بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

STUDY ON COMPONENTS, ACIDITY AND TOTAL BACTERIA COUNT OF FERMENTED MILK PRODUCT " MISH" PRODUCT IN KHARTOUM STATE.

دراسة المكونات والحموضة والعد البكتيري الكلي لمنتج الالبان المتخمرة المش المنتج بولاية الخرطوم

By:

Nahla Hassan Elmalik Mohamed

B.Sc. (Honor) Animal Production.Sudan University of Science and Technology (2001).

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Supervisor

Dr. Anas Mohamed Osman.

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الاستهلال

بسم الله الرحمن الرحيم

قال تعالي :

(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

سورة الإسراء الآية (85)

Dedication

For my dear Mother

Acknowledgment

I thank God Almighty for giving me the health, strength, patience and courage to accomplish this work.

I would like to express my gratitude, thanks and appreciations to my supervisor Dr. Anas Mohamed Osman, for his suggestions, guidance and valuable advices, as well as his continuous assistance during all the stages of this research.

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Research Abstract

The current research was conducted to study the components, acidity and total bacteria count of mish product Produced in Khartoum state.

Twenty four (24) samples of mish ready for sale and consumption produced by 4 different milk factories, were collected. The samples are divided in to 4 groups A, B, C and D, 6 samples per each group and milk factory. All samples were then subjected to laboratory analysis.

The average fat % obtained, 3.283 ± 0.095, 3.400 ± 0.073, 3.233 ± 0.056 3.417 ± 0.101 for group A, B, C, S respectively. The Statistical analysis revealed no significant difference between the averages of the samples of all groups.

Values obtained for protein % were 9.283 ± 0.496 8.567 ± 501, 9.200 ± 0.480, 8.600 ± 0.505 for group A, B, C and D respectively. Also no significant variation was recorded in this case.

The ash % of group A, B, C, D 2.300 ± 0.086, 2.183 ± 0.114, 2.350 ± 0.076, ,2.183± 0.108 respectively.

The Statistical analysis showed no significant difference hereby.

Values of total solids%, 23.869 ± 0.398, 22.900 ± 0.451, 23.317 ± 0.407, 23.100 ±534, for group A, B, C and D respectively. No significant difference was detected hereby.

The average acidity estimated as lactic acid % for group

A, B, C & D, was2.267±0.264, 2.633±0.348, 2.33±0.276, 2.600±0.313 respectively also no significant variation was recorded in this case.

The average total bacteria count determined was log 4.967±1.065, log4.467±0.882,log5.807±0.673,log5.807±0.673 for group A, B, C, D. The statistical analysis indicated no significant differencebetween the averages of samples of A, B C& D.

The obtained values and variation between the averages may be related to the factors influencing the manufacturing of mish, such as milk composition and quality the starter culture used, the fermentation process, the process procedures flavor added as well as packing and storage.

Finally contain recommendations are given.

ملخص البحث

أجري هذا البحث لدراسة المكونات والحموضة والعد الكلي للبكتريا لمنتج المش المسوق بولاية الخرطوم تم جمع (24) عينة مش معد للبيع والاستهلاك صنع بواسطة أربعة مصانع الألبان بولاية الخرطوم. حيث قسمت العينات لأربعة مجموعات A, B, C, D بواقع 6 عينات لكل مجموعة ولكل مصنع ومن ثم أجريت عليها الاختيارات المعملية.

نسبة الدهن% المتحصل عليها بلغ 0.095 ± 3.283، 0.073 ± 3.400، 0.056 ± 3.233، 0.101 ± 3.417 للعينات A, B, C, D علي التوالي ولم يظهر التحليل الإحصائي وجود فروقات معنوية بين متوسطات النسبة المئوية للدهن بلغت نسبة البروتين المئوية 0.496 ± 9.283، 0.541 ±8.567، 0.480 ± 9.200، 0.505 ± 8.600 لكل من A, B, C, D علي التوالي أيضاً لم يرصد أي فروق معنوية في هذه الحالة.

أما بالنسبة للمواد الصلبة للمجموعات A, B, C, D فكانت 0.398 ±23.267، 0.451 ± 22.900، 0.407 ± 23.317، 0.543 ± 23.100 حيث لم يتم رصد أي فرق معنوي. أيضاً نسبة الرماد المئوية بلغت 0.086 ±2.300، 0.114 ± 2.183، 0.076 ± 2.350، 0.108 ± 2.183، للمجموعات A, B, C, D علي التوالي ولم يرصد أي فرق معنوي بين متوسطات النسبة % للرماد.

الحموضة مقدرة على أساس النسبة المئوية لحامض اللاكتيك

للمجموعة A, B, C, D بلغت 0.264 ± 2.267، 0.348 ± 2.633، 0.276 ±2.233، 0.313 ±2.600 علي التوالي ايضاً لم يكن هناك فرق معنوي في هذه الحالة أما متوسط العد الكلي للباكتريا لعينات المجموعات A, B, C, D بلغت log 1.065 ± 3.967، 0.882 ±4.467، 0.673 ± 5.807، 0.673 ±5.807 حيث أبان التحليل الإحصائي عدم وجود فروق معنوية بين متوسطات عينات المجموعات , A, B, C& D

القيم المتحصل عليها والفروقات بين المتوسطات قد تعزى إلى العوامل التي تؤثر على صناعة المش مثل تركيب اللبن الخام ونوعيته، الأواني المستخدمة، عملية التخمر، الخطوات المتبعة عند التصنيع المنكهات المضافة التعبئة والتخزين.

أخيراً قدمت توصيات محددة.

Chapter One

1- Introduction:

Fermented milk products are cultured dairy products made from skim, whole or slightly concentrated milk that require specific lactic acid bacteria to develop their characteristics, flavor and texture (Thapa, 2000). According to Tamime (2006). A wide range of indigenous fermented milk products are traditionally made in rural areas worldwide and most of them rely primarily on spontaneous fermentation due to the presence of indigenous micro flora mainly lactic acid bacteria in the milk, but now a- days most fermented milks are manufactured under controlled conditions with specific starter culture.

Fernandes (2008) noticed, fermented milks have been produced by traditionally methods for many centuries and there are several hundred such products recorded around the world, and they are produced as a result of microbial souring of milk, usually from cow milk, but also milk of other species, e.g. sheep, goats and buffalo.

Osman (2007) explained, fermented milk products are processed from, whole, standardized or skim milk, after fermentation using selective micro organisms, that convert the milk sugar (lactose) into lactic acid, developing the acidity, coagulate casein and the fluid milk is changed into a semi- solid product, e.g. yoghurt, sour milk (mish), butter milk and others.

The nature of fermented products is different from one region to another. This is depending on the local indigenous micro flora, which in turn reflect the climatic conditions of the area (Savadogo et. al. 2004).

Naturally acidified milk may have been one of the first milk products and it is known by many names (Spreer (1998). According to Kurman et. al. (1992), around 400 generic names are applied to traditional and industrial fermented milk products, many of these products are known locally by different names.

Many people throughout Africa enjoy soured milk products. In these products, the lactic acid bacteria perform an essential role in preserving a highly nutritious food product (Beukes et. al. 2001).

One of the popula fermented milk products consumed by the different societies in Sudan is mish. It is processed either traditionally or by applying advanced methods and it has proved to have high nutritive values, and health- benefiting effects.

Elmardi (1980) noticed, Mish is one of the most fermented products almost known in all regions of the Sudan with different names and the intensity of spicing may differ from region to anther and even from family to another within the same district, it depends on spices availability and the taste of the preple.

The optimum utilization of mish as food, is reached when special attention is paid to its composition, natural properties and hygienic processing methods, that ensure no presence of pathogenic or deteriorating micro organisms. The current research studies these aspects.

Objectives:

- To study the composition, acidity and microbial quality of mish.

- To determine different possible variation between the averages of parameter studied.

Chapter two

2- Literature Review

2.1 Definition of Mish

Mish is atypical Sudanese sour milk product, obtained by acidifying the raw milk with selective lactic acid bacteria and addition of certain flavoring stuffs e.g. species, left to ripen, packed or consumed. (Osman 2007).

Dirar (1993) described mish as that product gained from milk, which first boiled, inoculated by starters after cooling and after souring seeds of black cumin or of fenugreek and perhaps few pods of green or red pepper are added and the product is fermented for two or more days before consumption.

2.2 Factors Influencing Mish Manufacture

The manufacture of mish is dependent on several factors, which may be given as follows:

2.2.1 Raw milk composition:

The composition of mish is similar to that of normal raw milk used to produce it. According to ELNimer (2007) the only difference between both is related to the action of the bacteria, which convert the form of the milk from raw to coagulated, whereby a slight concentration of the components is noticed as a result of heat treatment, which in turn decreases the volume of water and increases the total solids. Murshidi (1998) noticed, the fat content in the final product depends on the fat content of the raw milk used, if it is whole, standardized or fully skimmed.

The lactose % in the final product is decreased due to the fermentation process, by which the lactose is converted into lactic acid. (ELNimer 2007).

According to Walstra et. al. (2005), the composition of the product may be changed by such process steps as standardization, ultra filtration, addition of skim milk powder, caseinates, stabilizators and flavourings.

2.2.2. Starter Cultures:

Blume (2013) defined the starter cultures as selective strains of lactic acid bacteria used in the manufacture of a wide variety of milk products and classified by Lawrence et. al. (1976) in single strain, multi strains and mixed strains starters.

According to Sharma (2006), starter cultures are selected strains of lactic acid bacteria (LAB), (e.g. Str. cactis, Str- cremoris, Str. thermophilus, Leuconstoc dextranicum, Lactobacillus bulgaricus, lactobacillus acidophilus and lactobacillus helveticus) used singly or in combination of two or more species as starter cultures in the manufacture of several milk products; their function is to produce lactic acid and flavor compounds and bring about the coagulation of milk and desired changes in milk cream.

The lactic acid bacteria (LAB) are heterogeneous family of micro- organisms that can ferment a variety of nutrients, primarily into lactic acid (Poolman, 2002). (Hugenholz et. al. 2002). (Kleerebezem and Hugen- holz, 2003) explained, LAB

are mainly Gram- positive, anaerobic bacteria, non – sporulating and acid tolerant; Biochemicaly they include both homofer menters and heterofermenters, where by the former produce primarily lactic acid, while the latter yield also a variety of fermentation by- products, including lactic acid, acetic acid, athanol, co_2 and formic acid.

According to Tamime (2002), LAB are the main group of microorganisms that has been used successfully for decades for the production of fermented milks, and these organisms belong to the genera Lactococcus, Leuconostoc, Pediacoccus, Streptococcus and Lactobacilli, classified into cocci and rods, have a growth temperature of (20-30^{o}c) and (37-45^{o}c).

Lactic acid is formed by the action of many forms of bacteria upon sugars and there are two forms, called d- lactic acid and c-lactic acid receptively (Herrington. (2000).

All acidified milk products have one characteristic in common, which is the presence of lactic acid (Spreer 1998).

The growth of starter culture bacteria is inhibited by the presence of bacteriophages (viruses),the in-milk naturally found antibiotics like lactinin and agglutinin, beside antibiotics, bacteriosins, as noticed by Abdel hamid et. al. (2001) and Robinson (1997).

2.2.3 Fermentation Process

Fermentation is a mean of obtaining energy from carbohydrates without the presence of molecular oxgen i.e. in anaerobic

conditions; it will proceed wherever the appropriate carbohydrate substance is in contact with microorganisms under favourable conditions of pH and low oxygen and hence is an important adjunct to get the full nutritional value from carbohydrates rich foods (Solomon 2002).

Spreer (1998) defined fermentation is a conversion of a substance by a microorganism, by a vegetative or animal cell or by its enzymes into a product. In food production, the term fermented is applied to the value addition and conversion of raw materials by microorganisms and enzymes into a product ready for consumption.

Fermentation is one of the oldest technologies and a process dependent on the biological activity of microorganisms for production of a range of metabolites, which can surpress the growth and survival of undesirable micro flora in foods (Fox, 1993).

As given by Thapa (2000), controlled fermentation of milk produces acidity and flavor at desirable level, when preparing fermented milk products. The dramatic shift from food production for local communities to large scale food production to the requirements of expanding markets, led to the development of large scale of fermentation processes for commercial production of fermented foods with the most used microorganisms including lactic acid bacteria (LAB) for a

variety of dairy products as explained by Klanehammer and Fitzgerald (1994).

Ray and Daeschell (1992) mentioned, fermentation process involves the oxidation of carbohydrates to generate a range of products, which are principally organic acids, alcohol and co_2.

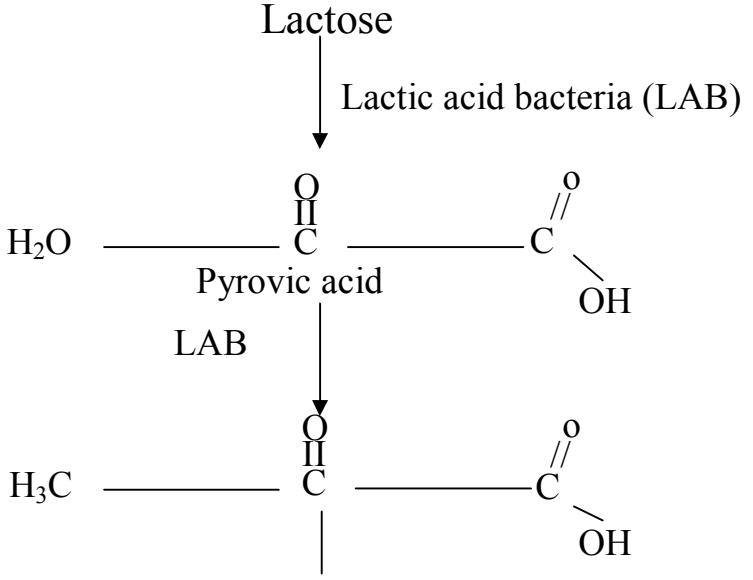
The production of fermented milks is based on the fermentation process performed by lactic acid bacteria.

When considering food fermentation, lactic acid bacteria are primarily responsible for many of the microbial transformation found in the more common fermented food products (Franz et. al. 1999).

The conversion of milk sugar (lactose) into lactic acid is given in the following figure.

Fig 1.

Conversion of lactose into Lacticacid.



(Lactic Acid)

To conclude, the production of mish is based on the fermentation of lactose by selective lactic acid bacteria that produce principally lactic acid, e.g Str. lactis, Str. cremoris.

2.2.4 Processing Methods:

Mish can be processed either by using traditional or industrial methods. The tradional methods are performed in small house holds for self consumption, while the industrial in milk factories for commercial purposes. The fermentation of the raw milk is common for the production of both. But, the difference between both methods is associated with the addition of starters. Starter cultures, which are found naturally in milk are dominating, when applying traditional methods. When preparing mish on industrial basis, artificial starters, which are a mixture of one or more pure microbial cultures are added to the raw milk. (Aada Hayat Tadris, 2010).

2.2.4.1 Traditional Methods:

Using this methods, raw milk is left till it gets sour either by lactic acid bacteria present in the milk or by adding a few amount of mish produced previously.

Then specific spices are added, the mixture is stirred thoroughly and left to ripen for one or two days, consumed as such or packed and kept in a cool store. However, by this method, the fermentation process should be controlled, otherwise defects will appear in the final product.

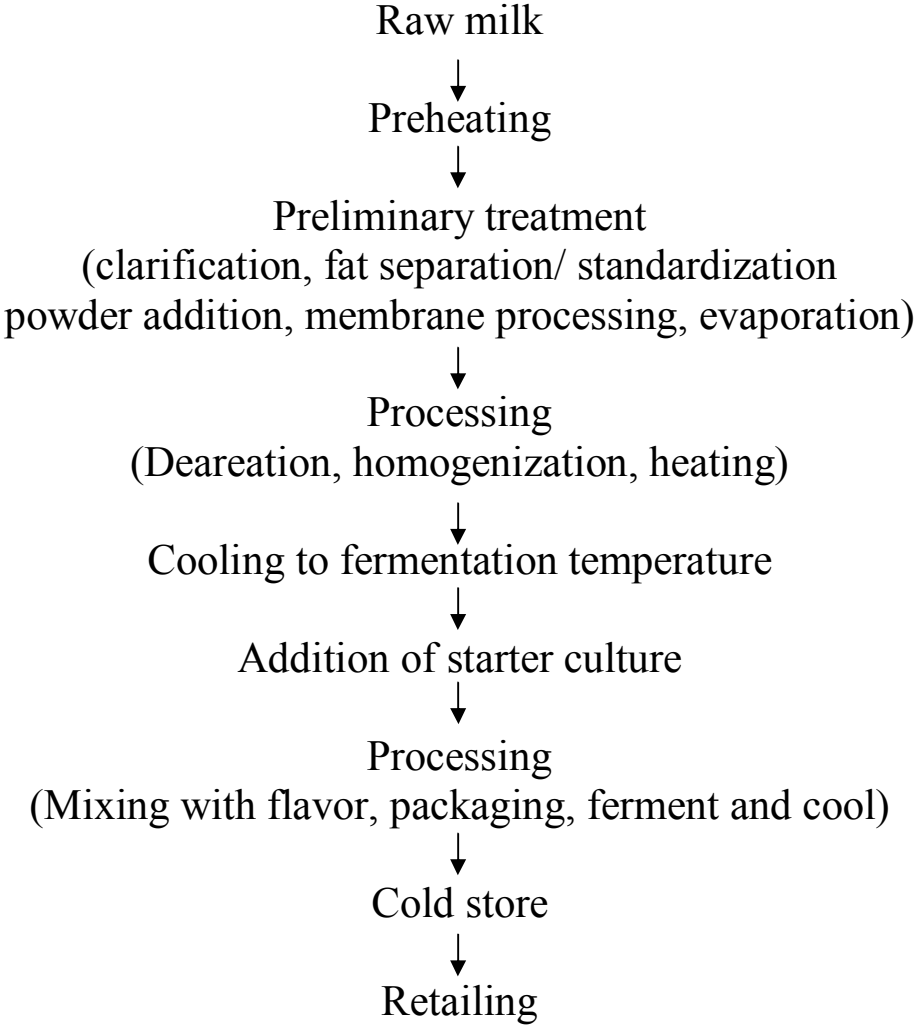
2.2.4.2 Industrial Methods:

Normally the steps followed by this methods are controlled.

The following figure shows the procedures of processing (adopted according to Kurman et. al., (1992).

Fig-2

Procedures of processing



2.2.2.4.3 Processing of mish in Sudanese milk factories:

Mohamed (2000), described the production of mish in the Nile Dairy Plant (Capo) and Kuku milk factory as follows:

a) Nile Dairy Plant:

After adding the starter, the skim or whole milk used for preparation of mish is left to form a curd, after which the whey is separated and spices like fenugreek, black cumin, garlic or hot pepper are added. Then, after packing, mish is left for 28 hours to ripen.

b) Kuku Milk Factory:

Mish is simply made from spoiled cow's milk and occasionally from surplus milk, raw fresh and often after being skimmed. Then brought into vats and left till curdling in an attempt to stop the growth of the starter bacteria. Afterwards half of the whey is drained off and spices (black cumin, fenugreek) and salt are added.

The mixture is left to ripen, after which the mish is recognized by taste and packed into plastic packs.

2.2.5 Spices:

The spices used in mish production contribute to the flavor, taste and have also health- benefiting effects. EL-Hussien (1980) mentioned, black cumin seeds are found to be acceptable without health hazards associated with their consumption. Similarly, garlic was used since ancient times as food, spice or remedy; in the Middle Ages it was used as antibiotic and is registered as a drug in many European countries, (Erinwald 1992).

In Asia, fenugreek seeds (Trigonella foenumgrocum) ae consumed as spices and also are medicines (Patil et.al. 1997).

2.3 Microbiology of fermented milks:

In general, the production of fermented milks is associated with the known traditional micro flora of the raw milk, namely lactic acid bacteria. But, now a-days many bacterial species known as nontraditional micro flora have been incorporated into applications and are used in the manufacture of fermented and other dairy products. Some examples belong to the genera Lactobacillus, Bifidiobacterium and Entero coccus, which showed health benefits for the consumer (Tamime, 2006).

Moreover, certain non – traditional species of lacto bacilli and yeasts are used in fermented milk products to contribute to special flavor and taste in such products (EL Nimer, 2007).

According to Murshidi (1998), the shelf- life of mish is dependent on the high acidity it contains, which inhibits the growth of typhoid-, paratyphoid- and coli form bacteria. Furthermore, he added, Tuberculosis and Bruclla microorganisms may survive in the product for weeks due to their resistance to high acidity.

Escherichia coli, Listeria monocytogenes and Yersinia enterocolitica are three of the most important food borne Bactria pathogens and can lead to food- borne diseases through consumption of contaminated milk and fermented milk products (Morgan et. al. 1993), (Mead et.al. 1999).

Mish samples analyzed by Abdel Hafiz (2001), showed a mean value of pH, total bacteria count, lactic acid bacteria, cocci and yeasts as 3.77 log, 5.9 log, 1.7 1og, 2.32 log and 5.07 respectively.

Abdalla and El zubeir (2006) reported that samples of mish produced by a dairy factory in Khartoum State revealed mean 10g for E. coli counts of 1.55± 2.42, Staph. aureus mean 10 g count of 1.00 ± 2.25 cfu ml^{-1}

Strptococcus spp. Mean 10 g count of 1.52±2.44 cfu ml^{-1} and salmonella spp mean 1og count of 1.11 ± 2.7cfu ml^{-1}.

As explained by Viljoin et. al. (2003) and Mayoral et. al. (2005), yeasts and molds are the main spoilage organisms found in cultured milk products, since the high acidity of such products inhibits many bacteria.

Roosht and Fleet (1966), Cadega et. al (2000), Carbo et. al. (2001) and Cadega et. al. (2001), linked the increasing presence of yeasts and molds in fermented dairy products to insufficient hygiene during the production, sanitation of the equipment, air contamination, insufficient heat treatment or inadequate microbiological quality of the supplements used.

2.4 Defects of fermented milk products:

Alkholi (1999) and Murshidi (1998) summarized the defects in fermented milk products as follows:

- Excessive whey: Due to low or less temperature applied during processing.

- Excessive acidity: Caused by adding big quantities of the starter cultures or that of bad quality, beside insufficient cooling and storage.
- Bitterness: When contaminated with microorganisms e.g. Bacillus spp., that hydrolyze proteins.
- Saltiness: Due to the presence of Nacl in the raw milk as preservative.
- Soft curd: Occurs when the milk salts are not balanced, (especially calcium), insufficient heat treatment and presence of substances influencing the growth of the starter culture.

Chapter 3

Materials and methods

3-1 Samples Collection:

Twenty four (24) samples of mish produced by 4 milk factories in Khartoum state and ready for consumption were collected. The samples were divided into 4 groups, 6 samples per each group and milk factory.

Then all samples were subjected to chemical (fat, protein, ash, total solids % according to dry matter), acidity and microbiological laboratory tests as follows:

3-2 laboratory Analysis:

3-2-1 Fat content:

Equipments and Materials:

- Gerber tubes.
- Centrifuge.
- Tubes holder
- H2 So_4 (89-90%)
- Amyl alchol.
- Mish samples.

The fat content was determined by Gerber method according to A.O.A.C. (2000).

Ten milliliters of sulfuric acid (density 1-815 gm/ml at 20c^{o}) were poured into a clean dry Gerber tube, followed by the addition of 10.9 gram of well mixed mish sample. One ml of amyl alcohol (density 0.814-0.816 gram at 20 C^{o}) and distillate

water (at $20c^0$) were added. The contents were then thoroughly mixed till no white particles could be seen. Gerber tubes were centrifuged at 1100 revolution per minute (rpm) for 3 minutes and the tubes were then transferred to a water bath at 65 c^o for 3 minutes. The fat percent was then read out directly from the fat column.

3.2.2 Protein content

Equipment:

- Distillator - Kijeldahl apparatus
- Puretts.

Materials

- H2 so_4 (0-1).
- Red methylin indicator
- H2 So 4 (40%)
- Cu So4 + K So4.
- Na oH solution.
- Boric acid (2%).
- Mish sample.

The protein content was determined by Kjeldahl method (A.O.AC. 2000).

In a clean dry Kjeldohl flask, l0 gm of mish were placed. Then 25 ml of concentrated H_2 So_4 were added followed by addition of two Kjeldahl tablets (Cu So_4). The mixture was than digested on a heater until a clear solution was obtained after 3 hours. The flask, were removed and left to cool. The digested sample was

poured into a volumetric flask (100ml) and diluted to 100ml with distilled water. Then 5 ml were taken, neutralized using 10ml of 40% sodium hydroxide and the neutralized solution was then distilled. The distillate was received in a conical flask containing 25ml of 4% boric acid plus three drop of indicator (bromo cresol green plus methyl red). The distillation was continued until the volume in the flask was 75ml the flask was then removed from the distillate was then litrated against o. In Hcl Until the end point was obtained (red color).

The protein was calculated as follows:

$$\text{Nitrogen\%} = \frac{\text{TX } 0.1 \text{ x } o.o14 \text{ x } 20 \text{ x } 100}{\text{Weight of sample}}$$

Protein % = Nitrogen % x 6.38

Where T = Titration figure.

0.1 = Normality of Hcl

0.2 0.014 = Dilution factor

0.3 6.38 = Conversion factor of milk into protein

3-2-3 Total Solids Content:

Equipments:

- Aluminum dishes.
- Oven.
- Wotes bath.
- Dissicator.
- Balance.

Material:

- Mish sample.

Total solids content was determined according to the drying over method of (A.O.A.C. 2000).

Three grams of mish were placed in a clean dried flat- bottomed aluminum dish and heated in steam bath for 10 minutes. The dishes were then dried in an air oven at 10 c^o for 3hr after which they were transferred to dissicahy rapid several times until the differences between two successive weightings was less than e.5 mg. the total solids content was calculated as follows:

Total solids (%) = $\frac{W1}{w2}$x100

Where w1 = weight of sample after drying.

W2= weight of sample original samples

3-2-4 Ash content:

Equipments:

- Drying oven.
- Dissr cator.
- Crucibles.
- Balance.

Materials:

- Mish samples.

Procedure:

The ash content was determined according to A.O.A.C. (2000). Five grams of mish were weighed into suitable clean dry crucibles which were then placed in a muffle furnace at 550 c^o for 3 hrs, cooled in a desiccators and weighed. The ash percentage was calculated as follows.

Ash% = $\frac{W1}{w0}$x100

Where w1 = weight of ash

Wo = weight of the original sample

3-2-5 Titratable acidity

Equipments:

- Sensitive balance.
- Conicat flask (250 ml).
- Pipettes.
- Test tubes.
- Buretts.

Materials

- Na oH
- Phenolphtalein Indicator.
- Distilled water

Procedure

Titratable acidity was determined according to A.O.A.C. (2000). Ten grams of mish were placed in a white and 5 drops of phenol Phalein indicator were added. The sample titrated against 0.1N

NaOH till afaint pink color was obtained. The acidity was calculated as follow:

Titratable acidity (%) Lactic acid = $\frac{Tx4}{w}$

Where T = Titratable figure

W = weight of sample

3.2.6 Microbiological Analysis

Equipment

1. Autoclave.
2. Incubator.
3. Oven.
4. water bath.
5. Colony counter.
6. Sensitive Balance.

Medi used

Plate count Agar.

Dilutents Used

0.1% peptene solution.

Total count of Bacteria:

It was carried out by using the plate count method as described by Campbell and Marshal (1975). Suitable medium for this purpose is plate count agar.

Preparation of serial dilutions:

Aseptically 10 grams of the sample were homogenized in 90ml of sterile dilution (0.1% peptone water). It was mixed well to give dilution (10^{-1}). By using sterile pipette 1ml was transferred especially from dilution (10^{-1}) to a test tide containing 9ml of sterile dilution and it was mixed well to give dilution (10^{-2}). In the same way the preparation of serial dilution was continued until the dilution (10^{-6}). One ml of each detection was transferred into sterile petri dish.

15ml of sterile melted plate count agar were added. The inoculum was mixed with medium and allowed to solidify.

The plates were incubated at 37^{o}c for 48 hours. A colony counter was used to count the viable bacterial colonies after incubated and. The results were expressed as colony- forming units [Cfu] per gram.

Statistical analysis:

The data one analysis statistically using ANOVA, Statistical Package for Social Sciences (spss, ver, 13) to determine the significant variation between the averages of the studied parameters.

Chapter Four

4. Results and Discussion

4.1 Results:

The laboratory analysis results are given in the following tables:

Table 1:

Average fat %

Group samples	Average	Significance
A	3.283 ± 0.095	NS
B	3.400 ± 0.073	
C	3.233 ± 0.056	
D	3.417 ± 0.101	

NS: Non significant at 0.05

Table 2:

Average protein%

Group samples	Average	Significance
A	9.283 ± 0.496	NS
B	8.567 ± 0.541	
C	9.200 ± 0.480	
D	8.600 ± 0.505	

NS: Non- significant

Table 3:

Average Ash %

Group samples	Average	Significance
A	2.300 ± 0.086	NS
B	2.183 ± 0.114	
C	2.350 ± 0.076	
D	2.183 ± 0.108	

NS: Non- significant

Table 4:

Average Total solids %

Group samples	Average	Significance
A	23.267 ± 0.398	NS
B	22.900 ± 0.451	
C	23.317 ± 0.407	
D	23.100 ± 0.543	

NS: Non- significant

Table 5:

Average acidity %

Group samples	Average	Significance
A	2.267 ± 0.264	NS
B	2.633 ± 0.348	
C	2.233 ± 0.276	
D	2.600 ± 0.313	

NS: Non- significant

Table 6:

Average Total bacteria count (log) %

Group samples	Average	Significance
A	4.967 ± 1.065	NS
B	4.467 ± 0.882	
C	5.807 ± 0.673	
D	5.807 ± 0.673	

NS: Non- significant

4.2 Discussion:

The obtained results indicate the following:

Fat content:

The average fat % of samples of group A, B, C and D was, 3.283 ± 0.095, 3.400 ± 0.073, 3.233 ± 0.056 and 3.417 ± 0.101 respectively.

The fat content of all samples vary very little. The statistical analysis revealed no significant difference between the average of the fat % of all groups. The low fat content in the final product may be related to partially skimming of the raw milk fat (Walstra et.al. 2005).

Protein content:

Average protein % obtained, 9.228 ± 0.446, 8.567 ± 0.511, 9.200 ± 0.480 and 8.600 ± 0.505 for samples of group A, B, C and D respectively. Group A and C samples showed the highest

protein content compared with B and D. No significant variation was recorded in this case.

The values obtained for the protein content is associated with the casein % found in the raw milk, since the fermentation process coagulates primarily the casein fraction of the milk (El Nimer, 2007; Osman, 2007; Tamime, 2006).

Ash content:

The average ash % obtained, 2.300 $\pm$ 0.086, 2.183 $\pm$ 0.114, 2.350 $\pm$ 0.076 and 2.183 $\pm$ 0.108 for samples of group A, B, c and D respectively. No significant variation was noticed here by. The value or the ash found in the final product, compared with that of raw milk, may be due to the concentration of the components in association with the fermentation process and heat treatment (El Nimer 2007).

Total solids %

Average values of 23.262 $\pm$ 0.393, 22.900 $\pm$ 0.451, 23.217 $\pm$ 0.407 and 23.100 $\pm$ 0.543 for the total solids % of group A, B , C, D, were obtained respectively. Also no significant variation hereby was recorded. The high total solids found in the product may be related to the rate of concentration and transformation of the raw milk from liquid to gel form due to the fermentation process and addition of flavourings during manufacture. (Sharma, 2006; Franz et. al 1999; Kurman et. al. 1992).

The acidity:

The average acidity as lactic acid % was 2.267 ± 0.34, for group A, 2.633 ± 0.343 for group B, 2.233 ± 0.276 for group C and 2.600 ± 0.313 for group D. Also no significant variation was recorded here with. The high acidity in the product is related to the presence of lactic acid as a result of the fermentation. (Fernandes, 2008; Tamime, 2006; Sharma, 2006; Spreer 1998).

Total bacteria count:

Average of total bacteria count obtained 4.967 ± 1.065, 4.467 ± 0.882, 5.807 ± 0.673 and 5.027 ± 0.977 log for samples of group A, B, C and D respectively. It was noticed that samples of group A showed the lowest count, followed by group B, while group C and D showed almost the same count. The statistical analysis recorded no significant difference between the averages of group A, B and Group C, D for the total count.

The total bacteria count of both group C and D was almost similar to that given by Abdel Hafiz (2001) for tested mish samples.

The variation in the total bacteria count may be related to the initial micro flora prevailing in the raw milk for mish preparation, since lactic acid bacteria are responsible for many microbial transformations found in fermented milk as given by Franz et. al (1999).

Chapter Five

Conclusion and recommendations

5.1 Conclusion:

The fermented milk product, mish is considered as one of most popular diet in the Sudan. It is consumed as such or with other meals. Also, it has been proved that mish has, beside its high nutritive value, positive health benefiting effects. Thus, it is of vital importance to prepare it under acceptable hygienic measurements, especially that produced by applying traditional methods. The different factors associated with mish manufacturing e.g. raw milk quality, fermentation processes, starter culture and supplements used, as well as packing and storing, should be regarded. The mish produced by the different milk factories in Khartoum State, current of this study, satisfied the standards required for this product.

5.2 recommendations:

- Raw milk used for processing of mish should be of high quality.
- It is recommended to consume mish produced by milk factories rather than that by traditional methods to avoid possible health hazards.
- Sanitation, proper handling of equipment and utensil (s), and adequate manufacturing practices should be conducted, when applying both traditional and industrial methods.

- More researches and studies should be carried out on physicochemical properties and microbial quality of mish.

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