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PRE-CONCENTRATION MILK ENHANCE WITH LEMON GRASS (CYMBOPOGON) FOR PRESERVATION

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ABSTRACT

Aims: In order to increase milk preservation time and transfer it from the remote agricultural areas raising cows to the dairy factory in the city center, and because of the lack of application of this technique in Iraqi factories or lack thereof, we have implemented this research.

Methodology and results: The milk was concentrated as a first stage by ultrafiltration for the purpose of standardizing its composition from the ratio of protein and total solids and later used in the manufacture of yoghurt and was preserved for several days by adding lemongrass (cymbopogon) extract, the results of the models taken from the ultra-filtration showed that the trance membrane pressure TMP and the speed of flow had a clear effect on the ratio of protein isolate and total solids and was (93% and 26%) respectively, and the milk was concentrated to 2.7 by membrane measuring 10 kDalton and the permeate flux was about 5-15 L/m^2.hr,

Conclusion, significance and impact of study: The results of the biological test of concentrated milk with two doses of lemongrass extract the first 100 mg/ml and 200mg/ml were non-existent after 24 hours and about (240CFU/ml) after 48 hours.

Keywords: Concentrated milk, Microbial conten ,Ultra-filtration technology,.

INTRODUCTİON

Concentrated milk is called differently in each country, but it serves the same sole purpose of being a preservative-free and versatile storehouse, whether for breakfast or dinner, and is used in the manufacture of various pastries and pastries to impart the distinct freshness and flavor desired by the consumer, as well as to its high nutritional value to promote overall health (Arunkumar and Etzel, 2015). Ultrafiltration technology purifies and separates liquids such as water and milk, which is a device for separating a solution using an ultrafiltration membrane as a filter medium and applying pressure difference on both sides of the membrane as a driving force. Ultra filtration (UF) is the process of separating components of a compound through fine membranes using pressure by cutting the molecular weight between 10 and 75 kg·mol−1used to separate Large molecules such as milk proteins are known as caseins, while small molecules (such as lactose and minerals) permeate through the membrane (Ferrer, et al., 2011; Arunkumar and Etzel, 2018). In recent years, milk has been concentrated through ultrafiltration filters and gained great demand in dairy technology, as well as the possibility of modifying milk functions and producing ingredients with high nutritional value (Bouchoux *et al*., 2014). Ultrafiltration of milk compared to other separation methods to reduce the risks and damages caused by microbial contamination and loss

of nutritional value of various dairy products or biological activity of milk [Ferrer, et al., 2011; ,8], low energy consumption, and this cycle reduces cost, helps to get rid of harmful waste due to the reduction or absence of organic matter in the disposed whey and retained through its passage in ultra-filtration technology, and the resulting water remains free of organic pollution, and the remaining water can be used in cleaning operations and irrigation (Abhiram and Etzel 2018). Membrane filtration technology is one of the modern techniques that have been introduced during the past thirty years in the dairy industry (Agarwal *et al*., 2015), especially in the cheese industry, and to split the components of milk into protein concentrates, lactose, fats, total solids...etc of the fine components of different proteins without the use of heat (Chenchaiah *et al.,* 2013). The method of concentrating milk by ultrafiltration membranes depends on the concentration of milk to a degree ranging from 5-7%, where a large amount of filtrate containing dissolved substances in water such as (salts and lactose) is removed, while proteins, fats and insoluble salts are retained in as concentrate. There are several types of ultrafiltration membranes, including tubular, spiral wind, and ceramic (Meyer *et al.,* 2015; Arunkumar, *et al.,* 20116). The use of ultra-high filtration technology in the manufacture of various dairy products has increased continuously in most developed countries, including neighboring countries, due to the many advantages that this technology possesses, the most important of which is to increase the yield of products and in terms of improving the value on the other hand, it has a nutritional value in order to retain the serum proteins within the curd of different products, whether concentrated milk, various types of cheese, milk and cream from various products (Marella *et al.,* 2013). Ultrafiltration membrane technology is used in the production of milk protein concentrate MPC with a low molecular weight (MWCP) ranging from 10 kDa to 30 kDa where the membranes retain the proteins and allow lactose and very small particles to permeate (Arunkumar and Etzel 2015). Milk contains about 8.6% of total solids, proteins represent 3.2%, total lactose, ash, non-protein nitrogen (NPN) and other small molecules account for about 5.4%, and the remainder is 91.4% water. Milk proteins are concentrated in casein and whey proteins, which have a molecular mass of 14.4-150 kDa. (Arunkumar *et al.,* 2016). Lemongrass *(Cymbopogon citratus)* is native to Asia and in all Asian regions but now grows all over the world, for its subtle flavor in its fresh and dried leaves common to Asian cuisine and use in curries, tea, soups and milk, it is also suitable for cooking with poultry, seafood and fish (Wifek *et al*., 2016). And it was also used in folk medicine (Bhoj Raj Singh *et al.,* 2011), which was mixed with tea as sedatives, and to treat many fever diseases, and as an immunostimulant in India, Egypt and Iran (Amirdivani & Baba, 2011; Tilaye *et al*., 2018). The antimicrobial activity of lemongrass is due to its high content of phytochemicals and essential oils (Calo *et al*., 2015; Zulfa **et al.,** 2016; Al-Hamdani, 2022). In this research, the initial concentration of milk was applied using an ultra-filtration teqnique and enhanced by adding concentrations of lemongrass extract in order to increase its preservation time and transfer it from the remote agricultural areas raising cows to the dairy factory in the city center, and because of the lack of application of this technique in Iraqi factories or lack thereof, we have implemented this research.

MATERIALS AND METHODS

Material

Five kilograms of raw cow's milk was purchased and brought from the College of Agriculture / University of Baghdad / Abu-Ghraib to the laboratory and placed in the refrigerator. Experimental testing was conducted after preparing the instruments in the laboratory of the Ministry of Science and Technology/Environment and Water Department, using flat sheet ultra-filter membrane with 10 K-Dalton made from polyethylsulphone (PES) and two percentage of lemongrass extract (100mg/ml, 200mg/ml).

Equipment

To complete the experiment, the milk concentration process was carried out using Membrane testing device manufactured by the American company as shown in Figure (1) below:

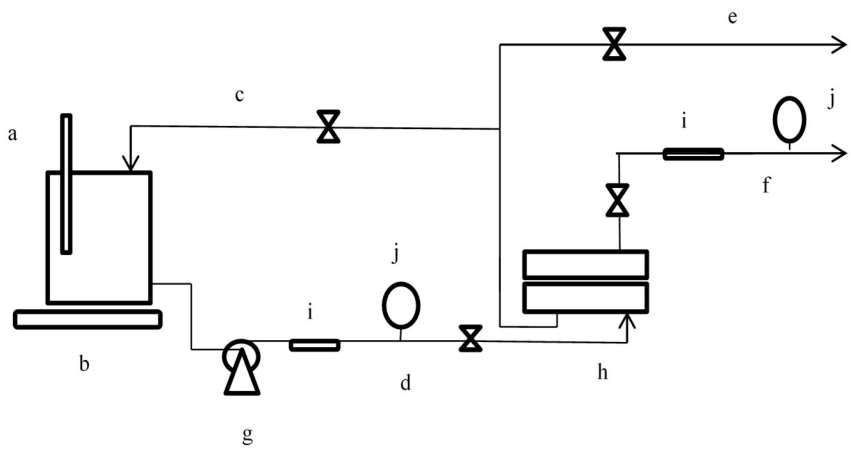

Figure 1: A simplified piping and instruments flow chart to concentrate milk using the membrane testing system: 1=Heat Sensor, 2=Tank, 3=Recycle, 4= Flow meter, 5= Permeate, 6= retntate, 7= Pump, 8= sepa, 9= Flow meter, 10= pressure gage.

Stages of operating the system

Where the process was carried out to reach a concentration of 2.7 times (calculated on the percentage of fat concentration due to its almost complete retention and estimated at a rate of 99%) and took about an hour and a half. Five kg of fresh milk was brought and used for this study and it was pasteurized at 73 ^{0}C / 15 min then cooled to a temperature of 35 ^{0}C, followed by the filtration process of milk at a temperature of 35±2 C and a pressure of 2 bar by pumping the milk into the sepa chamber as in Figure (1), and samples were taken in sterile glass containers of 50 ml of milk upon receipt, from the centre and the filtrate during the concentration process every 15 minutes, and it was frozen until analysis, and then the samples taken were also subjected to chemical tests to determine the changes in the composition between the milk and the concentrate compared it with the filtrate.

Also, five kg of fresh milk; was taken for concentrated milk by traditional method (boiling and continuous stirring). The experiments were conducted at room temperature. Membrane

ultrafiltration's experiment was conducted in four stages of operation: 1- Washing Stage, distilled water was used to wash the used membrane from the remnants of the solutions from previous runs and prepare it for the following filtration process by pumping 4 liters of distilled water at a flow rate of 1-1.5 L/min and at a pressure of 4 bars. 2- Filtration stage, the milk to be concentrated was pumped into the sepa membrane chamber and then the readings of the pressure change across the membrane (TMP) were recorded, at the place where the milk entered the feed chamber, through the output tube permeate and the flow level of the filtered solution every five minutes until the flow level stabilized and then samples were taken for chemical analysis. 3- Final washing stage is similar to the first washing stage and has the same operating conditions to be ensured that it is free of sediment and suspended matter. 4- The stage of cleaning and storing a caustic soda solution is pumped at 0.1 M at a flow rate of 1-1.5 L/min and a pressure of 2 bars for half an hour to wash the membranes and tubes unit according to (Meyer *et al.,* 2015). The membrane is stored in the same solution

Physicochemical composition

The yield %, moisture, total solid were determined (Hooi *et al*., 2011) as tabulated in Table 1. The pH, total acidity, total solids and ash were determined according to (AOAC 2012). The basis for the determination of acidity is that an alkaline solution has been added to the milk, until it reaches the equilibrium point, which is known as one of the reagents. After that, the amount of alkali used is recorded and divided by the amount of milk taken and the result is multiplied by 100, which indicates the acidity in the milk that is, as a percentage of lactic acid (AOAC, 2012).

Analytical Methods

Analyse were performed on milk, UF concentrated samples

- The Bradford method was chosen to measure the percentage of protein concentration in milk, which is a spectrophotometric method based on the principle of shifting the absorbance of the blue dye under acidic conditions from brown to blue. The spectrophotometer has a wavelength of 595 nm and the standard curve was made, then the readings were taken for the different models and the protein concentration was measured compared to the standard solution according to (Bradford, 1976).
- The percentage of lactose in whey was determined by the colorimetric method depending on its reducing sugar ability with the presence of substances that help reduce (copper sulfate solution, sodium tungsten solution, sulfuric acid, phenol, lactose standard solution) and then the absorbance was measured at a wavelength of 490 nm according to (Chollangi and Hossain, 2007).

Sensory Evaluation

Sensory assay was done using quantitative description analysis (QDA) with 9 trained and healthy members with experience in dairy products, and experienced in sensory analytics. Participants were trained on product definition and specific attributes using condensed milk. Participants were asked to gives score of each color, flavor, taste, mouth-feel and overall acceptance using a 5-point

Hedonic scale (5= like extremely and 1= dislike extremely). A reference sample of concentrated, canned milk available in the local markets was presented out of its original can in similar containers and given to be used for with other samples. Participants evaluated a maximum of 3 samples each time. Water was provided to clean the mouth between samples(Isis *et al.,* 2018). The sensory evaluations were determined according to the method described by (Blanc and Odet,1981).

Microbial Analysis

The resultant concentrated milk were microbiologically examined for total bacterial count, mold and yeasts count and coliform group according to American Public Health Association (Vinderola and Reinheimer, 2000).

Statistical Analysis

It was used a statistical analysis program (SAS, 201) to demonstrate the effect of the different parameters difference on the composition and sensory properties of fortified cheese by adding a specific concentration of spices used daily and healthy plants by the consumer, using the LLD test - which shows the least important difference and compares it with big differences Among the different coefficients in this study.

RESULTS AND DISCUSSION

Ultra-filtration measuring

From figure (2) observe the working efficiency of the Ultra-filtration measuring on 10kDalton to isolate milk protein in the form of concentrated milk and and exudes the lactose at pressure 2 bar.

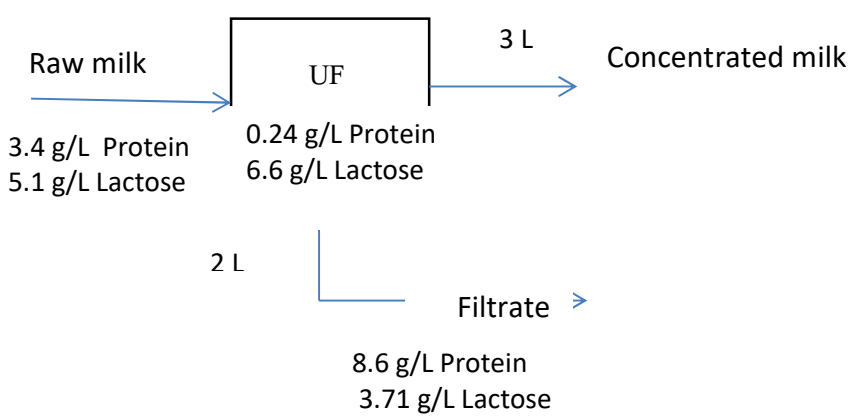

Figure 2: scheme of Ultra-filtration measuring at 10 kdalton

Change in the flow rate of the filtration with time

The flow rate of the milk was decreased over time due to the accumulation of the components of milk on the surface of the membrane. This appears about 25 minutes then, after that it reached almost equal value as in figure (3), which is called the stability state in which the accumulation

rate of particles on the surface of the membrane is equal to the removal rate of particles due to the type of the tangible runoff of the membrane surface.

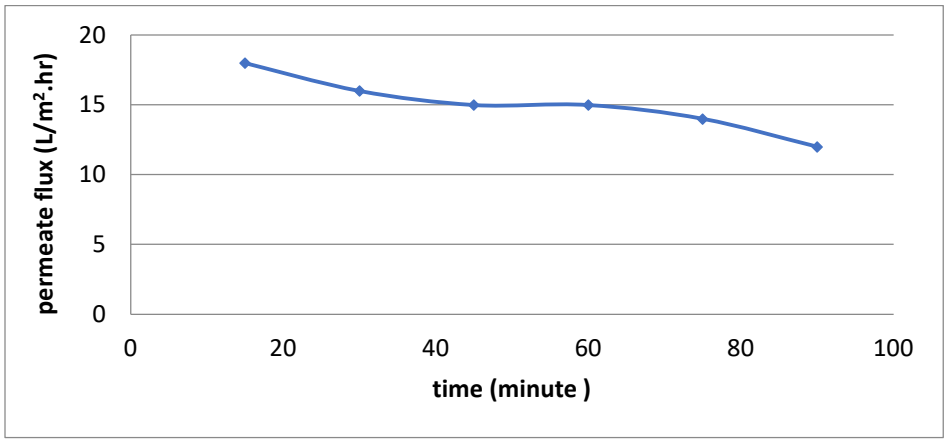

Figure 3: Change the flux of the filtration with time

Pressure Effect

The flow rate of the milk was increased gradually with the increase in the pressure difference across the membrane due to the small pores that do not allow the passage of protein molecules larger than 10 kDa such as α-lactalbumin and its molecular weight is 14 kDa β-lactoglobulin with a molecular weight of 18 kDa. The filtrate flows to its maximum at a pressure difference of 2 bar and then decreases back at higher pressures as shown in Fig 4. This could be a result of the gradual accumulation of protein and insoluble substances on the surface of the membrane (Ferrer *et al.,* 2011).

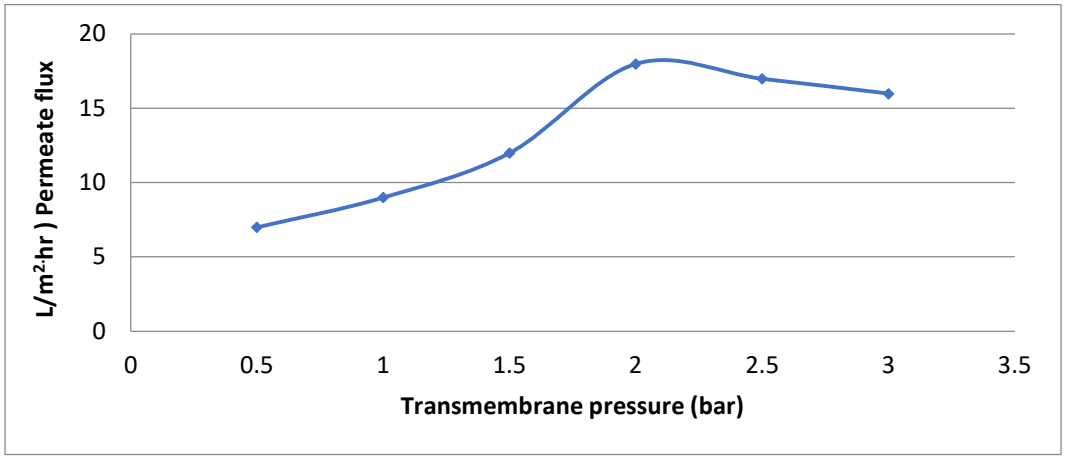

Figure 4: change of milk flux with trancemembrane pressure (TMB).

Change in protein concentration over time

Figure (5) shows the change in protein concentration during the ultra-high filtration process. The results showed a high percentage of total protein during the concentration process, where the ratio at the beginning of the process for raw milk reached 3.2, while the ratio reached at the end of the filtration process and after an hour and a half to 8.0, which indicates the efficiency of the filtration process using the ultrafiltration membrane of 10 kDa, and then the process of concentration indicating the stability of the filtration during this time period under the specified pressure 2 bar.

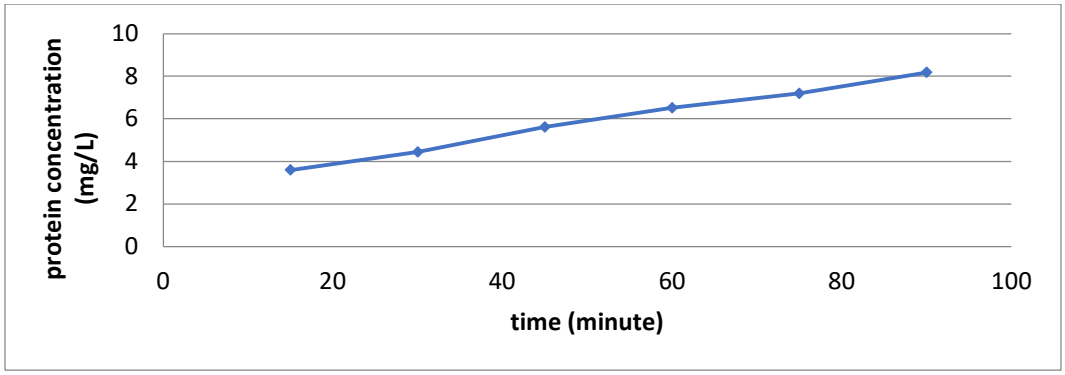

Figure 5: change of protein % of milk with time of filtration.

Change in protein concentration with transe-membran- pressure (bar)

Figure (6) shows the changes in protein content with pressure difference across the membrane in the ultrafiltration process used to concentrate milk in this study. The protein content was low in the beginning, so there is an urgent need for large protein molecules to cross while small protein molecules such as α-lactalbumin inside the pores of the membranes is to increase the pressure to 2 bar and the temperature is 35°C. All of these factors led to an improvement in the concentration process until a stable state was reached at a pressure difference of 2 bars and a temperature of and a temperature of 35°C.

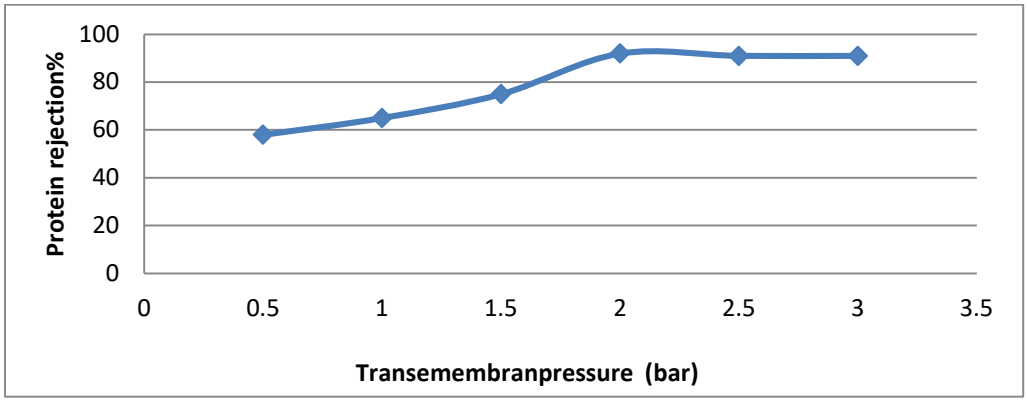

Figure 6: Change of protein rejection % with trancemembrane pressure (bar)

Change in total solids percentage with time

Figure (7) shows the change of percentage of total solids with time in minutes for milk concentration using ultrafiltration technique used in this study. Through the results, an increase in the percentage of total dry matter can be observed during the filtration process with a significant difference. The average rate at the beginning of the filtration process for raw milk reached 11%, while the average rate at the end of the filtration reached 24%. The reason is attributed to the presence of some dissolved components in the water such as mineral salts, lactose and others, which are included in the dry ingredients, which the membrane cannot seize. These results are similar to what was founded by (Miriam *et al.,* 2015).

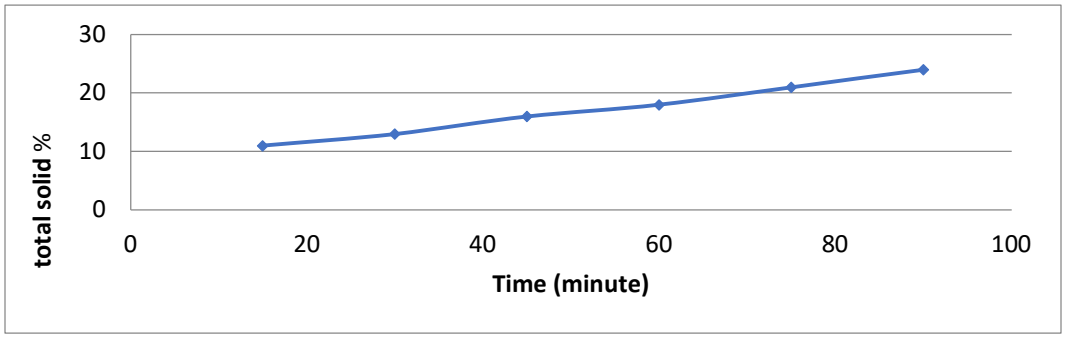

Figure 7: change of total solids% with time of filtration

Physicochemical composition of raw and evaporated milk

About 5 liters of raw milk was filtered for each treatment by ultrafiltration membranes and traditional open heating process, the volume of the evaporated milk output was about 2 liters, from this we conclude that the volume of the resulting milk is approximately 5 less than the volume of raw milk pumped at the beginning of the experiment. The moisture content of raw milk is usually between 80% and 90% of other components of raw milk (Domagala, 2012). The results of this study showed that the moisture content in raw milk was 86.2 % and decreased significantly to 28.0 and 25.0 in both concentrated milk by the traditional method and ultra-filtration, respectively, as shown in Table 1. It is noted that these result is lower than what was found (Li *et al*., 2017) as the moisture content in canned concentrate milk was 69.5. Milk usually contains 5.0 % of its weight is nitrogen, mostly in the form of proteins, and the rest in the form of Non- nitrogenous substances (Isis *et al.,* 2017), and milk proteins are proteins of complete nutritional value, comparable only to egg proteins (Ferrer *et al.,* 2011). The results of this study, which are shown in Table 1, showed that the percentage of protein in the raw milk sample is 3.6. While the percentage of protein increased significantly to in both samples of concentrated milk by the traditional method and ultrafiltration, respectively. Milk is more nutritious than low-fat milk, as recommended by the Food and Drug Administration Drug and FDA, PHS VS, that the percentage of fat in whole milk is not less than 3.25 (Li *et al.,* 2017). The results of this study found that the percentage of fat in

the raw milk sample was 3.7, while it increased to 6.5 and 8.0 in both conventional and ultrafiltration concentrated milk, respectively. It is inferred that the ash content expresses the amount of salts in the milk, which is less than 1% of the milk components, which represents the inorganic part of the milk (Isis *et al*., 2017). It is noted from Table 1, that the percentage of ash in raw and concentrated milk is 0.72, 0.73 and 1.8 respectively. Table 1, explain pH of the raw milk was 0.15, while was increased to 0.30 in condensed milk that processed by traditional method open heating, then decreased to 0.24 in condensed milk that processed with ultrafiltration membranes. This was comparable with result (Prafulla *et al.,* 2021). And results were lower than [29]. The acidity of the milk indicates the activity of bacteria in the milk and its freshness, and thus indicates its quality (Meyer *et al.*, 2015). Where the pH is less than 14 referred a higher quality, and the acidity of the milk affects the taste of the milk when the acidity exceeds 0.3 %, the milk becomes unpleasant in taste, while when the percentage exceeds 0.4 % milk becomes acidic, and when the percentage exceeds 0.6 %, the milk settles at the normal temperature and becomes unusable (Schiano *et al.,*2017).

Table 1: Physicochemical content in raw milk and processed milk using traditional (heating) and Ultrafiltration membrane

Milk composition	Raw milk	Concentrated milk with-traditional method	Concentrated milk with- UF	LSD value
Yield (L)	10.00	1.80	2.0	3.29 *
Moisture content %	87.2	28.0	25.0	6.88 *
Total Milk Solids %	13.1	25.0	28.6	4.03 *
Ash %	0.72	0.73	1.8	0.524 *
Milk Fat %	3.7	6.5	8.0	1.98 *
Protein %	3.6	6.0	8.0	1.75 *
pH	0.15	0.30	0.24	0.137 NS
Titratable Acidity (as lactic acid) %	0.17	0.29	0.25	0.071 *

* ($P \leq 0.05$).

The results of the study indicate that the concentration of the main components of the milk using ultra filtration increased by 5 times in the percentage of fat in the retaining material, and retained 45.97% of protein, 33.43% of lactose, and 03.60% of ash. The study demonstrated the efficiency of the Ultrafiltration membrane compared with traditional method. Also other study was founded an increase in the profitability of 39.19% when making mozzarella cheese from UF concentrates, and 52.21% when making mozzarella cheese from UF concentrates (Chenchaiah *et al.,* 2013).

Sensory analysis of concentrated milk

Since the early twentieth century, the brand has become a basic necessity for any food product, so companies began to pay attention to officially recognized quality standards to enhance their quality from the first step of production until it reaches the consumer. Sensory analysis parameters are the critical means of ensuring sensory quality, assessing acceptability, and identifying defects in milk and milk products (Blance and Odet, 1981). Usually the majority of sensory procedures are easy and gives excellent results when assessed correctly and in compliance with the conditions (Isis *et al.,* 2018). Sensory feedback data can also be paired with consumer feedback in a special dashboard known as preference mapping to better understand drivers of product liking (Schiano, *et al.,* 2017). Result of sensory evaluation presented in Table 3. It was founded most of sensory characters' color, flavor, taste, mouth feel and overall acceptance of produce condensed milk significantly acceptable by all panelists with ultrafiltration process.

Table 3: Sensory evaluation of condensed milk processed by traditional and Ultrafiltration membrane compared with Imported, Canned, condensed milk.

Treatments sample	Sensory characters				
	Color	Flavor	Taste	Mouth feel	Over all acceptance
Canned, Condensed milk (control)	4	4	4	4	4
Condensed milk by traditional process	2.5	3	3	2.5	3.0
Condensed milk by Ultrafiltration process	4	4	3.5	4	4
LSD value	1.63 *	1.07 NS	0.784 NS	1.28 *	1.162NS

* ($P \leq 0.05$), NS: Non-Significant.

Microbial assay

The contamination of membranes with microbes is the main concern in the dairy industry, which creates some limitations when using membrane filtration, such as blockage of pores and the formation of a layer of different sediments and bacteria present with milk, which is called (cake), reducing pollution and preserving milk after its concentration for a long time as possible, so an extract of the herb was prepared lemongrass with two concentrations: the first is 100 mg / liter and the second is 200 mg / liter and added to the milk concentrate. The two concentrations were planted in standard media for the purpose of conducting a biological examination. The result after 24 hours was very small for the first concentration and in the second concentration it was estimated at (240 CFU) as in Figure 8 and 9. Microbial count was within the limits permitted by the Iraqi standard. Molds and yeasts were also not detected in most of the samples, due to good ultrafiltration and lemongrass extract during concentration by UF teqnique.

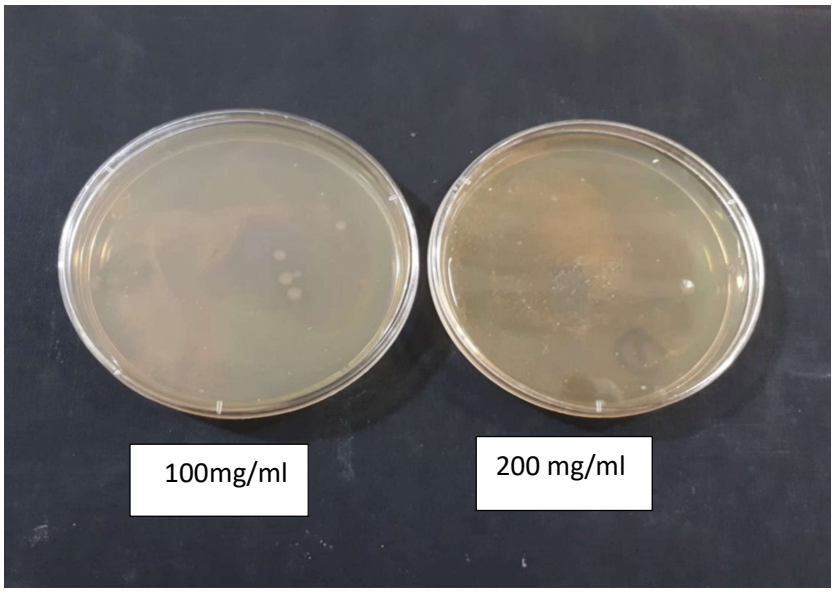

Figure 8: Microbial assay with Lemmon grass extract after 24 hour

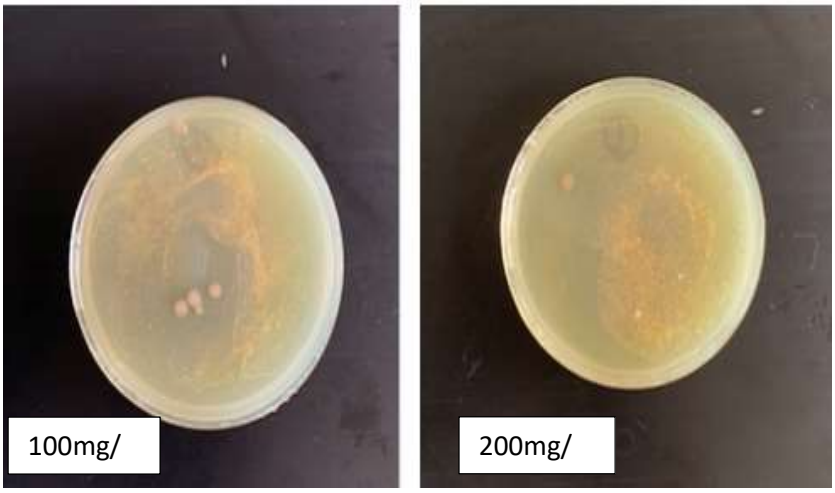

Figure 9: Microbial assay with Lemmon grass extract after 48 hour

CONCLUSION

There are several methods for concentrating liquid milk, including: evaporation, membrane filtration, and freezing. The last decades of this century witnessed the application of membrane separation technology in various biological treatments, including the dairy industry and its various products vital to consumer health with the increasing demand for these products as they are mainly used as ingredients in the manufacture of various food products, such as sweets and pastries. Therefore, in this study, an attempt was made to produce unsweetened concentrated milk using ultrafiltration membrane techniques in order to use it in small dairy farms, which are widely available in the outskirts of the city of Baghdad, the capital, and what it suffers from overcrowding and traffic congestion in order to provide an opportunity for the cost of transportation and to avoid

microbial contamination of fresh milk during its transportation to the center of the city where the main dairies are available there. The results of the study showed the absence of microbial contamination of concentrated milk, as well as the concentration of nutritional components important for health, as well as good sensory qualities. So the membrane separation technology continues to hold a key role in selective fraction and development of novel dairy ingredients. In our current study, it was proved that the concentration of milk with ultra-filtration technology in laboratory did not affect the quality of milk with regard to the physicochemical composition and sensory characteristics in terms of color, flavor, taste, mouth feel and general acceptance as well as reducing the percentage of microbial contamination and this was similar to what was found [32], Thus, there is a high, appropriate and quality possibility to use concentrated milk to produce different dairy products such as different types of cheese, powdered milk, cream...etc. of dairy products.

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