IASET: International Journal of Biology,
Biotechnology and Food Science (IASET: IJBBFS)
ISSN(P): Applied; ISSN(E): Applied
Vol. 1, Issue 1, Jan – Jun 2016; 7-22

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

INCORPORATION OF PAPAIN INTO ICE CREAM: IMPACT ON PAWPAW (*CARICA PAPAYA*) ICE CREAM QUALITY

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ABSTRACT

Pawpaw (*Carica papaya*) ice cream was produced and papain extracted from unripe pawpaw was added to it in order to inhibit ice crystal formation. Vanilla ice cream was used as a control. Proximate compositions, physiochemical, sensory and microbial properties of the ice cream samples were evaluated using standard methods. The proximate composition result indicated high moisture contents of the samples; pawpaw ice cream was significantly ($p<0.05$) lower (62.81%) than the control (65.64%). The ash and fat contents of vanilla ice cream (control) were significantly ($p<0.05$) higher (0.78%) and (7.77%) respectively than that of the pawpaw ice cream (0.45%) and (4.22%) respectively. However, protein and carbohydrate contents were significantly ($p<0.05$) higher in pawpaw ice cream (3.79% and 92.55%) than vanilla ice cream (2.79% and 87.55%) respectively. The total titratable acidity (tta) of both samples had no significant difference ($p>0.05$) (0.22% and 0.23%) respectively. However, they differed significantly ($p<0.05$) in total solids, pH, overrun and sugar contents. The meltdown rate of the pawpaw ice cream (0.02g/min) was lower than that of the control (0.03g/min), though they did not significantly ($p>0.05$) differ. In terms of all sensory attributes, the control sample was more preferred. The ice crystal perception test showed that the control had smoother and silky texture than the pawpaw ice cream sample which was slightly smooth and gritty/ coarse/grainy, slightly creamy and gummy. The microbial load of pawpaw ice cream was higher (3.6 x 103 cfu/ml) than the control (3.1 x 103 cfu/ml).

KEYWORDS: Ice Cream, Ice Crystals, Papain, Pawpaw, Physicochemical, Proximate, Sensory, Microbial, Vanilla

INTRODUCTION

Papain is an enzyme that is found naturally in unripe papayas (Shuren, 2008). Papain also known as papaya *proteinas*e is a cystein protease (ec 3.4.22.2) enzyme present in papaya (*Carica papaya*) and mountain papaya (*Vascencellea cundinamarcensis)* (Khouri, 1990). Historically, people harvest this enzyme by slashing the skin of unripe papaya and collecting the resulting sticky latex- like sap which collected. People also cook with unripe papayas relying on the green papaya to break up in the cooking process and release the enzyme (Dupras, 1990).

Commercially, papain is added to many preparation designed to tenderize meats. It is also used in wound debridement and other procedures and it is sometimes recommended as a dietary supplement for people who experience digestive problem as the papain supplement can help people break down food so that they are easy to digest (Shuren, 2008). However, in the dairy industry, the inhibition of ice- crystal growth in ice- cream mix by gelatin hydrolysate is produced by papain (Damodaran, 2007).

Ice cream which was derived from iced cream or cream ice is frozen desserts usually from dairy products such as milk and cream and often combined with fruit or other ingredients and flavors (Beeton, 1991). Most varieties contain sugar although some are made with other sweeteners. In some cases, artificial flavourings and colorings are used in addition to or instead of the natural ingredients (Beeton, 1991). When it begins to freeze, the mixture is beaten to stop the formation of ice crystals resulting in light and creamy product (Gorski, 1995).

Ice cream can also be defined as a smooth sweet, cold food prepared form a frozen mixture of cream, milk or evaporated milk (or both), to which non fatty milk solids are added. It also contain sugar (14% - 16%), emulsifiers, stabilizers, flavors and colorings, sometimes natural but most often artificial. The additives, which act as emulsifiers and stabilizers, are used to prevent heat shock and the formation of ice crystals during the production process. The yield is the increase in volume of a frozen product by adding air also known as “over run”. Air is added to ice cream to improve its ability to absorb flavorings and to facilitate serving. Without air, ice cream becomes heavy and soggy. On the other hand, too much air results in ice cream that is snowy and dry. Ice cream with an overrun of 20% - 50 % would yield a creamier ice cream that melts quickly (Larger *et.al.*, 1994).

Part of the fun of making ice cream is to make up new flavors and color and so fruits such as strawberry, banana, and apples are incorporated in the form of purees in ice cream mix for flavor and color. More so, ice cream manufactures continue to develop new flavoring using nuts, beers, coffee, tea, alaskas and fruit and one of the more recent has been the introduction of liqeur- flavoured ice- creams (Dickson, 1972).

Papaya fruit is a rich source of nutrients such as pro vitamin A *carotenoids*, Vitamin C, B vitamin, *Lycopene*, Dietary minerals and variety of phytochemicals. It is also rich in energy, boosting natural sugars, low in saturated fats and also rich in anti-oxidants such as beta-carotene, vitamin A, B, C, flavanoids, folate, and panthothenic acid. It also has small amount of calcium (Ca), Chloride (Cl), iron (Fe) phosphorus (P), potassium (k) silicon (si) and sodium (Na). Previous research has shown that pawpaw has remarkable medicinal virtues and is abundant in health benefits (Seagon, 2004).

Ice cream becomes an issue of concern when it forms ice crystals due to temperature fluctuations during storage and handling of frozen foods.

There could also be the problem of poor product development which in turn will affect the nutritional quality of the ice cream. There is also limited information on the use of papaya in ice cream production. The use of pawpaw in ice cream production will add to the variety of productions already in existence and the nutritional quality of the ice cream will be improved.

Therefore, the main aim of the study was to produce pawpaw ice cream and inhibit the formation of ice crystals using papain. The specific objectives were to extract papain form pawpaw (unripe) (*Carica papaya*), to produce pawpaw ice cream using papain as one of the ingredients, to carry out the sensory evaluation or the consumer acceptance of the pawpaw ice cream, to evaluate the proximate composition, physiochemical and microbial content of the pawpaw ice cream with the inclusion of papain.

MATERIALS AND METHODS

The pawpaw used for the analysis was obtained from Ndioro market in Ikwuano Local Government of Abia State. Laboratory equipments and other materials used for the laboratory work were obtained from Michael Okpara University of

Agriculture, department of Food Science and Technology Laboratory and Central Service Laboratory of National Root Crop Research Institute all in Umudike. All chemical reagents were of analytical grade.

Preparation of Papain

The process of papain preparation involved cutting the papaya (unripe) from top to bottom with razor blade making sure the cut was not more than 2mm deep; the unripe papaya was held over the collection dish, allowing the liquid to drop onto the dish and this was done for about 6 minutes (the papaya must not be squeezed to get the liquid out) the collection dish was then placed into the oven allowing the liquid papain to dry for five (5) hours. It was ready when it took a crumbly using appearance and it was then transferred into an air tight container using a stainless spoon and stored in a cool, dry place (Bailey, 2011).

Production of Ice Cream

The process involved the blending of the mixtures of eggs, milk, sugar, fat, and then pasteurizing to kill any existing bacteria and then homogenizing to produce a uniform texture. The mixture was cooled and rested to blend flavors and then poured into stainless bowls and then cooled to a soft serve consistency. The ripe pawpaw which has been washed sliced and blended into puree as well as 5g of papain were added together with other flavorings and then blended for even distribution. The finished product is then allowed to harden before storing in the freezer.

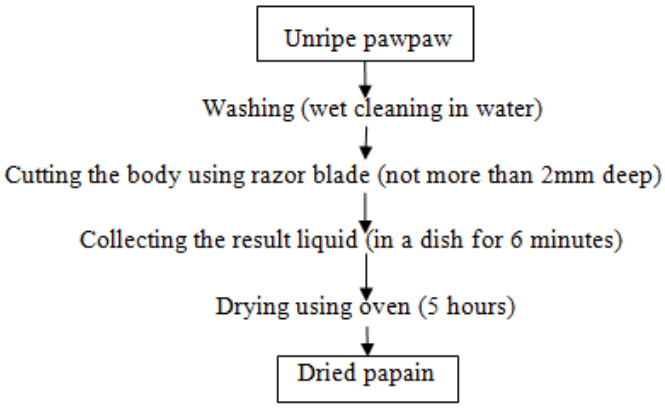

Figure 1: Flow Chart for the Preparation of Papain

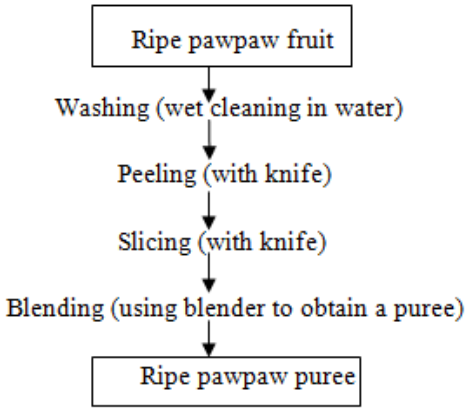

Figure 2: Flow Chart for Pawpaw Puree Production

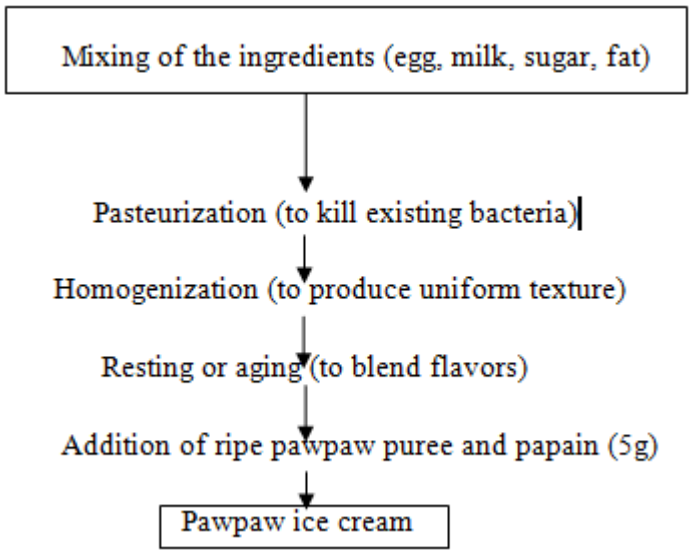

Figure 3: Flow Chart for the Preparation of Pawpaw Ice Cream Mix

The **proximate composition** of the pawpaw (*Carica papaya*) ice cream and a control (vanilla ice cream) were determined using the methods of Beeton (1991).

PHYSIOCHEMICAL ANALYSES

Over Run

Percentage overrun which is the added air to the product was determined according to Hui *et al*. (2004) as:

$$\text{Overrun} = \frac{\text{weight of cup mix} - \text{weight of cup of ice cream}}{\text{weight of cup of ice cream}} \times 100$$

Melting Rate

Melting rate was carried out for each sample according to Tharp *et al.* (1998) with slight modification. Melting property was analyzed for hardened ice cream (25g- 18^0C) which was placed on a sieve (2mm wide, square opening). The volume of the melted ice cream was measured and recorded at every 5min until the time of 60 min was reached. A plot was created of mass of drip loss divided by the total mass of the ice cream sample versus time and the slope of the main melting event was taken as the melting rate.

Total Titratable Acidity (TTA)

This determination was carried in accordance with the method by AOAC (1995), distilled water (100ml) was put into a conical flask. Five (5) ml of the sample and 5 drops of phenolphthalein indicator were added to the distilled water in conical flask. The mixture was titrated against 0.0IN solution of sodium hydroxide. The end point was reached when a change of colour was observed after adding a drop of the sodium hydroxide solution.

$$\% \text{ titratable acidity} = \text{average titre} \times 0.09008$$

pH

The pH was measures directly using a pH meter (Jens ways model) twenty (20) ml of the sample was put in a 50ml glass beaker. The electrodes of the pH meter were put inside the ice cream and pH reading was read directly from the screen of the meter when the pointer became steady.

Total Soluble Solids

This was determined using AOAC (1995) method. Two ml of the sample was measured with a previously weighed evaporating dish. The dish and sample content was put in a carbohydrate moisture extraction oven at 105^0C. The sample was evaporated to dryness and dried to a constant weight. It was allowed to constant weight was obtained.

Calculation

$$\% \text{ total solid} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where:

W1 = weight of moisture can

W2 = weight of can sample before drying

W3 = weight of can sample after drying.

MICROBIAL ANALYSIS

The microbial content of ice creams depends largely on the quality of the ingredients used for their manufacturing. Milk, cream, sugar, water, etc are normally blended and pasteurized and their microbial counts are generally low but flavourings, colorings agents' papaya fruit which were added to the mix after the pasteurization can also be a source of contamination. In addition, post- pasteurization can also occur from unclean equipments and air incorporation. The number and types of spoilage organisms will depend on the extent of post pasteurization contamination and one time temperature exposure. The present study evaluated the microbial quality of pawpaw ice cream and vanilla ice cream and 50ml of each sample of were taken to the laboratory in an insulated container and were tested immediately for microbial quality. Dilution of the melted ice cream samples were made up to 10^{-6} using 0.1% peptone water and selected dilution of each sample were used for the estimation of various microbial loads per gram of the sample. The microbial qualities were evaluated by estimating the total viable count (TVC) Lancette and Benette (2001). The count was expressed in $\log_{10}$ cfu/g. The data obtained from the above study were subjected to statistical analysis following the procedure described by Rangaswami (1995).

SENSORY ANALYSIS

Sensory analyses were carried out using the method described by Ihekoronye and Ngoddy (1985). A 20 semi trained panelist were used to rate the sensory attributes of appearance, taste, mouth feel, flavour (1 = dislike, extremely, 5 = neither, Neither like nor dislike, 9= like extremely) were used to score the product. In addition, to the sample, another sample (vanilla ice cream) a commercial ice cream produced by Crunchies Fried Chicken Umuahia was used as a control.

Ice Crystal Perception Test

The ice crystal perception test was carried out subjectively using trained panelists to perceive the ice cream texture (mouth feel).

STATISTICAL ANALYSIS

The result from proximate, microbial, physiochemical, and sensory were subjected to analyses of variance (ANOVA) according to Rangaswami (1995). Statistical analyses of data were carried out using ANOVA and SPSS version 15.

RESULTS AND DISCUSSIONS

The result of the proximate composition of pawpaw ice cream produced by incorporating papain to the ice cream mix is shown in table 1. Moisture content of the control (vanilla ice cream) (65.64%) was significantly higher ($p<0.05$) than that of pawpaw ice cream (62.81%). Moisture content is an index of water activity of many foods and the observed high moisture content in the ice cream samples is an indication that as expected they may have a short shelf life since spoilage micro organisms thrive in foods having high moisture and also indicates how total solid (Adepoju *et al.*, 2006). Ash content which is an index of mineral content in biota (Eneche, 2005) was lower 0.45% in the pawpaw ice cream and significantly differed ($p<0.05$) from the control 0.78%.

Also, pawpaw ice cream sample had a lower fat content (4.22%) than the control (7.77%) which could imply that the control (vanilla ice cream) requires a better storage condition to prevent rancidity. However, fats provide texture to foods, hold water, provides mechanism for heat transfer at higher temperatures and act as a carrier for fat soluble flavor molecules (Onimawo *et.al*, 2006). Fat also plays an important role in improving the consistency and mouth feel of ice cream (Christian and Greger, 1985). A study carried out by Meydani and Ha (2000) also reported that the consumption of low fats ice cream can promote weight loss. The control sample had significantly ($p< 0.04$) lower protein (2.79%) than the pawpaw ice cream sample (3.90%). This difference in protein could be as a result of the presence of papain incorporated in the pawpaw ice cream, as papain is a proteinous enzyme (Delbridge, 1988).

Carbohydrate content of the control ice cream sample was 87.55 % and 92.55% in the pawpaw ice cream. The significant difference ($p<0.05$) in the carbohydrate content of the samples might be as a result of the incorporation of the pawpaw puree to the ice cream. The protein content of the control was increased while its carbohydrate content was decreased and this was in line with increase in protein and subsequent reduction in carbohydrate (Khouri, 1990).

The physicochemical properties of the Pawpaw and Vanilla ice cream samples were shown in table 2. Total solids of the control (34.36%) was significantly lower ($p<0.05$) than that of pawpaw ice cream (37.19%). The values obtained for both samples were higher than that (20.77 – 24.27%) reported by Miller *et.al*. (2000). Li *et al.* (1997) found that ice creams with a high amount of total solids (39%) (the sum of the fat, milk solids-not-fat, sugars, stabilisers, and emulsifiers) melted faster than did those ice creams containing low amounts of totals solids (33%). This, they argued, was probably due to the effect of dissolved solids on lowering the freezing point. Similar results were reported by Kurultay *et al*. (2010) who reported that melting rapidly increased with increasing total solids from 20, to 30, to 40%. Ice cream mix with low total solids (high water content) has proportionately more water to freeze than a higher total solids mix (low water content) hardened to the same storage temperature. The total solids of the ice cream mix is directly related to ice crystal size

distribution (Flores and Goff, 1999) and lower total solids ice cream contains larger ice crystals (Donhowe *et al*., 1991).

There was no significance difference ($p > 0.05$) in the P^H of the vanilla ice cream and pawpaw ice cream (6.52% and 6.67%) respectively although, the control sample had lower acidity ($P^H = 6.52$). The result of this study is in line those reported by O' Neil *et al.* (1979); Sokolinska *et al.* (2004) who indicated that the pH values of ice cream decreased during the manufacturing process from (6.7 to 4.34). The pH of all samples were near neutral pH and since lower pH (acidic) in foods helps to reduce the activity of spoilage microorganisms it implies that all the ice cream samples may have low shelf stability. Thus, there is need for cold storage in order to extend its shelf stability (Umelo *et al*., 2014). There was no significant difference ($p > 0.05$) in the total titratable acidity (TTA) of vanilla ice cream (control) (0.23%) and pawpaw ice cream (0.22%). Ice cream with very low total acids could encourage the growth of proteolytic and lipolytic bacteria which are implicated for deterioration of ice cream not adequately refrigerated (Umelo *et al*., 2014).

The overrun result of the control was lower (63.71%) than that of the pawpaw ice cream (69.77%). Ice cream containing a high amount of air (high overrun) tends to melt slowly (Goff & Hartel, 2013). Air cells act as an insulator and slow the ability of heat to penetrate into the ice cream and melt the ice crystals, thus reducing the rate of meltdown (Sofjan & Hartel, 2004). Sakurai *et al.* (1996) found that ice creams with low overruns melted quickly, whereas ice creams with high overruns began to melt slowly and had a good melting resistance. This slower melting rate in the ice creams with high overrun was attributed to a reduced rate of heat transfer due to a larger volume of air but may also be due to the more tortuous path through which the melting fluid must flow (Hartel *et al*., 2003).

From the result as shown in figure 3, the pawpaw ice cream sample at (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes) had a lower melt down value (0.02g/min) than the vanilla ice cream (control) sample (0.03g/min) at the same time rate. This could be as a result total solids present in the pawpaw ice cream and conforms to the observation of Reuben (2016) for a desirable ice cream. According to Lin *et al*. (1974) melt down stability is important as it examines the effect of fat globule size and fat agglomerate size. The melt-down rate of ice cream is affected by many factors, including the amount of air incorporated, the nature of the ice crystals, and the network of fat globules formed during freezing (Muse and Hartel, 2003). A slow melting rate and good shape retention are generally considered desirable qualities in ice cream (Reuben, 2016). Ice cream has three main structural components: air cells, ice crystals, and fat globules, which are dispersed throughout a continuous phase of unfrozen solution (Muse & Hartel, 2003). These components affect the melting rate. Structural attributes include properties of the air phase (overrun and air cell size distribution), fat phase (total fat content, fat globule size distribution and extent of fat destabilisation), ice phase (ice phase volume and ice crystal size distribution), and the continuous phase (viscosity) (Hartel *et al.*, 2003).

Table 3 shows the sensory result of the vanilla and pawpaw ice cream. The control (vanilla ice cream) sample was more preferred in terms of taste, color, flavor, mouth feel and general acceptability than the pawpaw ice cream. All the sensory attributes were significantly different ($p<0.05$) in both samples. The difference in color of the ice cream samples could be as a result of the incorporation of the pawpaw fruit to the ice cream to make up new flavors and colors (Beeton, 1991). The difference in taste of pawpaw ice cream could be as a result of the peculiar pawpaw flavor as well as the papain which was added to inhibit ice crystal formation, which imparted a slightly bitter taste. Papain produces an unpleasant bitter sensation on the tongue and throat when taken (Shuren, 2008). Flavor difference could also be as a result of the natural odour associated with ripe pawpaw fruit which produces an unpleasant after smell when is exposed to air.

As shown in table 4, the pawpaw ice cream sample was smooth but slightly gritty, coarse or grainy and this could

be attributed to sudden temperature fluctuation (heat shock) during refrigeration storage which caused the initial tiny ice crystals that were formed and made it smooth to become enlarged as they melt and refreeze, thus becoming larger in size (Simonite, 2008) as well as probable lactose crystals, insoluble and milk solids suspended in the aqueous phase and the colloidal nature of the ice cream mixes. It could also be due the quantity of total solids in the sample (Reuben, 2016). The smoothness and creaminess of pawpaw ice cream could further be attributed to the incorporation of papain into the ice cream which has been reported to be effective in preventing large ice crystals formation (Damodaran, 2007) as well as the activities of the stabilizers. Stabilizers are hydrophilic colloids which impart a higher viscosity to the mix, maintain homogeneity and prevent the growth of large ice crystals during aeration/ freezing, which tend to make ice cream coarse (Klahorst, 1997).

The crunchiness and gumminess observed in the sample could result from the pawpaw used in the ice cream production which affected the viscosity. The vanilla ice cream sample was observed to be smooth and silky in texture probably due to a more controlled refrigeration temperature used as it was purchased from a commercial outfit (Crunches) which has a more stable light supply. It could also result from the type of stabilizers used in the product. During serving and consumption, stabilizers contribute to uniform meltdown, mouth feel and texture. A stabilized ice cream is one that resists or retards structural changes in a dynamic environment. Although a group of viscosity-producing gums and hydrocolloids exists (generally referred to as "the stabilizers"), it's important to note that other elements also contribute to ice cream stabilization: naturally occurring milk proteins; emulsifiers; and heat and mechanical processing (pasteurization, homogenization and freezing. (Klahorst, 1997). This invariably implies that ice cream stabilization depends on its composition and ratios. The quantity of total solids in the vanilla ice cream could also be responsible for its smoothness and silkiness (Reuben, 2016).

Table 5 shows the microbial load of the vanilla and pawpaw ice cream samples. In both ice cream samples, Lactobacillus spp were identified, however the microbial load was higher in the pawpaw ice cream sample (3.6×10^3 cfu/ml) than the vanilla ice cream (3.1×10^3 cfu/ml). The microbial load for both samples was within acceptable range for dairy products (Jay, 2000). Organisms that were identified in the samples include *Lactobacillus* spp, *Bacillus* spp and *Micrococcus* spp. *Lactobacillus bulgaricus, Streptococcus thermpophilis* were reported as beneficial bacteria found in ice creams which exert probiotic effect (Trindade, 2001). According to FAO/WHO (2001), ice cream which is a functional food must contain at least 10^6-10^7cfu/g probiotic bacteria (lactobacillus) because they confer health benefits to their hosts. Nevertheless, both ice cream samples were short of the required load for probiotic bacteria. . The decrease in the count was as a result of freezing and thawing which may seriously damage the cells, causing death (lethal effect), or inhibition of multiplication and/or interruption of metabolic activity (sub lethal effect), which could defeat the potential advantages of probiotics (Forest *et al*., 2008).

Ice cream is beneficial to human health because of the bacteria it contains (Fedela *et al.*, 2009) although quantitative standards for ice cream bacteria differ (Tamline and Death, 1980).

CONCLUSIONS AND RECOMMENDATIONS

This study has shown that pawpaw ice cream is nutritional and had good physiochemical and organoleptic properties which did not differ much from the commercial vanilla ice cream. Pawpaw ice cream production could add to already existing ice creams in terms of colour, flavor and variety. Further study should be carried out to determine the shelf

life of the pawpaw ice cream. Also study on how to inhibit the unpleasant slight bitter after taste associated with papain and the flavor in the pawpaw fruit. Finally but not the least, objective study on ice crystals inhibition in pawpaw ice cream.

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APPENDICES

Table 1: Proximate Composition of Pawpaw Ice Cream and Vanilla Ice Cream

Sample (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Pawpaw Ice Cream	$62.81^{b} \pm 0.04$	$0.45^{b} \pm 0.00$	$4.22^{b} \pm 0.02$	$3.79^{b} \pm 0.01$	$92.55^{a} \pm 0.01$
Vanilla Ice Cream	$65.64^{a} \pm 0.22$	$0.78^{a} \pm 0.00$	$7.77^{a} \pm 0.1$	$2.79^{a} \pm 0.08$	$87.55^{b} \pm 0.20$
Control $LSD_{(0.05)}$	0.012	0.001	0.024	0.045	0.017

Means with different superscripts are significantly different ($p<0.05$)

Table 2: Physiochemical Properties of Pawpaw and Vanilla Ice Cream

Samples	Total Solid (%)	P^{H}	TTA (%)	Overrun (%)	Sugar (^{o}Brix)	Meltdown (g / min)
Pawpaw Ice Cream	$37.19^{a} \pm 0.04$	$6.67^{a} \pm 0.02$	$0.22^{a} \pm 0.01$	$69.77^{a} \pm 0.10$	$46.82^{a} \pm 0.03$	$0.02^{a} \pm 0.06$
Vanilla Ice Cream	$34.36^{b} \pm 0.22$	$6.52^{b} \pm 0.00$	$0.23^{a} \pm 0.00$	$63.71^{b} \pm 0.64$	$38.47^{b} \pm 0.02$	$0.03^{a} \pm 0.03$
Control $LSD_{(0.05)}$	0.019	0.036	0.006	0.028	0.028	0.001

Means with different superscripts are significant different ($P<0.05$)

Table 3: Sensory Evaluation of the Pawpaw Ice Cream and Vanilla Ice Cream

Samples	Appearance	Taste	Flavor	Mouth Feel	Overall Acceptability
Pawpaw ice cream	$6.0^{b} \pm 1.72$	$5.6^{b} \pm 1.39$	$5.8^{b} \pm 1.91$	$6.0^{b} \pm 2.00$	$6.1^{b} \pm 1.29$
Vanilla ice cream	$7.5^{a} \pm 1.05$	$7.8^{a} \pm 0.83$	$7.1^{a} \pm 0.97$	$7.3^{a} \pm 0.98$	$7.0^{a} \pm 0.97$
Control $LSD_{(0.05)}$	0.261	0.185	0.117	0.103	0.1112

Means with different superscript are significantly different ($p< 0.05$)

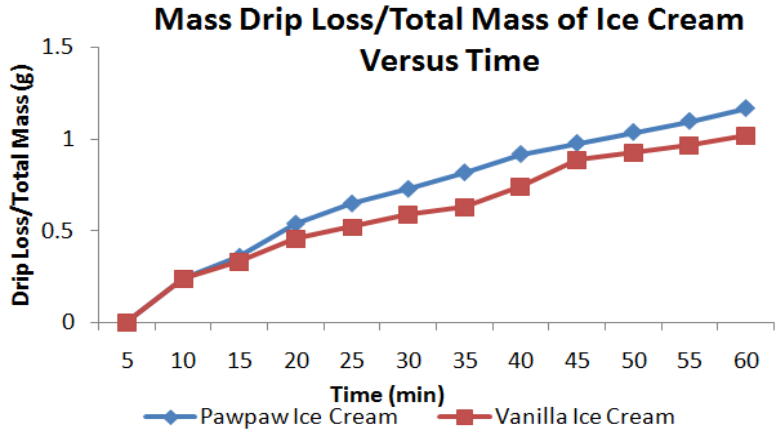

Figure 3: A Plot of Mass of Drip Loss/Total Mass of Ice Cream versus Time

Table 4: Ice Crystal Perception Test of Pawpaw and Vanilla Ice Cream

Samples	Observation
Pawpaw Ice Cream	smooth and slightly gritty, slightly creamy and gummy slightly coarse or grainy and crunchy
Vanilla Ice Cream	smooth, silky texture

Table 5: Microbial Analysis of Pawpaw Ice Cream and Vanilla Ice Cream

Samples	Cfu/ml	Organism Identified
Pawpaw Ice Cream	3.6×10^3	*lactobacillus spp, bacillus spp, streptococcus spp and micrococcus spp*
Vanilla Ice Cream	3.1×10^3	*lactobacillus spp, bacillus spp, streptococcus spp and micrococcus spp*