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Stability offrozen Nigerian soups as affected by freeze-thaw cycles

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ABSTRACT

The freezing and defrosting of frozen soups is a known practice among Nigerians and their stability is of utmost concern. Standard recipes were used to prepare selected traditional soups ('ila', 'ogbono', 'ewedu' and 'kuka' soups) of Nigerian origin. The influence of packaging materials (aluminium and plastics) and thawing conditions (microwave oven, ambient condition and hot water thawing) on the soups were determined by freezing them at -20 °C and thawing at the above conditions for four cycles at an interval of 5 days. At the end of each cycle, the free fatty acid, peroxide value, pH value, titratable acidity, viscosity, total plate counts and sensory attributes of the samples were determined using standard methods. Free fatty acid, peroxide value, pH, titratable acidity, viscosity and total plate counts of soups ranged from 0.56-3.09%, 5.82-14.54 mEq/kg, 6.53-7.17, 0.07-1.41%, 0.81-2.00 Pa·s and 1.50-2.92Log CFU/mL respectively. The nature of packaging materials and thawing conditions significantly influenced the stability indices of the soups at $p<0.05$. Limited deteriorative changes were observed in microwave-thawed soups in plastic containers, and were mostly preferred by panelists. The soups stability cannot be guaranteed if the number of cycle is above three, because of deteriorative increase in free fatty acid and peroxide values.

Introduction

Freezing is one of acceptable preservation methods used to extend the shelf life of food for a relatively long period of time (Pietrasik and Janz, 2009).The freezing process reduces the temperature of the food product generally below −18 °C, whichcrystallizespart of the free water and concentrates of some of the solutes (Leygonie et al., 2012). Freezing and frozen storage reduces the food quality, but the rate of quality loss can be minimized if the process is carefully controlled (Nilgun et al., 2013). During freezing, the activities of most of the pathogenic microorganisms are seized in frozen state to prevent spoilage (Bing and Da-wen, 2002). The nature of ice formed during freezing, the storage temperature, the packaging materials used and thephysiology of the frozen mass have significant influence on the quality and safety of frozen food products (Torres and Canet, 2001). Even though freezing highly retains the quality of frozen food, quality deterioration still occurs at a slower rate during frozen storage. Therefore, a better understanding of the effects of frozen storage is of utmost importance to the food industry.

Thawing of frozen materials is of great concern in food processing, since the quality of frozen food isclosely related to freezing and thawing processes.The rate of freezing and size of ice crystals formed during freezing are of utmost importance for reduction of tissue damage and drip loss during thawing (Bing and Da-wen, 2002). Thawing process induces chemical changes and physical damage to foods. Therefore, thawing time ought to be minimal

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to limit microbial proliferation, chemical eterioration and excessive loss of water caused by dripping or dehydration (Taher and Farid, 2001).

Traditional foods are extremely health protective and nutrient rich, some have been reported to possess high proteins, important fats like omega-3fatty acids and antioxidants (Kpikpi et al., 2009; Amadi et al., 2011). Nigeria is made of many tribes, having completely different traditional soups that are endemic to different ethnic groups and tribes. Dietary staple foods obtained from majorly cereals and tubers are consumed with the soups and each tribe has developed their different local dishes from cereals and tubers cultivated within their locality (Raji et al., 2016). However, repetitive freezing of soups after use as being practiced in modern homes has been reported as a better alternative to traditional periodical heating of soups after use, which results in losses of vitamins (Raji et al., 2015). This research work was carried out because literature is sparse on the stability, microbial safety and sensory properties of some freeze-thawed Nigerian soups.

Materials and methods

Preparation of soups

Chosen soups were prepared in accordance with the standard recipe (Table 1) described by FIIRO (2006).

Preparation of Ogbono soup

Fish and meat were washed thoroughly before and after cutting them into pieces. The meat and fish were placed in clean pot containing sliced onions and considerable amount of water. The mixture was boiled until the meat softened. Crayfish, periwinkles pepper, oil and *Ogbono* (*Irvingia gabonensis*) ground seeds were added to the mixture. The mixture was gently stirred and allowed to boil over a medium heat for 5 minutes. Seasoning and chopped vegetable were added to the mixture and was allowed to simmer for 5 minutes. Prior to freezing, the soup was allowed to cool down and then packaged into different containers.

Preparation of Ewedu soup

The freshly detached *Ewedu* (*Corchorus olitorus)* leaves from the parent stalks were washed thoroughly to remove dirt. Blending of the leaves was done in a blender containing considerable amount of water. Small piece of potash was added into the pot containing the blended leaves and it was boiled for 5 minutes**.** While the pot was kept open, melon seeds powder was sprinkled into it. It was simmered and gently stirred for 5 minutes. Before heating was stopped, ground crayfish, locust bean and table salt were added to it. Prior to freezing, the soup was allowed to cool down and then packaged into different containers.

Preparation of Okro soup

Fish and meat were washed thoroughly before and after cutting them into pieces. They were placed in clean pot containing sliced onions and considerable amount of water. The mixture was boiled until the meat softened. Milled crayfish, pepper, and oil were added to the mixture. The mixture was gently stirred and it was allowed to boil over a medium heat for 10 minutes. Seasoning and chopped okro (*Hibiscus esculentus*) fruits were added to the boiling mixture. The mixture was then allowed to simmer until the meat, vegetable and okro tendered, and the required consistency was reached. The soup was allowed to cool down and then packaged into different containers prior to freezing.

Preparation of Miyan Kuka soup

The pieces of fresh meat bought from the market were washed and placed into a clean pot. Clean water and sliced onions were added to it. The mixture was heated for 25 minutes. Washed stockfish (*Gadus morhua*)**,** curry, dried bonga fish (*Ethmalosa fimbriata*), ground pepper and water were added to the boiling mixture. It was further cooked for 20 minutes. Ground “kuka” (*Adansonia digitata*)leaves were added and it was gently stirred. The soup was allowed to steam for 15 minutes, to cool down and then it was packaged into different containers prior to freezing.

Freezing and thawing of soups

Freshly prepared soups packaged in aluminum and plastic containers (100g each) were frozen and stored in a laboratory scale chest freezer (Scanfrost chest; Model SFL-111, Hangzhou, China). The frozen soups were stored at −20°C for 5 days before being thawed (cycle 1). Soups were repeatedly frozen and thawed for four cycles using the thawing methods described below in order to mimic the thawing and refreezing processes that frozen soups experienced when some portions are consumed in modern homes.

Thawing methods

The soups were thawed using three different thawing methods stated below.

Table 1. The list of ingredients used for preparation of soups

Ogbono	***Ewedu***	***Ila***	***Kuka***
Bitter Leaf(20g)	Leaves(120g)	Okro(fruit(640g))	Meat (1000g)
Ogbono (ground (240g))	*Egusi*(ground(40g))	Pepper(14g)	Onion (75g)
Palm oil (80 mL)	Water (500 mL)	*Ugwu*(200g)	Dry fish (50g)
Water (2000 mL)	Crayfish(40g)	Meat(1000g)	Stockfish(500g)
Maggi (8g)	Potash(1g)	Crayfish(40g)	*Kuka* (Powder (150g))
Onion (3400g)	*Iru*(5g)	Onion(300g)	Maggi (10g)
Iru (locust beans (10g))	Salt(5g)	Salt(14g)	Salt(8g)
Fish(Smoked (310g))		Palm oil(40 mL)	Curry(3g)
Meat(1000g)		Water(2000 mL)	Thyme(1.4g)
Crayfish(ground (40g))		Maggi cube(8g)	Crayfish(30g)
Periwinkle(deshelled (94g))		*Iru*(locust beans(10g))	Water(2000 mL)
Salt(10g)			Palm oil(100 mL)
Pepper(ground (14g))			

Thawing in microwave oven

Thawing of soups was done in a microwave oven operated at frequency of 2.45 GHz (LG, MS2024W, 450 W max. power) using defrost setting, until the temperature at the centre of the soups reached 0^oC. Temperature variation was monitored with the aid of digital thermometer with a probe inserted at the centre of the frozen mass.

Thawing in hot water

The frozen soups were thawed in a water bath containing boiling water (100^oC) until the temperature at the centre of the soups reached 0^oC. Temperature variation was monitored with the aid of digital thermometer with a probe inserted at the centre of the frozen mass.

Thawing at ambient temperature

The frozen soups were thawed at ambient temperature (28–32^oC) until the temperature at the centre of the soups reached 0^oC. Temperature variation was monitored with the aid of digital thermometer with a probe inserted at the centre of the frozen mass.

Determination of free fatty acid content

The analyses described below were carried out at the end of every freeze-thawed cycle to determine the stability of the selected soup samples.

The percentage of the free fatty acid (FFA) was calculated for the soup samples using AOAC (2005). 1.0 gof soup sample was measured into 250 mL flask. 25 mL of alcohol and 1.0 mL of phenolphthalein indicator were added to it. The mixture was titrated with aqueous 0.5 N NaOH. The volume of NaOH used in the titration corresponded to the percentage of free fatty acid calculated, using equation 1:

$$\%\,FFA = \frac{Vol\ of\ the\ titrant(mL)\ x\ Normality\ of\ NaOH\ x\ 5.61}{Wt\ of\ sample\ used} \quad (1)$$

The FFA figure was calculated as oleic acid (1 mL of 0.1M sodium hydroxide = 0.0282g oleic acid).

Peroxide value

Peroxide value was evaluated according to the method of AOAC (2005). Two grams of soup sample was weighed into a tube and1g of powdered Potassium iodide with 20 mLof solvent mixture (glacial acetic acid and chloroform) was added.This was then placed into boiling water for 30s.The content was poured into a flask containing 20 mL of 5%iodide solution. The tube was then washed with 25 mL of distilled water and titrated with 0.002N Sodium thiosulphate solution using starch as indicator. A blank was prepared alongside with the soup sample. Peroxide value was obtained using the equation 2:

$$Peroxide\ value = \frac{2\,(V_1 - V_2)\ mEq/kg}{Wt\ of\ sample\ used} \quad (2)$$

Where: V_2 = Blank titre value, V_1 = Sample (s) titre value

pH determination

The pH of each soup was evaluated using a Crison MicropH 2002 potentio-meter and axerolyt electrode In gold Lot 406-MG- DXK-57/25, that was calibrated at pH 4.0 and pH 7.0. The probe on the electrode was inserted into the test samples and the pH was read directly from the potential meter.

Titratable acidity

Each soup sample (5 g) was diluted with 200 mL of CO_2 free distilled water inside the beaker.1.0 mL of phenolphthalein indicator was added and then titrated with 0.1M NaOH solution until pink colour was observed (AOAC, 2005).

Viscosity determination

The method described by Akusu and Kiin-Kabari (2013) was used to determine the viscosity of each soup. The viscosity of the soups was obtained at ambient temperature (30 ± 2°C) with the aid of a rotary digital viscometer (NDJ – 85, China) with spindle number 3 at 30 rpm and read in Pa·s.

Microbial analyses

The microbiological analyses described below were carried out on the selected soups samples.

Total bacteria count

Conventional colony count method for the enumeration of bacteria in food was adopted as described by Jasson et al. (2010). Samples (1.00 mL/g) were taken from the soups. Decimal dilutions were performed for all the samples and 0.1 mL of 10^{-1} dilutions were inoculated on nutrient agar plates and incubated at 37°C for 24h. After incubation, the colonies were counted by using colony counter (Subra Scientific Co., India). The final counts of colonies in petri dishes took into account the dilution factors depending on the seeded volume and dilution, thus providing the initial number of the cells. Analysis was replicated three times and the arithmetic mean was reported as the final result. Inactivation was expressed as $LogN_0/N$, where N_0 is the number of microorganisms initially contained in the fresh samples and N is the corresponding number of microorganisms after freezing and thawing.

Isolation procedure

Various colonies observed on the plates were examined for colonial differences using the method described by Fawole and Oso (1998). Representatives of different colonies were streaked on sterile nutrient and mac-conkey agars. Pure cultures resulting from the isolations were subcultured and preserved on agar slants.

The characterization of the colonies

The isolated bacteria were identified and characterized using the following morphological and biochemical tests:

Morphological test

Gram reaction and cell morphology of the isolates were examined from their heat fixed smears, using the methods described by Prescott et al. (2005).

Biochemical tests

Theoxidase, indole, catalase and coagulase tests were carried out on the isolates using the methods described by Prescott et al. (2005).

Fungi counts

The fungi count was determined using the method of Raper and Fennel (1973) as reported by Atanda et al. (2009). 1 mL of each sample was aseptically transferred into 9mL distilled water and properly homogenized. The samples were subsequently decimally diluted. All samples were analysed in triplicate on potato dextrose agar containing 0.001% of chloraphenicol and allowed to incubate at ambient temperature (30°C±2) for 48h.

Sensory evaluation

The effect of freeze-thaw cycles on the sensory properties of the selected soups subjected to freezing and thawing conditions was tested at the end of the fourth cycle by comparing them with the freshly prepared soups. Twenty trained panelists were selected from the staff and students (12 females and 8 males) of the Kwara State University, Malete, Nigeria on the basis of interest, availability and familiarity with the soups. The samples were coded with 3-figure random numbers and presented in random order to each panelist at ambient room conditions (25-30°C).A 9-point hedonic scale (where 1 represents dislike extremely and 9 represent like extremely) was used by the panelists to score for colour, taste, aroma, consistency and overall acceptability of the soups.

Statistical analysis

All analyses were performed in triplicate. Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) V. 17.0. Following ANOVA, means were separated using Duncan Multiple Range Test (DMRT) at 95% confidence level ($p \leq 0.05$).

Table 2. The stability of free fatty acid in selected soups as affected by freeze-thaw cycles (%)

Sample	*Ogbono*	*Ewedu*	Okro(*Ila*)	*Kuka*
Cycle 0				
FP	1.70±0.01^{i}	0.56±0.01^{i}	1.13±0.01^{d}	1.13±0.10^{j}
Cycle 1				
AH	1.77±0.06^{e-i}	0.61±0.06^{i}	1.21±0.02^{d}	1.16±0.01^{g-j}
AR	1.85±0.04^{ef}	0.61±0.06^{i}	1.22±0.10^{d}	1.17±0.01^{g-i}
AM	1.79±0.05^{e-i}	0.61±0.05^{i}	1.21±0.01^{d}	1.16±0.00^{g}-i
PH	1.74±0.06^{g-i}	0.58±0.05^{i}	1.16±0.00^{d}	1.15±0.00^{h-j}
PR	1.83±0.06^{e-g}	0.61±0.07i	1.21±0.04^{d}	1.18±0.01^{gh}
PM	1.72±0.07^{hi}	0.57±0.04i	1.14±0.02^{d}	1.14±0.01^{ij}
Cycle 2				
AH	1.79±0.06^{e-i}	0.80±0.02^{h}	1.24±0.02^{d}	1.16±0.01^{g-j}
AR	1.86±0.04^{e}	0.81±0.02^{h}	1.25±0.01^{d}	1.18±0.00^{gh}
AM	1.81±0.06^{eh}	0.81±0.01^{h}	1.25±0.01^{d}	1.17±0.01^{g-i}
PH	1.76±0.05^{f-i}	0.82±0.02^{h}	1.27±0.02^{d}	1.16±0.01^{g-j}
PR	1.86±0.03^{e}	0.83±0.02^{h}	1.27±0.04^{d}	1.18±0.01^{g}
PM	1.73±0.06^{hi}	0.77±0.05^{h}	1.19±0.07^{d}	1.15±0.01^{h-j}
Cycle 3				
AH	2.28±0.06^{b-d}	1.99±0.05^{g}	2.71±0.08^{c}	2.21±0.02^{ef}
AR	2.35±0.02^{ab}	2.06±0.02^{fg}	2.81±0.13^{bc}	2.26±0.01^{a-c}
AM	2.31±0.05^{a-c}	2.06±0.02^{fg}	2.79±0.22^{bc}	2.24±0.03^{c-e}
PH	2.22±0.07^{d}	2.10±0.01^{f}	2.79±0.42^{bc}	2.22±0.01^{ef}
PR	2.35±0.04^{ab}	2.33±0.06^{e}	3.02±0.42^{ab}	.28±0.02^{a}
PM	2.20±0.07^{d}	1.99±0.01^{g}	2.69±0.11^{c}	2.21±0.01^{f}
Cycle 4				
AH	2.32±0.05^{a-c}	2.38±0.08^{de}	2.78±0.07^{bc}	2.23±0.01^{d-f}
AR	2.39±0.04^{a}	2.41±0.02^{cd}	2.83±0.06^{bc}	2.27±0.01^{ab}
AM	2.35±0.03^{ab}	2.46±0.06^{bc}	2.86±0.12^{bc}	2.25±0.02^{b-d}
PH	2.25±0.07^{cd}	2.50±0.03^{b}	2.90±0.11^{a-c}	2.21±0.01^{ef}
PR	2.39±0.02^{a}	2.77±0.08^{a}	3.09±0.32^{a}	2.26±0.05^{a-c}
PM	2.21±0.07^{d}	2.39±0.04^{c-e}	2.79±0.09b^{c}	2.20±0.06^{f}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Results and discussions

Degradative Changes during Freezing and Refreezing of Soup Samples

The results of the free fatty acid, peroxide value, pH, titratable acidity and viscosity of the freeze-thawed soup samples were presented in Tables 2-6.

Free Fatty Acid (FFA)

The soups` free fatty acid values varied from 0.56 ±0.01 to 3.09±0.32% (Table 2). Free fatty acid is formed due to the hydrolysis of triglycerides and is responsible for off flavor development during storage.

High temperature, moisture, and presence of active lipase are responsible for the production of FFA in fat containing food products (Kumar et al., 2009). Among the freshly prepared soups, *ewedu* had the least FFA value (0.56±0.01%). This suggested that *ewedu* might be more stable than others during storage. Further study revealed that the highest free fatty acid values were observed in soups packaged in both containers (plastics and aluminium) and thawed at ambient condition, while microwave thawed soups in plastic containers had the lowest FFA contents (Table 2). Variation in FFA values of the freeze-thawed soups indicated that the cycle significantly affected lipid hydrolysis. The increase in FFA was found markedly higher during the third freeze-thaw cycle of the 4-cycle experiment. This might be due to maximal lipase release from liposomes during the third freeze-thaw cycle (Sikorski and Kolakowski, 2000), making the enzyme close the substrate. Since the formation of FFA does not directly lead to nutritional losses, its assessment is deemed important when considering rancidity occurrence in food. However, the pro-oxidant effect of FFA on lipid matter was based on the catalytic effect of thecarboxyl group on the development of free radicalsby the decomposition of hydro-peroxides (Yoshida et al., 1992). In addition, it has been established that interaction between FFA and proteins leads to texture deterioration (Mackie, 1993).

Peroxide Value

The soups` peroxide values ranged from 5.82±0.22 to 14.54±0.25 mEq/kg (Table 3). The peroxide value (PV) which is a biochemical lipid oxidation index

(peroxide value) was used o evaluate the development of rancidity in the soups subjected to freeze-thaw cycles. Lipid peroxidation products, especially aldehydes, cause rancidity and food deterioration (Undeland, 2001). It was observed that the peroxide value increased as the freeze-thaw cycle increased. However, the least PV at every freeze-thaw cycle was observed in microwave thawed soups in plastic containers. The observed increase indicated that hydrolysis of lipids occurred during storage.
Egan et al. (1981) reported that acidity and a rancid taste were noticed in food when peroxide value was in the range of 20 to 40 mEq/kg. A moderate peroxide formation was detected during the frozen storage period, except for the end of the experiment when the highest mean values were obtained. This was similar to the findings of Akusu and Kiin-Kabari (2013) who reported on the effect of storage period on selected functional, chemical stability and sensory properties of bush mango (Irvingia gabonensis) seed flour. Room temperature thawed soups in plastic containers had the highest values at each cycle and the values obtained were approaching the unacceptable limits of 20 mEq/kg at the fourth cycle. This implies that the shelf life of room temperature thawed soups in plastic containers cannot be guaranteed beyond the fourth freeze- thawed cycle.

pH

The soups` pH values ranged from 6.53±0.06 to 7.17±0.06 (Table 4). It was observed that the pH decreased as the freeze-thaw cycle increased.This was due to the differences in the thawing methods as well as the packaging materials. The highest pH values reductions were obtained in room temperature thawed soups in plastic containers at every freeze-thaw cycle. Since pH is a measure of the amount of free hydrogen ions (H^+) in a solution, denaturation of buffer proteins could be caused by freezing as a result of exudates production, which might lead to the release of hydrogen ions and a subsequent decrease in pH (Leygonie et al., 2012).
A further explanation for this finding might be due to microbial or enzymatic deamination of proteins that might result into the release of hydrogen atoms (Leygonie et al., 2011).

Table 3. The stability of peroxide values in selected soups as affected by freeze-thawe cycles (meq/kg)

Sample	***Ogbono***	***Ewedu***	**Okro(*Ila*)**	***Kuka***
Cycle 0				
FP	8.60±0.10^{g}	7.17±0.05^{h}	5.82±0.22^{e}	6.09±0.09^{gh}
Cycle 1				
AH	8.88±0.29^{e-g}	7.81±0.68^{gh}	6.30±0.11^{e}	6.15±0.05^{e-h}
AR	9.30±0.20^{e}	7.87±0.59^{g}	6.36±0.05^{e}	6.21±0.01^{e-h}
AM	9.01±0.27^{e-g}	7.80±0.51^{gh}	6.30±0.47^{e}	6.17±0.03^{e-h}
PH	8.74±0.30^{fg}	7.46±0.52^{gh}	6.02±0.02^{e}	6.11±0.03^{f-h}
PR	9.19±0.29^{ef}	7.81±0.70^{gh}	6.30±0.23^{e}	6.26±0.03^{ef}
PM	8.66±0.33^{fg}	7.35±0.48^{gh}	5.94±0.12^{e}	6.07±0.05^{h}
Cycle 2				
AH	9.02±0.30^{e-g}	9.77±0.01^{fg}	6.45±0.11^{e}	6.15±0.06^{e-h}
AR	9.35±0.12^{e}	10.17±0.15^{ef}	6.53±0.05^{e}	6.25±0.04^{e-g}
AM	9.12±0.29^{e-g}	10.17±0.12^{ef}	6.51±0.03^{e}	6.20±0.06^{e-h}
PH	8.84±0.26^{e-g}	10.22±0.15^{ef}	6.59±0.08^{e}	6.15±0.05^{e-h}
PR	9.36±0.13^{e}	10.51±0.32^{e}	6.62±0.20^{e}	6.30±0.03^{e}
PM	8.70±0.28^{fg}	9.35±0.54^{g}	6.20±0.35^{e}	6.11±0.06^{f-h}
Cycle 3				
AH	13.91±0.36^{b-d}	11.38±0.01^{d}	11.68±0.41^{cd}	9.80±0.07^{cd}
AR	14.10±0.10^{a-c}	12.13±0.12^{bc}	12.17±0.45^{cd}	10.10±0.10^{a}
AM	14.09±0.29^{a-c}	12.17±0.16^{bc}	12.00±0.85^{cd}	9.91±0.12^{bc}
PH	13.52±0.42^{d}	12.39±0.03^{b}	11.90±1.31^{ab}	9.76±0.07^{cd}
PR	14.32±0.27^{ab}	13.76±0.39^{a}	13.18±1.31^{ab}	10.08±0.07^{a}
PM	13.42±0.44^{d}	11.73±0.10^{cd}	11.38±0.69^{d}	9.73±0.12^{d}
Cycle 4				
AH	14.14±0.28^{a-c}	12.04±0.39^{bc}	11.98±0.48^{cd}	9.80±0.11^{cd}
AR	14.54±0.25^{a}	12.19±0.02^{bc}	12.03±0.28^{cd}	10.00±0.08^{ab}
AM	14.34±0.20^{ab}	12.43±0.24^{b}	12.31±0.44^{b-d}	9.91±0.12^{bc}
PH	13.73±0.43^{cd}	12.62±0.10^{b}	12.37±0.52^{bc}	9.77±0.05^{cd}
PR	14.53±0.13^{a}	13.99±0.33^{a}	13.57±1.03^{a}	9.99±0.23^{ab}
PM	13.46±0.40^{d}	12.06±0.16^{bc}	11.58±0.96^{cd}	9.71±0.07^{d}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different (p<0.05). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Table 4. The stability of ph in selected soups as affected by freeze-thaw cycles

Sample	***Ogbono***	***Ewedu***	**Okro(*Ila*)**	***Kuka***
Cycle 0				
FP	7.10 ± 0.10^{a}	6.97 ± 0.06^{ab}	7.10 ± 0.10^{a}	7.17 ± 0.06^{a}
Cycle 1				
AH	7.10 ± 0.10^{a}	7.00 ± 0.00^{a}	7.07 ± 0.12^{ab}	$6.93\pm0.06^{b\text{-}d}$
AR	$6.87\pm0.06^{c\text{-}e}$	6.87 ± 0.06^{cd}	$6.87\pm0.06^{c\text{-}f}$	$6.90\pm0.00^{b\text{-}e}$
AM	$6.87\pm0.06^{c\text{-}e}$	6.87 ± 0.06^{cd}	$6.87\pm0.06^{c\text{-}f}$	$6.87\pm0.06^{c\text{-}f}$
PH	6.93 ± 0.06^{bc}	$6.93\pm0.06^{a\text{-}c}$	$6.93\pm0.06^{b\text{-}d}$	$6.90\pm0.10^{b\text{-}e}$
PR	$6.90\pm0.00^{b\text{-}d}$	$6.93\pm0.06^{a\text{-}c}$	$6.93\pm0.06^{b\text{-}d}$	$6.87\pm0.06^{c\text{-}f}$
PM	7.10 ± 0.00^{a}	7.00 ± 0.01^{a}	7.10 ± 0.10^{a}	7.13 ± 0.06^{a}
Cycle 2				
AH	7.00 ± 0.00^{ab}	7.00 ± 0.00^{a}	$6.97\pm0.06^{a\text{-}c}$	$6.87\pm0.06^{c\text{-}f}$
AR	6.73 ± 0.06^{fg}	6.77 ± 0.06^{ef}	$6.73\pm0.06^{f\text{-}i}$	$6.73\pm0.06^{e\text{-}i}$
AM	$6.80\pm0.10^{d\text{-}f}$	6.77 ± 0.06^{ef}	$6.80\pm0.10^{c\text{-}g}$	$6.80\pm0.10^{c\text{-}g}$
PH	$6.77\pm0.06^{e\text{-}g}$	6.77 ± 0.06^{ef}	$6.77\pm0.06^{e\text{-}h}$	$6.77\pm0.06^{d\text{-}h}$
PR	6.60 ± 0.00^{hi}	6.67 ± 0.06^{gh}	$6.63\pm0.06^{h\text{-}j}$	6.60 ± 0.00^{hi}
PM	$6.87\pm0.06^{c\text{-}e}$	$6.90\pm0.00^{b\text{-}d}$	$6.90\pm0.10^{c\text{-}e}$	7.07 ± 0.06^{ab}
Cycle 3				
AH	$6.77\pm0.06^{e\text{-}g}$	6.77 ± 0.06^{ef}	$6.77\pm0.06^{e\text{-}h}$	$6.70\pm0.10^{f\text{-}i}$
AR	6.57 ± 0.06^{hi}	6.63 ± 0.06^{hi}	$6.63\pm0.06^{h\text{-}j}$	6.57 ± 0.06^{i}
AM	6.73 ± 0.06^{fg}	6.73 ± 0.06^{fg}	$6.73\pm0.06^{f\text{-}i}$	$6.73\pm0.06^{e\text{-}i}$
PH	6.73 ± 0.06^{fg}	6.73 ± 0.06^{fg}	$6.73\pm0.06^{f\text{-}i}$	$6.77\pm0.06^{d\text{-}h}$
PR	6.53 ± 0.06^{i}	6.63 ± 0.06^{hi}	6.57 ± 0.06^{j}	6.60 ± 0.17^{hi}
PM	6.83 ± 0.06^{fg}	6.87 ± 0.06^{cd}	$6.90\pm0.10^{c\text{-}e}$	$6.97\pm0.^{15bc}$
Cycle 4				
AH	$6.73\pm0.06f^{g}$	6.73 ± 0.06^{fg}	$6.73\pm0.06^{f\text{-}i}$	$6.73\pm0.06^{e\text{-}i}$
AR	6.53 ± 0.06^{i}	6.60 ± 0.00^{hi}	6.57 ± 0.06^{j}	6.60 ± 0.17^{hi}
AM	6.73 ± 0.06^{fg}	6.73 ± 0.06^{fg}	$6.73\pm0.06^{f\text{-}i}$	$6.73\pm0.06^{e\text{-}i}$
PH	6.67 ± 0.06^{gh}	6.67 ± 0.06^{gh}	$6.67\pm0.06^{g\text{-}j}$	$6.73\pm0.06^{e\text{-}i}$
PR	6.53 ± 0.06^{i}	6.57 ± 0.06^{i}	6.60 ± 0.17^{ij}	$6.63\pm0.21^{g\text{-}i}$
PM	$6.83\pm0.07^{c\text{-}f}$	6.83 ± 0.06^{de}	$6.87\pm0.06^{c\text{-}f}$	$6.93\pm0.06^{b\text{-}d}$

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Slight decrease in the pH of the soups indicated that fermentation might likely occur in the future. The pH changes are in agreement with the findings of Sigurgisladottir et al. (2000) who reported on the effects of freezing and thawing on the microstructure and the texture of smoked Atlantic salmon, but contrary to the findings of Simeonidou et al. (1998) who researched on the quality assessment of seven Mediterranean fish species during storage on ice and Arannilewa et al. (2005) who reported on the effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (Sarotherodun galiaenus). The changes in pH indicated a direct relationship with sensory and microbiological results, and reflected similar trends in other storage stability parameters (titratable acidity, peroxide value, free fatty acid and viscosity).

Titratable Acidity

The soups` titratable acid values varied from 0.07±0.01 to 1.39±0.00% (Table 5). The highest values of titratable acidity were observed in room temperature thawed soups in plastic containers at every freeze-thaw cycle. An increase in the titratable acidity values paralleled with the decrease in the pH of the freeze-thawed soups, as significantly influenced by the thawing methods and types of packaging materials. This finding was in agreement with the observation of Adewusi (1999) who reported the effect of processing on total organic acids content and mineral availability of simulated cassava-vegetable diets.

Viscosity

Viscosity values of the soups ranged from 1.31±0.00 to 2.00±0.00 Pa·s (Table 6). The viscosity values obtained decreased as the freeze-thaw cycle increased. The lowest viscosity values reductions were obtained in microwave thawed soups in plastic containers at every freeze-thaw cycle. The values obtained in this study was in line with those reported by Akusu and Kiin-Kabari (2013) on ‘ogbono’ soups made from stored bush mango (Irvingia gabonensis) seed flour (0.88-1.35 Pa·s) and Eze and Akubor (2012) who did the research on the effect of drying methods and storage on the physicochemical properties of okra and observed that the viscosity of okra samples varied from 1.50-1.60 Pa·s, with the fresh sample having the highest value of viscosity. From the previous findings, it could be deduced that the rate of moisture loss corresponded with the decrease in the viscosity of all the samples at every freeze-thaw cycle. This was correspondence with the findings of Eze and Akubor (2012) who reported that the rheological and hydrophilic properties of starch and gums in food systems might be altered by heat.

Freezing and thawing effects on microbiological quality of soups samples

There was no evidence of fungal contamination on the soup samples analyzed and this might be due to cooking temperature (100 °C) that was able to eliminate the initial fungal population. Bacterial population was recorded in the samples subjected to plate count method. The bacterial counts obtained ranged from 1.50±0.17 to 2.92±0.02 Log CFU/mL (Table 7).However, the homogeneity of the bacteria present in the soups samples and the impact of thawing on the soups were assumed to be randomly distributed across the samples and were therefore not factored into the analysis. Freshly prepared okro (ila) and ogbono soups cooled down to room temperature had the highest bacterial counts 2.92±0.02 Log CFU/mL and 2.80±0.02 Log CFU/mL, respectively. Lower bacterial counts were recorded for freshly prepared ewedu and kuka soups (2.51±0.30 Log CFU/mL and 2.34±0.01 Log CFU/mL). The results obtained showed that all the soup samples had bacterial count of < 104CFU/g (4.0 Log CFU/g). Some bacteria were suspected to be present in the soups. and the suspected bacteria were *Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli*. It was observed that *Bacillus* spp were the dominant organisms in ogbono, ewedu and ila soups, while the major organism in ewedu soup was *Escherichia coli* (Tables 8 and 9).Most strains of *Escherichia coli* are harmless and opportunistic in nature (Rodríguez-Lozano et al., 2010). The presence of *Escherichia coli* in foods is not alwaysalarming because most strains are harmless and opportunistic in nature (Cohen et al., 2006).However, the bacterium is well known as an indicator of fecal contamination level during handling and processing (Sachey et al., 2001).*Bacillus* spp are sporulated and thermal resistant bacteria that are widely distributed in nature, and often associated with different food products. Spore formation confers to bacteria resistance to pasteurisation treatments (Rodríguez-Lozan et al., 2010).

Table 5. The stability of titratable acidity in selected soups as affected by freeze-thaw cycles (%)

Sample	***Ogbono***	***Ewedu***	**Okro (*Ila*)**	***Kuka***
Cycle 0				
FP	0.21±0.01^{g}	0.11±0.01^{e}	0.07±0.01^{g-i}	0.07±0.01^{c}
Cycle 1				
AH	0.22±0.01^{e-g}	0.11±0.01^{e}	0.08±0.00^{f-i}	0.08±0.00^{b}
AR	0.23±0.01^{e}	0.10±0.01^{e}	0.08±0.00^{f-h}	0.08±0.00^{b}
AM	0.22±0.01^{e-g}	0.11±0.01^{e}	0.08±0.00^{f-i}	0.08±0.00^{b}
PH	0.22±0.01^{fg}	0.10±0.01^{e}	0.07±0.00^{hi}	0.08±0.00^{b}
PR	0.23±0.01^{ef}	0.11±0.01^{e}	0.08±0.00^{f-i}	0.08±0.00^{b}
PM	0.21±0.01^{fg}	0.10±0.00^{e}	0.07±0.00^{i}	0.08±0.00^{b}
Cycle 2				
AH	0.22±0.01^{e-g}	0.20±0.00^{d}	0.08±0.00^{fg}	0.08±0.00^{b}
AR	0.23±0.01^{e}	0.22±0.02^{d}	0.08±0.00^{fg}	0.08±0.00^{b}
AM	0.23±0.01^{ef}	0.20±0.00^{d}	0.08±0.00^{fg}	0.08±0.00^{b}
PH	0.22±0.01^{e-g}	0.20±0.00^{d}	0.08±0.00^{f}	0.08±0.00^{b}
PR	0.23±0.00^{e}	0.21±0.01^{d}	0.08±0.00^{f}	0.08±0.00^{b}
PM	0.22±0.01^{fg}	0.19±0.01^{d}	0.07±0.00^{g-i}	0.08±0.00^{b}
Cycle 3				
AH	0.30±0.01^{b-d}	0.25±0.07^{c}	0.12±0.00^{de}	0.13±0.00^{a}
AR	0.29±0.01^{cd}	0.36±0.01^{b}	0.12±0.00^{c-e}	0.13±0.06^{a}
AM	0.30±0.01^{a-c}	0.36±0.01^{b}	0.12±0.00^{cd}	0.13±0.00^{a}
PH	0.29±0.01^{cd}	0.36±0.00^{b}	0.12±0.00^{bc}	0.13±0.00^{a}
PR	0.31±0.01^{ab}	0.40±0.01^{a}	0.14±0.00^{a}	0.13±0.00^{a}
PM	0.29±0.01^{ab}	0.34±0.00^{b}	0.12±0.00^{e}	0.13±0.00^{a}
Cycle 4				
AH	0.30±0.01^{ab}	0.35±0.01^{b}	0.12±0.00^{c-e}	0.13±0.00^{a}
AR	0.31±0.01^{a}	0.36±0.00^{b}	0.12±0.00^{c}	0.13±0.00^{a}
AM	0.31±0.00^{ab}	0.36±0.01^{b}	0.12±0.00^{bc}	0.13±0.00^{a}
PH	0.29±0.01^{b-d}	0.37±0.00^{b}	0.13±0.00^{b}	0.13±0.01^{a}
PR	0.31±0.00^{a}	0.41±0.01^{a}	0.14±0.00^{a}	0.13±0.00^{a}
PM	0.29±0.01^{d}	0.35±0.01^{b}	0.12±0.00^{c-e}	0.13±0.00^{a}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Table 6. The stability of viscosity in selected soups as affected by freeze-thaw cycles (Pa·s)

Sample	*Ogbono*	*Ewedu*	Okro (*Ila*)	*Kuka*
Cycle 0				
FP	2.00 ± 0.00^{a}	1.40 ± 0.00^{a}	1.80 ± 0.00^{a}	0.90 ± 0.00^{a}
Cycle 1				
AH	1.99 ± 0.00^{cd}	1.39 ± 0.00^{c}	1.80 ± 0.00^{a}	0.89 ± 0.00^{c}
AR	1.96 ± 0.00^{l}	1.38 ± 0.00^{ef}	1.76 ± 0.01^{ef}	0.88 ± 0.01^{gh}
AM	1.98 ± 0.00^{gh}	1.38 ± 0.00^{f}	1.78 ± 0.01^{c}	0.89 ± 0.00^{d}
PH	1.99 ± 0.00^{b}	1.39 ± 0.00^{de}	1.78 ± 0.01^{bc}	0.90 ± 0.00^{b}
PR	1.98 ± 0.00^{gh}	1.39 ± 0.00^{d}	1.79 ± 0.01^{b}	0.88 ± 0.00^{e}
PM	2.00 ± 0.01^{b}	1.40 ± 0.00^{ab}	1.80 ± 0.00^{a}	0.89 ± 0.00^{c}
Cycle 2				
AH	1.98 ± 0.00^{h}	1.38 ± 0.00^{ef}	1.79 ± 0.01^{b}	0.88 ± 0.00^{e}
AR	1.92 ± 0.00^{o}	1.37 ± 0.01^{gh}	1.73 ± 0.00^{h}	0.86 ± 0.00^{k}
AM	1.97 ± 0.00^{k}	1.36 ± 0.00^{j}	1.76 ± 0.00^{ef}	0.88 ± 0.00^{g}
PH	1.99 ± 0.00^{de}	1.37 ± 0.00^{h}	1.77 ± 0.00^{d}	0.89 ± 0.00^{c}
PR	1.97 ± 0.00^{k}	1.38 ± 0.00^{g}	1.77 ± 0.01^{de}	0.87 ± 0.00^{i}
PM	1.99 ± 0.00^{c}	1.40 ± 0.00^{b}	1.78 ± 0.00^{b}	0.89 ± 0.00^{d}
Cycle 3				
AH	1.97 ± 0.00^{d}	1.37 ± 0.00^{gh}	1.79 ± 0.00^{b}	0.88 ± 0.00^{g}
AR	1.89 ± 0.00^{p}	1.35 ± 0.00^{k}	1.69 ± 0.00^{i}	0.83 ± 0.00^{m}
AM	1.95 ± 0.00^{m}	1.33 ± 0.00^{m}	1.75 ± 0.00^{g}	0.87 ± 0.00^{j}
PH	1.98 ± 0.00^{fg}	1.36 ± 0.00^{j}	1.76 ± 0.01^{ef}	0.89 ± 0.00^{d}
PR	1.95 ± 0.00^{m}	1.37 ± 0.00^{i}	1.76 ± 0.01^{f}	0.85 ± 0.00^{l}
PM	1.99 ± 0.00^{k}	1.40 ± 0.00^{bc}	1.79 ± 0.00^{b}	0.88 ± 0.00^{f}
Cycle 4				
AH	1.97 ± 0.00^{k}	1.36 ± 0.00^{i}	1.78 ± 0.00^{bc}	0.87 ± 0.00^{ij}
AR	1.85 ± 0.00^{q}	1.33 ± 0.00^{n}	1.65 ± 0.01^{j}	0.81 ± 0.00^{n}
AM	1.94 ± 0.00^{n}	1.31 ± 0.00^{o}	1.73 ± 0.00^{h}	0.85 ± 0.00^{kl}
PH	1.98 ± 0.00^{i}	1.34 ± 0.00^{l}	1.76 ± 0.00^{f}	0.88 ± 0.00^{e}
PR	1.93 ± 0.00^{n}	1.35 ± 0.00^{j}	1.74 ± 0.00^{g}	0.84 ± 0.00^{m}
PM	1.99 ± 0.00^{f}	1.39 ± 0.00^{bc}	1.78 ± 0.01^{bc}	0.87 ± 0.00^{h}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Table 7. Bacterial Examination of the freeze-thaw Nigerian Soups

Samples	***Ogbono*** **(Log 10 CFU/mL)**	***Ewedu*** **(Log 10 CFU/mL)**	***Ila*** **(Log 10 CFU/mL)**	***Kuka*** **(Log 10 CFU/mL)**
Cycle 0				
FP	2.80 ± 0.02^{a}	2.51 ± 0.30^{a}	2.89 ± 0.06^{ab}	2.34 ± 0.01^{ab}
Cycle 1				
AH	2.79 ± 0.01^{a}	2.44 ± 0.31^{b}	2.88 ± 0.02^{ab}	$2.31\pm0.0^{a-d}$
AR	2.79 ± 0.02^{a}	2.37 ± 0.30^{cd}	2.92 ± 0.02^{a}	2.35 ± 0.02^{a}
AM	2.75 ± 0.02^{ab}	$2.35\pm0.30^{c-e}$	$2.85\pm0.01^{b-e}$	$2.29\pm0.01^{a-e}$
PH	$2.64\pm0.03^{a-d}$	$2.29\pm0.28^{d-f}$	$2.82\pm0.01^{c-f}$	$2.26\pm0.01^{b-f}$
PR	$2.73\pm0.05^{a-c}$	2.42 ± 0.29^{bc}	$2.85\pm0.01^{b-d}$	$2.30\pm0.00^{a-e}$
PM	$2.58\pm0.03^{c-f}$	2.20 ± 0.28^{g}	2.64 ± 0.01^{k}	2.13 ± 0.02^{hi}
Cycle 2				
AH	$2.73\pm0.02^{a-c}$	2.31 ± 0.27^{de}	2.79 ± 0.01^{fg}	$2.29\pm0.03^{a-e}$
AR	2.79 ± 0.01^{a}	2.34 ± 0.29^{de}	2.86 ± 0.02^{bc}	$2.33\pm0.02^{a-c}$
AM	$2.67\pm0.03^{a-c}$	2.33 ± 0.29^{de}	$2.81\pm0.01^{e-g}$	$2.25\pm0.03^{c-g}$
PH	$2.62\pm0.02^{b-e}$	2.33 ± 0.33^{de}	$2.81\pm0.02^{e-g}$	$2.17\pm0.02^{g-i}$
PR	$2.70\pm0.00^{a-c}$	2.36 ± 0.29^{cd}	$2.81\pm0.02^{d-g}$	$2.27\pm0.01^{a-e}$
PM	2.22 ± 0.07^{gh}	2.18 ± 0.29^{gh}	2.55 ± 0.05^{l}	1.97 ± 0.03^{j}
Cycle 3				
AH	$2.67\pm0.03^{a-c}$	2.28 ± 0.27^{ef}	$2.74\pm0.04^{h-j}$	$2.24\pm0.01^{d-g}$
AR	$2.65\pm0.08^{a-c}$	2.32 ± 0.28^{de}	2.79 ± 0.01^{fg}	$2.26\pm0.00^{b-f}$
AM	$2.71\pm0.02^{a-c}$	2.32 ± 0.31^{de}	$2.78\pm0.00^{f-h}$	$2.22\pm0.02^{e-g}$
PH	$2.49\pm0.01^{d-f}$	2.36 ± 0.32^{cd}	2.73 ± 0.01^{ij}	2.10 ± 0.02^{i}
PR	$2.64\pm0.03^{a-d}$	$2.35\pm0.30^{c-e}$	2.80 ± 0.03^{fg}	$2.22\pm0.03^{e-g}$
PM	2.10 ± 0.17^{h}	2.11 ± 0.38^{hi}	2.56 ± 0.03^{l}	1.82 ± 0.07^{k}
Cycle 4				
AH	2.45 ± 0.08^{f}	2.23 ± 0.28^{fg}	$2.74\pm0.01^{h-j}$	$2.19\pm0.03^{f-k}$
AR	$2.58\pm0.03^{c-f}$	$2.30\pm0.27^{d-f}$	$2.77\pm0.01^{g-i}$	$2.24\pm0.03^{d-g}$
AM	$2.48\pm0.00^{e-f}$	$2.30\pm0.30^{d-f}$	$2.77\pm0.01^{f-i}$	$2.17\pm0.02^{g-i}$
PH	2.25 ± 0.13^{g}	2.28 ± 0.39^{ef}	2.71 ± 0.01^{j}	1.94 ± 0.06^{j}
PR	2.22 ± 0.07^{gh}	$2.29\pm0.32^{d-f}$	2.79 ± 0.01^{fg}	$2.24\pm0.03^{d-g}$
PM	1.83 ± 0.30^{h}	2.08 ± 0.53^{i}	2.54 ± 0.05^{l}	1.50 ± 0.17^{l}

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly Prepared, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

Ideally, the cooking temperature should be able to destroy all viable microorganisms, but the bacterial load recorded could be as a result of the presence of thermo-resistant micro organisms (Gould, 2000) that were able to withstand the cooking temperature (100 °C).The viscosity of the soups or other ingredients from the soup recipes such as oil might protect the identified organisms against the lethal effect of the heat treatment (Rodríguez-Lozan et al., 2010). *Bacillus* enzymes are resistant to pasteurisation treatment (Barefoot and Adams, 1998; Rodríguez-Lozan et al., 2010) and are usually destroyed at sterilization temperature of 120 °C for 10-15 min (Gould, 2000; Pflug and Gould, 2000).

Freezing of the soups packaged in aluminum and plastic containers, followed by freezing and thawing using different thawing methods at 5 days interval up to 20 days frozen storage caused significant reduction in the number of bacterial counts when compared to freshly processed samples, regardless of thawing conditions and packaging materials. The methods of thawing frozen foods determine the rate at which microbial population would grow during and after thawing (Leygonie et al.,2012).The thawing methods employed in this study had significant influence on the microbial growth and these were presented in Figure 1.

Table 8. Biochemical reactions of isolated bacteria in freshly prepared soups

Samples	Coagulase test	Indole test	Catalase test	Oxidase test	Gram staining test	Suspected organisms
Ogbono 1		-	+	+	-	*Bacillus cereus*
Ogbono 2		-	+	+	-	*Bacillus cereus*
Ogbono 3		-	+	+	-	*Bacillus cereus*
Ewedu 1	-	+	+	-	-	*Escherichia coli*
Ewedu 2	-	+	+	-	-	*Escherichia coli*
Ewedu 3	-	+	+	-	-	*Escherichia coli*
Ila 1		-	+	+	-	*Bacillus cereus*
Ila 2	-	-	+	-	-	*Bacillus subtilis*
Ila 3	-	-	+	-	-	*Bacillus subtilis*
Kuka 1		-	+	+	-	*Bacillus cereus*
Kuka 2		-	+	+	-	*Bacillus cereus*
Kuka 3		-	+	+	-	*Bacillus cereus*

(+) present; (-)absent

Table 9. Biochemical reactions of isolated bacteria in freeze-thawed soups

Sample	Coagulase test	Indole test	Catalase test	Oxidase test	Gram staining test	Suspected organisms
Ogbono						
AH		-	+	+	-	*Bacillus cereus*
AR		-	+	+	-	*Bacillus cereus*
AM		-	+	+	-	*Bacillus cereus*
PH		-	+	+	-	*Bacillus cereus*
PR		-	+	+	-	*Bacillus cereus*
PM		-	+	+	-	*Bacillus cereus*
Ewedu						
AH	-	+	+	-	-	*Escherichia coli*
AR	-	+	+	-	-	*Escherichia coli*
AM	-	+	+	-	-	*Escherichia coli*
PH	-	+	+	-	-	*Escherichia coli*
PR	-	+	+	-	-	*Escherichia coli*
PM	-	+	+	-	-	*Escherichia coli*
Ila						
AH	-	-	+	-	-	*Bacillus subtilis*
AR	-	-	+	-	-	*Bacillus subtilis*
AM		-	+	+	-	*Bacillus cereus*
PH		-	+	+	-	*Bacillus cereus*
PR		-	+	+	-	*Bacillus cereus*
PM	-	-	+	+	-	*Bacillus cereus*
Kuka						
AH		-	+	+	-	*Bacillus cereus*
AR		-	+	+	-	*Bacillus cereus*
AM		-	+	+	-	*Bacillus cereus*
PH		-	+	+	-	*Bacillus cereus*
PR		-	+	+	-	*Bacillus cereus*
PM	-	-	+	+	-	*Bacillus cereus*

PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature. (+) present; (-) absent

The obtained microbial inactivation values varied from 0 to 42.0% of the initial population in okro (ila) soup samples, 0-44.40% of the initial population in ewedu soup samples,0 - 85.0% of the initial population in ogbono soup samples and 0-100.0% of the initial population in ewedu soup samples. This observation is consistent with the results presented by Georgsson et al. (2006) who described the fragility of the organisms relative to the freezing process. The repetitive mechanical freezing process or desiccation of the frozen product over the storage period might be responsible for the considerable reduction in the bacterial counts (Georgsson et al., 2006). The structural disarray caused by the freezing process might result into loss of fluid that is rich in proteins, vitamins and minerals (Leygonie et al., 2012), which consequently provides an excellent medium for microbial medium for microbial growth. The bacteria counts of < 104CFU/g (4.0 Log CFU/g) indicated a tolerable level of contamination (FAO, 1979; Ossai, 2012) and this might not pose any health risk if the storage and handling practices are under strict hygienic control. Microwave-thawed soups in plastic containers had high rate of microbial destruction when compared with other procedures. This happened because microwave heating is a rapid process and heat distribution during microwave heating is more uniform than conductive heating (Karel and Lund, 2003), which is less uniform, time consuming and encourages microbial build up (Leygonie et al., 2012).

Sensory qualities of freshly prepared and freeze-thawed selected soup

The results of sensory evaluation of re-frozen soups are presented in Tables 10-13. Statistical analysis of the freeze-thawed soups assessed for organoleptic characteristics (taste, colour, aroma, consistency and overall acceptability) revealed significant differences ($p<0.05$) in the organoleptic characters within the column (Tables 10-13). Freshly prepared soups were rated significantly ($p<0.05$) higher than others and they were the mostly preferred. Room temperature thawed soups in aluminium containers had the least scores for all the sensory attributes. Panelists rated microwave thawed soups in plastic containers significantly ($p<0.05$) higher than other freeze-thawed soups for taste, colour, aroma, consistency and overall acceptability.

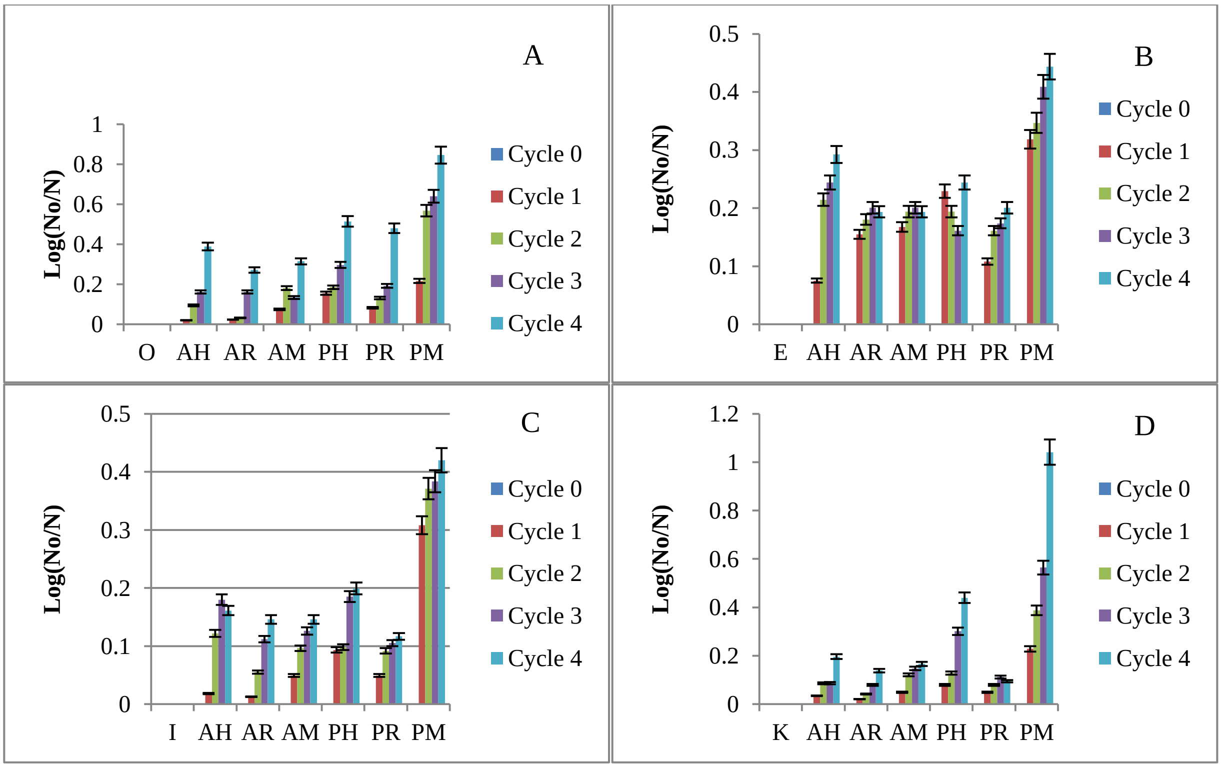

Fig. 1. Destruction of bacterial cells in A -*ogbono* soups; B - *ewedu* soups; C - *ila* soups; D - *kuka* soups subjected to freeze-thawed cycles. N_o= Initial microbial population N= Final microbial population O= Freshly prepared Ogbono soup, PH= Packaged in plastics but thawed with hot water, AH= Packaged in aluminium but thawed with hot water, PM= Packaged in plastics but thawed with microwave AM= Packaged in aluminium but thawed with microwave, AR= Packaged in aluminium but thawed at room temperature and PR= Packaged in plastics but thawed at room temperature.

The nature of the packaging materials and thawing conditions hada significant influence on the organoleptic characteristics of the soups. This is similar to the findings of Goral (2008) who reported the effect of different techniques of freezing and defrosting on the quality of spice vegetables after a long-term frozen storage. Plastic containers absorbed heat and retained it to thaw the soups packed in them at a considerable rate in hot water and microwave thawing conditions, while aluminum containers conducted heat away from the soups they contained, creating less effective thawing rate in hot water and microwave thawing conditions (Raji et al., 2016). The slowest rate of defrosting was obtained in soups packaged in aluminium containers and thawed under atmospheric condition. In addition, the reductions in the organoleptic attributes might be due to activities of spoilage agents (Oriakpono et al., 2011) and biochemical changes occurring therein with increasing thawing time(Alizadeh et al., 2007).

Table 10. Sensory qualities of freshly prepared and freeze-thawed *ogbono* soups after 4thcycle

Sample	Taste	Colour	Aroma	Consistency	Overall Acceptability
FP	8.50±0.69^{a}	8.15±0.93^{a}	8.10±0.97^{a}	8.35±0.75^{a}	8.25±0.79^{a}
AH	5.45±0.83^{d}	5.30±0.80^{d}	5.15±0.93^{d}	5.30±0.87^{d}	5.20±0.83^{d}
AR	3.45±0.89^{e}	3.50±1.00^{e}	3.45±1.10^{f}	3.70±1.03^{e}	3.80±1.10^{e}
AM	5.65±0.81^{d}	5.70±1.08^{d}	5.55±1.10^{d}	5.65±0.99^{d}	5.65±0.67^{d}
PH	6.40±0.75^{c}	6.50±1.05^{c}	6.05±0.61^{c}	6.20±0.70^{c}	6.35±0.59^{c}
PR	3.60±0.75^{e}	3.85±0.67^{e}	4.15±0.75^{e}	3.85±0.81^{e}	4.20±0.77^{e}
PM	7.20±0.83^{b}	7.25±0.85^{b}	7.50±0.83^{b}	7.35±0.81^{b}	7.55±0.61^{b}

Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly prepared, PH= Packaged in plastics, thawed with hot water, AH= Packaged in aluminium, thawed with hot water, PM= Packaged in plastics, thawed with microwave AM= Packaged in aluminium, thawed with microwave, AR= Packaged in aluminium, thawed at room temperature and PR= Packaged in plastics, thawed at room temperature.

Table 11. Sensory qualities of freshly prepared and freeze-thawed *ewedu* soups after 4th cycle

Sample	Taste	Colour	Aroma	Consistency	Overall Acceptability
FP	8.45±0.69^{a}	8.20±0.83^{a}	8.05±0.95^{a}	8.40±0.75^{a}	8.25±0.79^{a}
AH	5.55±0.89^{d}	5.35±1.04^{d}	5.10±0.91^{d}	5.40±1.10^{d}	5.20±0.83^{d}
AR	3.50±0.95^{e}	3.60±1.00^{e}	3.40±1.10^{f}	3.65±1.00^{e}	3.80±1.11^{e}
AM	5.75±0.85^{d}	5.55±1.00^{d}	5.45±1.10^{d}	5.80±0.95^{cd}	5.65±0.67^{d}
PH	6.40±0.75^{c}	6.45±1.00^{c}	6.10±0.64^{c}	6.30±0.66^{c}	6.35±0.59^{c}
PR	3.60±0.75^{e}	3.90±0.72^{e}	4.10±0.79^{e}	3.85±0.67^{e}	4.20±0.77^{e}
PM	7.30±0.80^{b}	7.30±0.73^{b}	7.45±0.89^{b}	7.65±0.59^{b}	7.55±0.61^{b}

Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly prepared, PH= Packaged in plastics, thawed with hot water, AH= Packaged in aluminium, thawed with hot water, PM= Packaged in plastics, thawed with microwave AM= Packaged in aluminium, thawed with microwave, AR= Packaged in aluminium, thawed at room temperature and PR= Packaged in plastics, thawed at room temperature.

Table 12. Sensory qualities of freshly prepared and freeze-thawed *ila* soups after 4th cycle

Sample	Taste	Colour	Aroma	Consistency	Overall Acceptability
FP	8.45±0.69^{a}	8.00±0.97^{a}	8.05±0.95^{a}	8.00±0.92^{a}	8.20±0.83^{a}
AH	5.55±0.89^{d}	5.25±0.97^{d}	5.10±0.91^{d}	5.12±0.75^{d}	5.20±0.83^{d}
AR	3.50±0.95^{e}	3.65±1.04^{e}	3.40±1.10^{f}	3.45±1.00^{e}	3.85±1.09^{e}
AM	5.75±0.85^{d}	5.30±1.03^{d}	5.45±1.10^{d}	5.40±0.75^{d}	5.60±0.68^{d}
PH	6.40±0.75^{c}	6.40±0.94^{c}	6.10±0.64^{c}	6.35±0.67^{c}	6.45±0.61^{c}
PR	3.60±0.75^{e}	3.90±0.72^{e}	4.10±0.79^{e}	3.80±0.77^{e}	4.25±0.85^{e}
PM	7.30±0.80^{b}	7.35±0.81^{b}	7.45±0.89^{b}	7.40±0.75^{b}	7.50±0.61^{b}

Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly prepared, PH= Packaged in plastics, thawed with hot water, AH= Packaged in aluminium, thawed with hot water, PM= Packaged in plastics, thawed with microwave AM= Packaged in aluminium, thawed with microwave, AR= Packaged in aluminium, thawed at room temperature and PR= Packaged in plastics, thawed at room temperature.

Table 13. Sensory qualities of freshly prepared and freeze-thawed *kuka* soups after 4th cycle

Sample	Taste	Colour	Aroma	Consistency	Overall Acceptability
K	8.35±0.81^{a}	8.45±0.61^{a}	8.15±0.88^{a}	8.10±0.85^{a}	8.00±0.86^{a}
AH	5.20±0.77^{d}	5.30±1.17^{c}	5.15±0.99^{d}	5.20±0.83^{d}	5.30±0.57^{c}
AR	3.35±0.88^{e}	3.35±0.81^{d}	3.60±1.05^{e}	3.65±1.18^{e}	3.80±1.11^{e}
AM	5.50±0.83^{d}	5.50±0.95^{c}	5.40±0.94^{d}	5.40±0.75^{d}	5.55±0.51^{c}
PH	6.50±0.83^{c}	6.60±1.00^{b}	6.05±0.76^{c}	6.35±0.81^{c}	6.25±0.55^{b}
PR	3.55±0.76^{e}	3.85±0.67^{d}	4.15±0.81^{e}	4.15±0.88^{e}	4.45±0.69^{d}
PM	7.45±0.83^{b}	7.10±0.64^{b}	7.30±0.80^{b}	7.40±0.68^{b}	7.65±0.59^{a}

Mean values having different superscripts within a column are significantly different ($p<0.05$). FP = Freshly prepared, PH= Packaged in plastics, thawed with hot water, AH= Packaged in aluminium, thawed with hot water, PM= Packaged in plastics, thawed with microwave AM= Packaged in aluminium, thawed with microwave, AR= Packaged in aluminium, thawed at room temperature and PR= Packaged in plastics, thawed at room temperature.

Conclusions

The results of this research revealed that deterioration of the soups increased as the number of freeze-thaw cycle increased. Both types of packaging materials as well as methods of thawing, besides the number freeze-thaw cycles, had a significant influence on the stability and sensory quality of the soups. Microwave-thawed plastic packed soups had limited deteriorative changes and the highest rate of microbial inactivation at each cycle when compared with other thawing methods. In addition, microwave-thawed plastic packed soups were mostly preferred by panelists. However, their stability cannot be guaranteed if the cycle is above three, because of deteriorative increase in free fatty acid and peroxide values.

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