triplicates; errors were recorded as standard deviation from the mean. Data were subjected to analysis of variance using SPSS 15 computer programme and means separated using New Multiple Range Test. Significance was accepted at 5% level of probability.

Experimental design: Recipes of yogurt and ice cream were allocated to taste panelists using a factorial design. Responses were scaled using 9-point hedonic scale from like extremely to dislike extremely.

X_{e1}	X_{e2}	X_{e3}	X_c
treatment	**Treatment**	**Treatment**	**control**

RESULTS AND DISCUSSION

Physicochemical Properties of Yoghurt: Table 1 shows some physicochemical properties of yogurt prepared from protein isolates extracted from *Lagenaria siceraria* seed flours. The pH of the yogurt samples varied significantly ($P \leq 0.05$). The pH of the yogurt ranged from 4.02 to 4.60. This range in pH compared with the range of 4.55 to 4.62 reported for low fat probiotic yogurt (Mazloomi *et al*., 2011). Addition of protein isolates generally reduced the pH of resultant yogurt. This may be due to increased protein content in the yogurt resulting into increased proteolysis by the proteolytic bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) thereby causing production of more lactic acid compared to the control.

The titratable acidity of *Lagenaria siceraria* protein isolate produced yogurt and the control ranged from 0.68 to 0.72 (% lactic acid). This range of values compared fairly with 0.68 – 0.77% titratable acidity reported for inulin added low fat probiotic yogurt (Mazloomi *et al*., 2011). Trachoo (2002) reported that the titratable acidity of yogurt should not be less than 0.5% lactic acid. The percentage total solid of the yogurt samples ranged from 8.96 to 12.02%. Harwalkar and Kalab (1986) reported that non fat yogurt is normally low in total solid and consequently suffers from whey separation or syneresis. Slocum *et al*. (1988) reported the range of 10.0 to 17.5% total solid in yogurt. The total solid of 100% milk yoghurt (12.02%) is significantly higher ($P \leq 0.05$) than protein isolate substituted yoghurt

(8.96 – 9.98%).

Sensory evaluation of yogurt: The sensory evaluation of yogurt produced from *Lagenaria siceraria* protein isolates is depicted in Tables 2 and 3. All the yogurt produced with *Lagenaria siceraria* variety 1 (LS_1) protein isolates are inferior to the control in all the sensory parameters evaluated (Table 2). However yogurt with LS_1 protein isolates at 25% level of substitution was rated significantly superior in taste to other higher levels of substitution. Yogurt produced with 25% LS_2 protein isolates show no significant difference in taste from the control (Table 3), however it showed noticeable difference in colour, aroma and overall acceptability. Yogurt samples produced with LS_2 protein isolates at higher levels of substitution were inferior to the control in all the sensory parameters. LS_2 (Bottle gourd seed) protein isolate may be useful in enhancing the protein content of yogurt up to 25% level of substitution.

Physicochemical Properties of Ice Cream: Table 4 shows the effect of adding *Lagenaria siceraria* protein isolates on the characteristics of resultant ice milk mixes. The pH of ice cream samples ranges from 5.81 to 6.20. The range of pH values of the ice cream compared favourably with the range of values from 5.8 to 6.1 reported for ice milk mixes produced from some oil seed protein concentrate substituted at various levels (Salama *et al*., 2007). The pH varied significantly with different levels of substitution in *Lagenaria siceraria* protein isolates substituted ice cream. Similar observation was reported in protein concentrate substituted ice milk mixes (Salama *et al*., 2007). The overrun of the ice cream samples ranged from 48.61 to 42.68%.

The overrun of 100% milk ice cream (control) is significantly higher than all the protein isolates substituted ice cream. The overrun values of the ice cream samples generally decreases as the protein isolate level increases ranging from 46.52 to 42.68% in LS_1 and 45.11 to 43.21% in LS_2, from 25 to 75% level of substitution respectively. Thomson *et al*. (1983) reported the range of values of overrun from 38.0 to 46.9% in ice cream prepared from various levels of whey concentrate or succinylated whey concentrate with or without stabilizers. The specific gravity of the ice cream increases with addition of protein. The decrease in overrun and increase in specific gravity of ice cream by increasing substitution levels of protein isolates may be attributed to increment of the mix's viscosity which affects the whipping rate of the mixes (Arbuckle, 1977). Similar observation was reported by El-Samahy *et al*. (2009) for ice cream substituted with concentrated cactus pear pulp. Salama *et al*. (2007) also reported increased specific gravity of ice cream mixes with increased level of substitution with some oil seed protein concentrate.

Table 1: Physicochemical Properties of Yogurt samples.

Yogurt Sample	pH	Titratable Acidity (%)	Total Solid (%)
Control (100% Milk)	4.52 ± 0.05^{ab}	0.71 ± 0.01^{ab}	12.02 ± 0.02^{a}
25% LS_1	4.60 ± 0.10^{a}	0.68 ± 0.01^{c}	8.96 ± 0.01^{e}
50% LS_1	4.24 ± 0.05^{c}	0.70 ± 0.01^{abc}	9.95 ± 0.01^{b}
75% LS_1	4.02 ± 0.05^{d}	0.69 ± 0.01^{bc}	9.55 ± 0.02^{c}
25% LS_2	4.50 ± 0.10^{ab}	0.72 ± 0.02^{a}	8.77 ± 0.02^{f}
50% LS_2	4.44 ± 0.05^{b}	0.68 ± 0.01^{c}	9.36 ± 0.03^{d}
75% LS_2	4.21 ± 0.05^{c}	0.70 ± 0.01^{abc}	9.98 ± 0.02^{b}

Values with different superscript on the same column are significant ($P \leq 0.05$).

Table 2: Sensory Evaluation of Yogurt produced with LS1 protein isolates.

Parameters	25%	50%	75%	Control
Taste	5.80 ± 0.79^{b}	5.20 ± 1.62^{c}	4.10 ± 1.79^{c}	8.30 ± 0.95^{a}
Colour	5.90 ± 1.66^{b}	5.40 ± 1.76^{b}	5.10 ± 2.38^{b}	8.40 ± 0.97^{a}
Aroma	5.50 ± 2.27^{b}	4.90 ± 1.59^{b}	4.70 ± 1.89^{b}	8.40 ± 0.70^{a}
Overall acceptability	4.40 ± 2.22^{b}	5.00 ± 1.89^{b}	4.10 ± 2.28^{b}	8.30 ± 1.06^{a}

Values with different superscripts on the same row are significant ($P \leq 0.05$).

Table 3: Sensory Evaluation of Yogurt produced with LS2 protein isolates.

Sensory Parameters	25%	50%	75%	Control
Taste	6.20 ± 0.63[a]	4.80 ± 1.75[b]	4.70 ± 2.11[b]	7.40 ± 0.96[a]
Colour	5.10 ± 1.91[b]	5.90 ± 0.74[b]	5.60 ± 1.58$_b$	7.90 ± 0.57[a]
Aroma	5.00 ± 2.05[b]	4.50 ± 1.78[b]	4.70 ± 1.06[b]	7.70 ± 0.67[a]
Overall acceptability	5.20 ± 2.10[b]	5.60 ± 1.24[b]	5.80 ± 1.48[b]	7.70 ± 0.82[a]

Values with different superscripts on the same row are significant (P ≤ 0.05).

Table 4: Physicochemical Properties of Ice Cream Samples.

Ice cream sample	pH	Overrun (%)	Specific gravity
Control (100% milk)	6.20±0.04[a]	48.61±0.05[a]	0.79±0.01[e]
25% LS_1	6.20±0.02[a]	46.52±0.02[b]	0.81±0.01[de]
50% LS_1	6.00±0.02[c]	43.28±0.04[d]	0.88±0.02[bc]
75% LS_1	5.81±0.01[e]	42.68±0.02[g]	0.90±0.01[ab]
25% LS_2	6.10±0.04[b]	45.11±0.05[c]	0.82±0.01[d]
50% LS_2	6.00±0.02[c]	43.89±0.04[e]	0.86±0.01[c]
75% LS_2	5.90±0.02[d]	43.21±0.03[f]	0.92±0.02[a]

Values with different superscripts on the same column are significant (P ≤ 0.05).

Sensory Evaluation of Ice Cream: Tables 5 and 6 show the sensory evaluation of ice cream produced from *Lagenaria siceraria* protein isolates. Addition of 25% LS_1 protein isolates to ice cream showed no significant difference (P ≤ 0.05) from the control in all the sensory parameters examined (Table 5). However at higher levels of substitution of milk solid nonfat with LS_1 protein isolates, there was generally noticeable difference from the control in all the sensory parameters. Salama *et al.* (2007) reported that ice cream produced with 25% sesame and almond protein concentrate were rated significantly higher in texture, flavour, melting properties and appearance than other used at higher level of substitution. This shows that LS_1 protein isolates may be used up to 25% of msnf as stabilizer in the production of ice cream.

Table 6 shows that addition of LS_2 protein isolates at the level of 25% substitution of the milk solid nonfat showed no significant difference from the control (100% milk solid) in all the sensory parameters examined. In addition there was no significant difference in the colour of ice cream produced with LS_2 protein isolates up to 75% level of substitution. However, at levels higher than 25% there was noticeable difference from the control in the taste, aroma and mouthfeel of ice cream produced. LS_2 protein isolates may also be used as stabilizer and enhancement of nutritional value in ice cream at levels up to 25% substitution.

Table 5: Sensory Evaluation of Ice cream produced with LS1 Protein Isolates.

Sensory Parameters	25%	50%	75%	Control
Taste	7.70 ± 0.95[a]	5.00 ± 2.58[bc]	3.50 ± 2.27[c]	6.50 ± 2.17[ab]
Colour	6.30 ± 1.70[ab]	4.60 ± 2.50[b]	5.70 ± 2.11[ab]	7.00 ± 1.15[a]
Aroma	6.80 ± 1.62[ab]	5.40 ± 2.71[ab]	4.90 ± 2.08[b]	7.10 ± 1.29[a]
Mouthfeel	7.80 ± 1.03[a]	3.70 ± 2.41[b]	3.40 ± 2.37[b]	7.90 ± 0.99[a]

Values with different superscripts on the same row are significant (P ≤ 0.05).

Table 6: Sensory Evaluation of Ice cream produced with LS2 Protein Isolates.

Sensory Parameters	25%	50%	75%	Control
Taste	7.70 ± 1.06[a]	5.60 ± 1.82[b]	3.80 ± 2.53[c]	7.50 ± 1.51[a]
Colour	6.50 ± 1.78[a]	5.80 ± 1.40[a]	4.90 ± 2.42[a]	6.80 ± 2.57[a]
Aroma	7.30 ± 1.32[a]	5.50 ± 2.51[b]	5.70 ± 1.83[ab]	7.20 ± 1.14[a]
Mouthfeel	7.30 ± 0.67[a]	4.60 ± 2.41[b]	3.40 ± 1.78[b]	7.30 ± 1.77[a]

Values with different superscripts on the same row are significant (P ≤ 0.05).

CONCLUSION

This work has shown that *Lagenaria siceraria* protein isolates may be substituted for milk solid non fat up to 25% in the production of yogurt and ice cream. The seeds protein isolates may be used in ice cream as stabilizer and protein supplement.

REFERENCES

AOAC. 2005. Official method of analysis of Association of Official analytical Chemist; 18th Ed. Washington D. C.

Arbuckle, W.S. 1977. Ice cream. 3rd ed. AVI Publishing Co. INC Westport. Connecticut, USA.

El-Samahy, S. K., Youssef, K. M. and Moussa-Ayoub, T. E. 2009. Producing ice cream with concentrated cactus pear pulp: A preliminary study. J. PACD. Vol. 11: 1 – 12.

Hammad, Y.A., Hamzawi, I. F., Zedan, A. N. and Klader, A. E. 1985. The use of defatted soy flour in ice cream manufacture. Mnufiya J. Agric Research. Vol 10: 833-840.

Harwalkar, V. R. and Kalab, M. 1986. Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted nonfat dry milk. Food Microstruct.5; 287 – 294.

Larmond, E. 1985. Laboratory methods for sensory evaluation of foods. Canada Agriculture Canada.

Mazloomi, S. M., Shekarforoush, S. S., Ebrahimnejad, H. and Sajedianfard, J. 2011. Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yoghurt. Iranian J. Vet. Res. Vol. 12, No 2 (Ser. No. 35); 93 – 98.

Ruck, J. A. 1969. Chemical methods for analysis of fruits and vegetable product Summerland B.C 14-33.

Salama, H., Hammad, Y., Hamzawi, L. F. and Hassan, Z. M. R. 2007. Functional properties of some oil seed protein concentrate and its utilization in healthy functional imitation ice milk. Proc. 10th Egyptian Conf. Dairy Sci & Technol. 503 – 516.

Saleem, R. N., Shaker, K. A. and El-Wacely, S. M. 1989. The use of soymilk in ice cream mixes. Egyptian J. Dairy Sci., vol 17: 281-288.

Slocum, S. A., Jasinski, E. M. and Kilara, A. 1988. Processing variables affecting proteolysis in yoghurt during incubation. J. Dairy Sci. Vol. 71: 596 – 603.

Thomson, L. U., Reniers, D. J., Baker, L. M. and Siu, M. 1983. Succinylated whey protein concentrate in ice cream and instant puddings. J. Dairy Sci. Vol. 66 (8): 1630-1637.

Trachoo, N. 2002. Yogurt: The fermented milk. A Review article. Songklanakarin J. Sci. Technol. Vol. 24 (4): 727 – 737.