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**Original Research Article** 

# **Studies on the Production of Cream Liqueur Using Whiskey and Milk Cream**

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**Abstract:** The quality of the liqueurs and their organoleptic properties depend on the characteristics of spirits or milk cream used as well as on the preparation procedures, including maceration and maturation processes. Hence the aim of this study is to produce a cream liqueur using whiskey as the spirit base and observe its shelf life stability. The specific objectives of this study were to produce cream liqueurs of different concentrations using whisky and milk cream, to evaluate the physicochemical properties of the cream liqueurs, to determine the shelf stability of the produced cream liqueur after three months of storage, to carry out sensory evaluation on the cream liqueurs in comparison with a commercial cream liqueur and to determine the acceptance of the liqueurs statistically based on parameters tested using One-Way ANOVA at P $\leq$ 0.05. Ten (10) samples of cream liqueur were produced using standard method with different concentrations of sodium caseinate and were labelled Samples A to J. The cream liqueur samples were kept and stored at 30°C for 3 months for shelf study. The result of organoleptic evaluation showed that there was no significant (P>0.05) difference in taste, aroma, appearance and overall acceptability between the cream liqueur samples and a commercial cream liqueur. The presence of alcohol in the cream liqueur helps in the preservation of the liqueur and ensures maximum microbial stability and extended shelf life. It can be concluded therefore, that whiskey and cream milk are good raw materials for cream liqueur production.

Keywords: Cream liqueur, whiskey, shelf life, milk cream, organoleptic, *Lactobacillus*, *Streptococcus* and *Acetobacter*. Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

#### **INTRODUCTION**

Cream liqueurs are products that mostly contain cream, colors, sodium caseinate, alcohol, flavors, low- molecular-weight surfactants and sugar. Carbohydrate source in alcoholic containing beverages includes honey, lactose, sucrose, dextrin, corn syrups, molasses; maltose, ribose, galactose, modified starch and glucose have been proposed for use (Banks *et al.*, 2010). Cream liqueur products that are well known, such as Bailey's Irish Cream are basically emulsions formed from a blend of cream and aqueous alcoholic spirits. Emulsion stability is a recurrent problem with such products, which is the ability of the 2 (two) phases of the emulsion to resist change for a long time and/or stress. During storage, the main problem facing cream liqueur is physical instability, and homogenization is a key technological step in producing cream liqueurs with very small diameters of fat globules and thereby ensuring the product's long-term stability. In the manufacture of cream liqueurs, the most important step is homogenization, which is imperative to produce a subtly dispersed emulsion and also to ensure the long term shelf stability of cream liqueurs (Banks *et al.*, 2010).

The homogenization technology employed has a profound effect on shelf life of the resulting cream liqueur. When the fat globules is more stable in the aqueous phase of the emulsion, coalesce is less likely and also devoid of creaming or the unsightly neck plug as seen in extremely aged cream liqueurs that occurs in

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the bottle necks. During homogenization, the fat globules size is reduced and sodium caseinate transfers from the serum phase to the newly- exposed fat globule surface to stabilize the product. The distribution of droplet size is a very essential characteristic of an emulsion, influencing stability (Heffernan *et al.*, 2009). Cream Liqueur of high quality can have a shelf life of 2 (two) years when stored in ambient condition and the sensory quality can be maintained even further when stored or kept under refrigeration conditions. The formation of ethyl esters is a major limiting factor to the sensory quality of cream liqueurs which increases in concentration after a while. These compounds are formed when fatty acids and alcohol reacts and appears as fruity notes in the cream liqueur (Heffernan *et al.*, 2009).

In the production of whiskey, the methods varies based on the country it originates from, the style being made, and other factors, but generally, the procedure in most cases remains the same (Agu, et al., 2006). Whisky begins as raw grain, which has to be specially treated in order to access its sugars. The barley is moistened, and then allowed to partially germinate or sprout, a process called malting which secretes or releases an enzyme that converts the grain starches to sugars. Germination is halted when the grain is dried by kilning. Before fermentation, the sugar contained in the grain must be extracted through mashing. The grains being used like barley, corn, wheat, oat or rye are ground up, poured in large tank called mash tun or tub, with hot water and agitated. In order to help catalyze the conversion of starches to sugars, some ground malted barley is usually added. The mixture gotten as a result resembles porridge. When sugar has been extracted, the mixture now known as mash or wort then goes to the fermentation stage (Agu, et al., 2006). The fermentation occur when the mash/wort meets yeast; Saccharomyces Cerevisiae, which gobbles up all the sugars in the liquid and converts them to alcohol. This takes place in giant vats, often called wash backs. The process normally takes between 48 to 96 hours, at different fermentation times and yeast strains that result in a spectrum of diverse flavours. The resulting beerlike liquid called distiller's beer clocks in at around 7% - 10% ABV before going into the still. Distillation increases the alcoholic content of the liquid and emits volatile components, both good and bad. Stills for distillation usually are made of copper, which in turn helps to strip spirit of unwanted flavours and aroma compounds. The two common types of stills are pot and column, both functions differently.

#### **MATERIALS AND METHODS**

The materials used for this study include; whiskey, low fat milk, sodium caseinate (Emulsifier), cloudy agent, phosphoric acid (good grade), aspartame (sweetener), sodium benzoate (preservative) and distilled water. Media used included: Nutrient agar, MacConkey agar, De Man, Rogosa and Sharpe (MRS) agar and Sabouraud Dextrose agar.

Reagents used included Gram stain reagent, Kovac's reagent, lactophenol cotton blue reagents.

#### **Sources of Materials**

All the materials were purchased from Onitsha main market in Anambra state. Media and reagents were obtained from Conraws Nigeria Limited. Other equipment was supplied/provided by the Laboratory of the Department of Applied Microbiology and Brewing, ESUT.

#### METHODOLOGY

#### **Preparation of Cream Milk**

According to Muir, 2012., Creamer was prepared from low fat milk by boiling for 3-5 minutes, after which, the milk is set aside for 2 hours in the fridge so that thick malai gets collected at the top. The malai was carefully collected with a spoon and put in a clean container. The malai was stored in a fridge until ready to use.

#### Preparation of Cream Liqueurs with Ten (10) Concentrations of Creamer

A total of ten (10) samples of cream liqueur were prepared in the following order

Sample A = prepared with 10ml of sodium caseinate (emulsifier)

Sample B = prepared with 20ml of sodium caseinate (emulsifier)

Sample C = prepared with 30ml of sodium caseinate (emulsifier)

Sample D = prepared with 40ml of sodium caseinate (emulsifier)

Sample E = prepared with 50ml of sodium caseinate (emulsifier)

Sample F = prepared with 60ml of sodium caseinate (emulsifier)

Sample G = prepared with 70ml of sodium caseinate (emulsifier)

Sample H = prepared with 80ml of sodium caseinate (emulsifier)

Sample 1 = prepared with 90ml of sodium caseinate (emulsifier)

Sample J = prepared with 100ml of sodium caseinate (emulsifier)

The sodium caseinate (emulsifier) samples were measured with measuring cylinder and made to dissolve in 200ml of water at  $65^{\circ}$ C. One hundred Milliliters (100ml) of creamer (milk) was then added to each conical flask at a continuous high speed to form a cream base. Then, 2ml of aspartame (sweetener) was dissolved in 150ml of whisky and mixed thoroughly to form pre-emulsion. The pre-emulsion formed was mixed thoroughly with the previously formed cream base at  $65^{\circ}$ C. A total of 100ml of ethanol was measured and mixed vigorously with the cream base. Caramel (3ml) was then added to the cream base to improve the colouring of the cream liqueur. The cream liqueur was allowed to cool at room temperature for 30mins.

### Physiochemical Analysis of the Cream Liqueur Samples

The following are the physicochemical analysis carried out on the cream liqueurs according to Banks, and Muir, (2015). These parameters are percentage alcohol, pH, sugar level, viscosity and total reducing sugar.

#### Percentage Alcohol (v/v)

An alcohol meter was dipped into 100ml of the cream liqueur in a measuring cylinder and the percentage alcohol (v/v) was read-off from the alcohol meter.

#### Percentage Sugar

The sugar percentage in the cream liqueur samples was determined using Brix refractometer. A drop of each cream liqueur sample was placed on the glass prism of the equipment and the sugar level was read from the eye piece lens.

#### pН

A pH meter was used for this analysis. The pH meter was first dipped in a buffer solution to standardize it. It was removed from the buffer solution and then inserted into the conical flask containing the cream liqueur. The stable figures on the display of the pH meter were read as the pH value.

#### Viscosity

The instrument used was rotational viscometer which is used to measure the absolute viscosity of the cream liqueur. Prior to measurement of absolute viscosity, liqueurs were transferred to a 20°C water bath for 1 hour. They were then transferred to the NV concentric cylinder measuring system of a Haake RV 20 rotational viscometer (Haake, Karlsruhe, Germany), which was also maintained at 20°C by circulating water from a temperature controlled water bath (Haake). A rotating apparatus known as the spindle was submerged within the cream liqueur in a beaker. The shear rate was increased from 0 to 20S<sup>-1</sup> over a 5 min period and maintained at 20S<sup>-1</sup> for a further 5 mins, during which time the mean shear stress was determined. The torque on the rotating shaft of the spindle measures the resistance of the cream liqueur to flow.

#### **Total Reducing Sugar**

Ten percent (10%) dilution of cream liqueur was prepared by adding 10ml of cream liqueur to 100ml of water and the dilution used in filling 50ml burette. Using 20ml measuring cylinder, 25ml each of Fehling's solution A and B was measured into a narrow necked boiling flask for each sample. A drop of methylene blue indicator was added to the mixture which was titrated against the dilution of wort. The content of each flask sample was heated over wire gauze in a moderate ebullition for 2 mins without removal from the flame and the colour changes were noted. Red precipitate was formed and the reading was recorded accordingly as the end point.

The Lane and Eynon formulae were used for the calculation.

Reducing sugar =  $\underline{\text{Lane and Eynon factor x 10}}$ Titre

Lane and Eynon factor = Reading from the table Titre = Reading obtained after titration

#### Shelf Life Study

The cream liqueur samples were kept and stored at  $30^{\circ}$ C for 3 months to determine its shelf stability.

#### **Microbiological Analysis**

After three (3) months of storage, microbiological analysis was done on the cream liqueur samples to determine their shelf stability.

#### Media Preparation

#### Nutrient Agar

The medium was prepared according to specification of the manufacturer. The nutrient agar powder (2.8g) was dissolved in 100ml of distilled water; the medium was sterilized in the autoclave at  $121^{\circ}$ C and 15p.s.i. for 15 mins and cooled to  $45^{\circ}$ C. The medium was dispensed in about 20ml amount into each sterile Petridis, allowed to solidify and later dried the surface at  $30^{\circ}$ C in a hot air oven to get rid of moisture prior to inoculation.

#### MacConkey Agar

This was prepared according to its specification by the manufacturer by dissolving 3.85g in 100ml of distilled water and sterilized in the autoclave at  $121^{\circ}$ C and 15p.s.i. for 15 min and cooled to  $45^{\circ}$ C. The medium was dispensed in about 20ml amount into each sterile Petri dish, allowed to solidify and later dried the surface at  $30^{\circ}$ C in a hot air oven to get rid of moisture prior to inoculation.

#### MRS Agar (De Man, Rogosa and Sharpe Agar)

This medium was prepared according to its manufacturer's instructions. A total of 6.7g of the powder of MRS agar (De Man, Rogosa and Sharpe agar) was dissolved in 100ml of distilled water. The medium was made to dissolve by heating. It was sterilized by autoclaving at 121°C for 15 minutes and 15psi for 15mins. The sterilized medium was allowed to cool down to about 45°C-50°C, it was properly mixed and poured into sterile petri dishes.

#### Sabouraud Dextrose Agar

A total of 6.5g of the powder of Sabouraud Dextrose Agar was suspended in 100ml of distilled

water. The medium was made to dissolve by heating while agitating. It was autoclaved at  $121^{0}$ C at 15psi for 15minutes. The sterilized medium was allowed to cool down to about  $45^{0}$ C- $50^{0}$ C. About 0.5ml of chloramphenicol was added to augment antibacterial effect and agitated for uniform mixture. Then poured into sterile petri dishes in about 15ml – 20ml each. The medium was allowed to solidify on these plates and were thereafter used.

#### Culturing and Incubation

The cream liqueur samples were homogenously mixed and 1ml of each was taken and tenfold serial dilution up to  $10^{-5}$  was made. Streak plate method was used for the inoculation. Using a sterilized wire loop, one millilitre of the sample was inoculated into already prepared Nutrient (enumeration of other bacteria), MacConkey (enumeration of coliform), De Man Regosa Sharp (MRS) (enumeration of lactic acid bacteria) and Sabouraud Dextrose agar (fungi enumeration) plates. After the inoculation of the samples into petri dishes, the petri dishes were turned upside down in other to avoid the growth of other organism which could arise from the condensation of heat from the prepared media. The inoculated Nutrient agar (NA), and MacConkey agar plates were aerobically incubated overnight at 30<sup>°</sup>C for 48 hr, MRS agar plates were incubated at 37°C for 48 hr while that of Sabouraud Dextrose Agar (SDA) were incubated at 30°C for 2-5 days. Developed discrete colonies were counted using Colony counter prior to sub-culturing into different fresh sterile media plates to obtain pure cultures. The grown cultures were later subjected to identification.

#### **Identification of Isolates**

Isolates were identified using standard morphological characteristics and identification keys. The tests used in the identification of bacteria include morphology, gram reaction, and biochemical test and sugar fermentation.

#### Morphological Analysis of the Bacterial Isolates

The morphological appearances of the organisms (colour, size, opacity etc.) were recorded. The isolates were further subjected to more identification tests as stated below:

#### **Gram Staining Procedure**

The prepared smear was air-dried and heatfixed. The slides were flooded with crystal violet for 60sec and washed off with water, and then each smear again was flooded with iodine solution for 1 minute and was washed off with water. Thereafter, the slide was decolorized with acetone until the solvent draining from the slide appeared colourless and was immediately washed with water. It was counterstained with safranin for 30sec and washed off with water. The slides were air dried and a drop of immersion oil was added before observation under oil immersion objectives lens (x100).

## Biochemical Analysis to Identify the Bacteria Isolates

#### **Catalase Test**

A drop of hydrogen peroxide was made on one side of clean microscopic slide and a drop of water on the other side as the control. A colony was then collected with a sterile applicator stick and smeared on the slide containing hydrogen peroxide and was also done for the control. The presence of bubbles within 10 sec which indicated positive result was observed and recorded.

#### **Oxidase Test**

Using the wet filter paper method, a strip of filter paper was soaked with a little freshly made 1% of Kovac's oxidase reagent. A distinct colony was then collected with a sterile applicator stick and rubbed on the filter paper that is already containing the reagent. A positive reaction was indicated by an intense deeppurple hue which appeared 5-10 seconds after the application. For a 'delayed positive' reaction, colouration took 10-60 seconds to appear and for a negative reaction, there was absence of coloration on the filter paper.

#### Indole Test (Kovac's Method)

Peptone water (5ml) was dispensed into test tubes and sterilized at  $121^{0}$ C/15 p.s.i for 15 minutes. A loopful inoculum of each isolate was introduced into each test tube and for 3 days at  $37^{\circ}$ C. Kovac's reagent (3 drops) was added into the cultures in the test tubes after incubation. The presence of red ring which indicated a positive result was observed and recorded.

#### **Sugar Fermentation Test**

Peptone water (54g) was dissolved in 360ml of sterile water with 1.8g of bromothymol blue. Durham tubes were inserted invertedly in to the test tubes. A total of 9ml of mixed peptone water was added to each of the tubes and sterilized in an autoclave. To prepare the sugars, 1g of each sugar was added to 10ml of sterile distilled water and purified using membrane filter. The medium was allowed to cool (45-40°C) before the addition of 1ml of each sugar solution. Thereafter the different isolates were inoculated into the sugar fermentation medium and incubated at 37°C for 24h and observed for the formation of acid and gas.

### Physicochemical Analysis of the Stored Cream Liqueur Samples

The physicochemical analysis (percentage alcohol, pH, sugar level, viscosity and total reducing sugar) of the cream liqueurs after three months shelf study was determined using the method stated earlier.

#### **Organoleptic Analysis**

#### Sensory Test

Sensory test was carried out on the cream liqueur samples stored for two months in comparison

with a commercial cream liqueur. The quality attributes valuated for includes colour, taste, mouth feel and general acceptability. A group of ten panelists tested the cream liqueur samples and recorded their inferences and insights about the product. The samples were evaluated using a 9-point hedonic scale where 1 represents "dislike extremely" and 9 represents "liked extremely" for parameters (colour, taste, mouth feel and general acceptability).

#### **Data Analysis**

The data collected from sensory test of the cream liqueur samples were subjected to a one-way

analysis of variance (ANOVA) at 95% confidence level and means that differed were considered significant at P < 0.05.

#### **RESULTS**

The physicochemical properties of the cream liqueur samples produced as recorded in (Table 1) showed percentage alcohol range from 15.2 to 15.9v/v, sugar level 7.5to 8.4<sub>o</sub>Brix, pH 6.5to 6.8, viscosity 22to31cP for all samples, while all samples also have same glucose of 54.73mg/l and maltose of 88.79mg/l.

Samples	% Alcohol (v/v)	Sugar level ( <sup>°</sup> Brix)	pH	Viscosity (cP)			
_			-		Glucose	Maltose	
А	15.2	8.4	6.7	31	54.73	88.79	
В	15.3	8.2	6.5	31	54.73	88.79	
С	15.4	8.2	6.8	30	54.73	88.79	
D	15.3	8.1	6.7	28	54.73	88.79	
Е	15.5	7.9	6.7	28	54.73	88.79	
F	15.6	7.9	6.8	27	54.73	88.79	
G	15.5	7.8	6.5	25	54.73	88.79	
Н	15.8	7.7	6.6	24	54.73	88.79	
Ι	15.7	7.6	6.7	23	54.73	88.79	
J	15.9	7.5	6.8	22	54.73	88.79	

Table 1: Result of the Physicochemical Properties of the Cream Liqueur Samples

#### Key:

A = prepared with 10ml of sodium caseinate (emulsifier)

B = prepared with 20ml of sodium caseinate (emulsifier)

C = prepared with 30ml of sodium caseinate (emulsifier)

D = prepared with 40ml of sodium caseinate (emulsifier)

E = prepared with 50ml of sodium caseinate (emulsifier)

F = prepared with 60ml of sodium caseinate (emulsifier) G = prepared with 70ml of sodium caseinate (emulsifier)

H = prepared with 80ml of sodium caseinate (emulsifier)

1 = prepared with solid of sodium casemate (emulsifier) 1 = prepared with 90ml of sodium casemate (emulsifier)

J = prepared with John of sodium caseinate (emulsifier) J = prepared with 100ml of sodium caseinate (emulsifier)

The physicochemical properties of the cream liqueur samples produced after three months of shelf studies as recorded in Table 2 showed percentage alcohol range from 15.4to 16.0v/v, sugar level 7.2to

8.2°Brix, pH 5.9to 6.1, viscosity 19to27cP for all samples, while all samples also have same glucose of 54.73mg/l and maltose of 88.79mg/l.

Table 2: Result of the Physicochemical Properties of the Cream Liqueur Samples after Three Months of Shelf
Studies

Samples	% Alcohol (v/v)	Sugar level ( <sup>o</sup> Brix)	pН	Viscosity	Reducing	Sugar
					Glucose	Maltose
А	15.4	7.2	5.9	27	54.73	88.79
В	15.4	7.5	6.0	26	54.73	88.79
С	15.5	7.7	6.1	26	54.73	88.79
D	15.5	7.8	6.0	25	54.73	88.79
Е	15.6	7.8	6.2	24	54.73	88.79
F	15.6	7.9	5.9	23	54.73	88.79
G	15.7	7.9	5.8	22	54.73	88.79
Η	15.8	7.9	5.8	21	54.73	88.79
Ι	15.9	8.1	6.1	20	54.73	88.79
J	16.0	8.2	5.9	19	54.73	88.79

A = prepared with 10ml of sodium caseinate (emulsifier) B = prepared with 20ml of sodium caseinate (emulsifier) C = prepared with 30ml of sodium caseinate (emulsifier) D = prepared with 40ml of sodium caseinate (emulsifier) E = prepared with 50ml of sodium caseinate (emulsifier) F = prepared with 60ml of sodium caseinate (emulsifier) G = prepared with 70ml of sodium caseinate (emulsifier) H = prepared with 80ml of sodium caseinate (emulsifier) 1 = prepared with 90ml of sodium caseinate (emulsifier)

J = prepared with 100ml of sodium caseinate (emulsifier)

After shelf study for three months, the cream liqueur samples showed good microbiological stability with minimum microbial load. Bacterial count as shown in (Table 3) ranged from  $1.0 \times 10_6$  and  $1.9 \times 10_6$ , lactic

acid bacteria load ranged from  $1.0 \times 10^{5}$  and  $1.7 \times 10^{5}$  for all samples while no coliform and fungal were present in any sample.

Samples	Bacterial count	Coliform count	Lactic acid bacteria count	Fungal count
	(Nutrient agar)	(MacConkey agar)	(MRS agar)	(SDA)
А	$1.4 \times 10^{6}$		$1.2 \times 10^5$	
В	$1.1 \times 10^{6}$		$1.6 \times 10^5$	_
С	$1.6 \times 10^{6}$		$1.2 \times 10^5$	_
D	$1.1 \times 10^{6}$		$1.0 \mathrm{x} 10^5$	_
E	$1.4 \times 10^{6}$		$1.1 \times 10^5$	_
F	$1.3 \times 10^{6}$		$1.6 \times 10^5$	_
G	$1.0 \times 10^{6}$		$1.4 \mathrm{x} 10^5$	_
Н	$1.7 \times 10^{6}$		$1.7 \mathrm{x} 10^5$	_
Ι	$1.2 \times 10^{6}$		$1.1 \times 10^5$	
J	$1.9 \times 10^{6}$		1.3x10 <sup>5</sup>	

Key:

A = prepared with 10ml of sodium caseinate (emulsifier)

B = prepared with 20ml of sodium caseinate (emulsifier)

C = prepared with 30ml of sodium caseinate (emulsifier)

D = prepared with 40ml of sodium caseinate (emulsifier)

E = prepared with 50ml of sodium caseinate (emulsifier)

F = prepared with 60ml of sodium caseinate (emulsifier)

G = prepared with 70ml of sodium caseinate (emulsifier)

- H = prepared with 80ml of sodium caseinate (emulsifier)
- 1 = prepared with 90ml of sodium caseinate (emulsifier)

J = prepared with 100ml of sodium caseinate (emulsifier)

The isolates identified includes; Lactobacillus, Streptococcus and Acetobacter species as shown in (Table 4).

Isolates	Growth Appearance	Biochemical test					Possible				
type	of Media	Microscopic	Cat	Ind	Oxi	Suga	Sugar fermentation			organisms	
		features				Gl	F	Ml	Ma	La	
Α	Large mucoid creamy	+ short rod in			_	AG	Α	А	А	А	Lactobacillus
	colonies on MRS Agar	chains									sp.
В	Small creamy mucoid	+ cocci in	+	_		А	Α	AG	А	А	Streptococcus
	colonies on MRS Agar	chains									sp.
С	Large slimy, spherical	Gram –ve long	+	+	+	AG	Α	AG	А	AG	Acetobacter
	shaped pale colonies on	rod									sp.
	nutrient agar										

#### KEY:

MRS = Man Regosa Sharpe Agar, Cat = Catalase test, Ind = Indole test, Oxi = Oxidase test, Gl = Glucose, F = D-Fructose, Ml= Maltose, Ma = Mannitol. The table below showed the percentage of occurrence of microbial isolates from the cream liqueur samples with *Lactobacillus* species being the most prevalent at 100% followed by *Streptococcus* species at 80% while *Acetobacter* species had least occurrence at 60%.

Tuble 2. Occurrence i uttern of Milerobian Isolates							
Isolate types	No. of samples	Occurrence	Percentage (%)				
Lactobacillus sp.	10	10	100				
Streptococcus sp.	10	8	80				
Acetobacter sp.	10	6	60				

Table 5: Occurrence Pattern of Microbial Isolates

The table below showed the null hypothesis (Ho) is accepted as there was no significant difference in all the parameters (colour, taste, and mouth feel and general acceptability) tested at  $P \le 0.05$  level of

significance, since all the calculated values of F are less than the F-Table values. The samples are said to be good according to statistical analysis and can be marketed.

Table 6: The Sensory Evaluation Table								
	Colour Taste Mouth feel General acceptability							
F-Value	30.15	16.06	2.79	14.21				
F-Tabulated	3.354	3.354	3.354	3.354				
LSD	0.243	0.389	0.467	0.405				

#### **DISCUSSION**

These preliminary studies investigated and established; formulation and manufacturing procedures for small- scale manufacture of cream liqueur using whiskey and milk cream. Initially, skim milk was used as the emulsifying agent in commercial cream liqueurs, but age gelation occurred in the resulting products after short storage times. Stability can be improved by replacing skim milk by sodium caseinate, but rapid increases in viscosity and gelation of the products occurred at high ambient temperature. The quality of the liqueurs and their organoleptic properties depend on the characteristics of spirits or milk cream used as well as on the preparation procedures, including maceration and maturation processes.

The emulsion stability of cream liqueur products are known to be improved by adding stabilizing agents such as the potassium and/or sodium salts of citric acid. Cream liqueur's emulsion stability is a result of its mode of preparation and composition. It is also known that, apart from the preparation, processing and composition of cream liqueur products, e.g. the order of mixture influences emulsion stability, hence, the shelf life.

The physicochemical properties of the cream liqueur samples produced as recorded in Table 1 showed percentage alcohol range from 15.2 to 15.9v/v, sugar level 7.5to  $8.4^{\circ}$ Brix, pH 6.5to 6.8, viscosity 22to31cP for all samples, while all samples also have same glucose of 54.73mg/l and maltose of 88.79mg/l. This result indicated moderate alcohol concentration which is almost the same to commercial cream liqueur and similar to the results of Banks *et al.*, (2010). The alcohol content preserves the cream liqueur; therefore, no preservatives are required. The moderate sugar content gave the cream liqueur their desirable sweet

taste and the characteristics high viscosity also makes the cream liqueur desirable.

The physicochemical properties of the cream liqueur samples after three months of shelf study as recorded in (Table 2) showed percentage alcohol range from 15.4to 16.0v/v, sugar level 7.2to 8.2oBrix, pH 5.9to 6.1, viscosity 19to27cP for all samples, while all samples also have same glucose of 54.73mg/l and maltose of 88.79mg/l. This result is similar to the findings of Muir (2012). Viscosity is an important quality control factor in the organoleptic assessment of cream liqueurs. This can be simply controlled by varying the amount of added sodium caseinate (creamer) in the formulation. In agreement with the work of Banks and Muir (2015) and Muir (2012), products manufactured by a two- step process (alcohol/sugar added after homogenization) were less stable than one-step products (alcohol added before homogenization). Gelation/thickening was promoted by homogenization, probably due to a change in the conformation of the protein and/or an increase in the effective reactive protein concentration.

The sample cream liqueurs showed little changes in their physicochemical properties after three months of shelf study. There are noticeable decrease in sugar level, pH and viscosity and observable increase alcohol in each of the samples. These changes occur naturally and sometimes can be as a result of microbial activities. The decreased viscosity observed can be attributed to chemical activities that occur during storage and homogenization which forms emulsion. These findings are in consonance with the findings of Heffernan *et al.*, (2019).

After shelf study for three months, the cream liqueur samples showed good microbiological stability with minimum microbial load. Bacterial count as shown in Table 3 ranged from  $1.0 \times 10^6$  and  $1.9 \times 10^6$ , lactic

acid bacteria load ranged from  $1.0 \times 10^5$  and  $1.7 \times 10^5$  for all samples while no coliform and fungal were present in any sample. This result indicated that the alcohol present in the cream liqueur is adequate enough to store the cream liqueurs up to 3 months. This result is similar to the results gotten by other researchers (Ganbaro *et al.*, 2017; Georgescus *et al.*, 2012). Lactic acid bacteria (LAB) in the cream liqueur are from the milk cream used and their proliferation up to three months in the cream liqueur is as a result of their ability to withstand harsh conditions such as alcohol and their ability to utilize alcohol to produce lactic acid.

The isolates identified includes; *Lactobacillus*, *Streptococcus* and *Acetobacter* species (Table 4). The organisms have also been isolated by other researchers (Abbott and Savage, 2013). Furthermore, the isolation of *Lactobacillus* sp. and *Saccharomyces* sp. corroborates the earlier report of Lynch and Mulvihill (1997). The LAB present in the cream milk samples might be responsible for the observable change in pH towards acidity.

During shelf-life testing of emulsions, visual observation at ambient temperature best characterized creaming. The presence of cream rings or collars at the top of bottles or extensive watery serum at the bottom of bottles are signs of creaming and may be unacceptable in certain circumstances. This creaming occurred relatively quickly (in the first week) and was due to inadequate homogenization and/or clustering. It was more obvious at ambient temperature since incubation at 45 °C thickened the liqueur and thus inhibited creaming. The percentage of occurrence of microbial isolates from the cream liqueur samples with Lactobacillus species being the most prevalent at 100% followed by Streptococcus species at 80% while Acetobacter species had least occurrence at 60% as shown in (Table 5).

Sensory evaluation as shown in (Table 6) shows that the sample of cream liqueur produced from whiskey and milk cream is acceptable when compared with a commercially available cream liqueur. The result of organoleptic evaluation showed that there was no significant (P>0.05) difference in taste, aroma, appearance and overall acceptability between the cream liqueur samples and a commercial cream liqueur. In this sense, commercial cream liqueur appeared to have demonstrated greater consumption potential than that of than the locally produced samples.

#### **CONCLUSION**

From the results obtained in this research, it can be concluded that whiskey and cream milk are good raw materials for cream liqueur production. The presence of alcohol in the cream liqueur helps in preservation of the liqueur and ensures maximum microbial stability and extended shelf life. The cream liqueur samples produced compare favourably with a commercial cream liqueur when analyzed for colour, taste, mouth feel and general acceptability. The cream liqueur samples produced with higher concentration of creamer demonstrated greater emulsifying potential than samples produced with lower concentration of creamer.

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#### **REFERENCES**

- Abbott, P. H. E., & Savage, R. J. B. (2013). An investigation into the manufacture of cream-based liqueurs. *International Journal of Dairy Technology*, 38(2), 47-48.
- Agu, R. C., Bringhurst, T. A., & Brosnan, J. M. (2006). Production of grain whisky and ethanol from wheat, maize and other cereals. *Journal of the Institute of Brewing*, 112(4), 314-323.
- Banks, W., & Muir, D. D. (2015). Effect of alcohol content on emulsion stability of cream liqueurs. *Food Chemistry*, 18, 139-152.
- Banks, W., Muir, D. D., & Wilson, A. G. (2010). Extension of the shelf life of cream-based liqueurs at high ambient temperatures. *Journal of Food Technology*, 16, 587-595.
- Gámbaro, A., Ivankovich, C., Roascio, A., Amarillo, M., Miraballes, M., & Araya-Quesada, Y. (2017). Job-sorting assignment to assess cream liqueur brand image. *Beverages*, 3(1), 11-14.
- Georgescu, L., Martin, S., Milos, O., Berbente, F., David, I., & Bujancă, G. (2012). Acurate evaluation of liqueur cream. Agricultural Management/LucrariStiintificeSeria I, Management Agroecology, 14(2), 15-24.
- Heffernan, S. P., Kelly, A. L., & Mulvihill, D. M. (2009). High-pressure-homogenised cream liqueurs: Emulsification and stabilization efficiency. *Journal of Food Engineering*, 95(3), 525-531.
- Heffernan, S. P., Kelly, A. L., Mulvihill, D. M., Uwe, L., & Schuchmann, H. P. (2019). Efficiency of a range of homogenization technologies in the emulsification and stabilization of cream liqueurs. *Innovative Food Science and Emerging Technologies*, 12, 628-634.
- Lynch, A. G., & Mulvihill, D. M. (1997). Effect of sodium caseinate on the stability of cream liqueurs. *International Journal of Diary Technology*, 50(1), 1-7.
- Muir, D. D. (2012). Cream liqueur manufacture: Assessment of efficiency of methods using a viscometric technique. *Dairy Industries International*, 52, 38-40.