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PRODUCTION OF SOFT CHEESE BY USING NANOFILTRATION (NF) MEMBRANE SEPARATION TECHNOLOGY

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Abstract

This work aimed to harness the most advanced and modern technology, nano-filtration technology, in order to overcome the obstacles facing the most important and most common dairy derivative and the most common and used around the world, soft cheese. Therefore, cow's milk was obtained from a reliable source and two soft cheese were prepared from it: (NC) was prepared from natural milk while (CC) was prepared by nano-filtration technology. The results indicated the feasibility of using concentrated milk using the nano-filtration technique in the production of soft cheese, as the use of concentrated milk with the nano-filtration technique reduced the time required for cheese from 35 to 8 minutes and increased the cheese yield from 12.11 to 26.51% when adding the same percentage of rennet to the same amount from natural milk or concentrated milk. Also, the chemical composition of (CC) was richer in nutrients than natural cheese (NC), the values of protein, fat, lactose and ash were (21.41, 26, 4.16 and 4.52%) for the first cheese and were (13.30, 18.25, 2.17 and 3.16%) for the last cheese respectively. In addition, the total microbial number was ($0.11 x 10^2$) for (CC), while it was ($0.23 x 10^2$) for (NC), molds and yeasts, spore-forming bacteria and Chloform bacteria were ($0.19 x 10^2$), ($0.31 x 10^2$) and (-) respectively while they were ($0.15 x 10^2$), ($0.23 x 10^2$) and (-) for (CC) respectively.This is despite the high selectivity of the nano-filtration technology and its preservation of the nutrients in the milk. Finally, from the perspective of sensory properties, (CC) had a higher acceptance than the (NC) by the judges, the scores of texture, flavor, aroma and general acceptance were (8.3, 8.2, 8.4 and 8.2) for (CC) compared to (6.8, 6.8, 7 and 7) respectively for (NC).

Key words: nanofiltration, membrane separation, soft cheese.

1. Introduction

Soft cheese is one of the most common dairy products ever, as a large number of consumers around the world depend on it mainly, due to its high content of nutrients such as protein, calcium, sodium and vitamins with its low calories, and it is characterized by its salt content, which makes its taste palatable to consumers **(Ouyang *et al.*, 2022)**. The manufacture of soft cheese in the traditional method depends on the curing of natural milk with a microbial initiator, then filtering the resulting curd to get rid of the whey and reach the curd to the required moisture. Although this method has proven its effectiveness over many years, it has some disadvantages. It depends on a large amount of natural milk, and thus the large size of the cheese factories to provide enough space to store raw milk on the one hand and it also needs to use a large amount of the starter and salt on the other hand **(Al Musa and Al Garory, 2022)**. Also, the traditional method causes loss of whey proteins, which represent 20% of milk proteins and about 10% of milk fat, in addition to the rest of the water-soluble components of milk. Commercially, this leads to a decrease in the yield of cheese, and nutritionally, the nutritional value of the cheese produced by the traditional method decreases due to the high nutritional value of whey proteins and the nutrients lost with whey. Environmentally, the whey produced by this industry is considered a source of pollution because it contains vital materials, and its disposal is a burden on producers **(Gamlath *et al*., 2022)**. Microbially, Soft cheese is rich in many nutrients such as proteins, fats, minerals and vitamins, as well as its high moisture content, which makes it an environment suitable for the growth of microbes such as bacteria and some yeasts and molds. When unpasteurized or insufficiently pasteurized milk is used, or cheese is contaminated during its production stages, however, it cannot be heat treated in order to preserve the activity of the rennet, which pose a threat to human health due to its ability to produce some toxic substances **(Kajak *et al.,* 2022)**. Use of evaporated milk in the manufacture of soft cheese is an effective method, but exposing milk to high heat leads to several negative effects, such as denaturation of proteins, the oxidation of fats and their decomposition into free fatty acids, as well as the decomposition of sugars. In addition, the proteins interact with the sugars in the milk and form brown materials due to the so-called Maillard reaction; this interaction changes the sensory characteristics of the milk thus, it negatively affects the chemical and sensory qualities of the cheese made from it **(Xiang *et al.,* 2021)**.

On the contrary, the membranes are characterized by unique mechanisms therefore have a very high selective ability. Also, the diversity of the mechanism has made the membranes suitable for many applications in the dairy field, while traditional methods such as heat treatment are often of one purpose, and it must be mentioned, despite the ability of the membranes to perform many tasks, but it is characterized by its small size due to its compact design, which makes it suitable in small factories with different designs without the need to modify the factory and also this feature granted the possibility of expansion in those factories with ease, as it does not they conflict when added to the existing production lines, the membranes require very low maintenance, and the way to operate them is very easy and simple, and therefore it does not require highly specialized knowledge to operate it to obtain the desired objective **(Gao *et al.,* 2021)**. Use of nanofiltration technology offers a wide range of advantages to producers. nanofiltration technology is a non-thermal and environmentally friendly technology, and it is an ideal way to use it in the dairy industry as it is used to remove unwanted harmful components such as microorganisms or impurities stuck in the milk, which have a negative impact on the quality of raw milk as well as dairy products and thus increases its shelf life. It is also characterized by not showing the negative effects resulting from high temperature such as denaturation of milk proteins and the change in the sensory characteristics of the product resulting from the interaction of proteins with milk sugars in the presence of heat, which makes the product more acceptable by the consumer **(Mollahosseini**

***et al*., 2021)**. Therefore, the aim of this work was to overcome the obstacles facing the manufacture of soft cheese by the traditional method, and to exploit the multiple advantages of nano-filtration technology in this important industry.

2. Materials and methods

2.1. Preparation of milk sample:

Milk used in this study was full fat milk obtained from the college dairy plant, which is belonging to College of Agricultural Engineering Sciences, University of Baghdad. The milk samples were kept at room temperature for at least 30 min before treated.

2.2. Preparation of membranes:

The membranes were prepared according to the method described by **Kujawski *et al.* (2020)**. The composition of mixtures used for the preparation of the investigated membranes is summarized in the following form.

Table 1: Composition of the (PPSU) and (PVP) membranes

Membranes	PPSU (mg)	NMP (ml)	PVP (mg)	PPSU%
PS1	5	20	-	20% PPSU
PS2	5.75	19.25	-	23% PPSU
PS3	6.25	18.75	-	25% PPSU
PS4	6.25	18.5	0.25	25%PPSU+1 % PVP
PS5	6.75	18.25	-	27% PPSU
PS6	6.75	18	0.25	27% PPSU+1% PVP

2.3. Membrane analysis:

2.1.1. Contact angels (CA):

Contact angle (CA) was measured according to **Xiao *et al.* (2017)**.

2.3.2. Porosity:

The membrane porosity was measured by the immersion method using distilled water as the medium according to **Jayalakshmi *et al.* (2012)**.

2.3.3. Scanning Electron Microscope (SEM):

The morphology and microstructures of membranes was explored using method described by **Kujawski *et al*. (2020)**.

2.3.4. Atomic Force Microscope (AFM):

The surface topography was tested by atomic force microscope (AFM) according to the method described by **Kujawski *et al.* (2020)**.

2.3.5. Fourier Transform Infrared Spectroscopy (FT-IR):

The functional groups of the membrane were tested by Fourier transform infra-red spectroscopy (FT-IR) according to **Kanagaraj *et al.* (2015)**.

2.4. Preparation of concentrated milk by nanofiltration technology:

Milk concentration by nanofiltration (NF) was carried out with eight membranes in the module. Milk concentration was performed at 10 °C using 1 kg of whole milk in each run at a lower transmembrane pressure (TP) of 7 to 9 bar and Concentration factor (CF) in a range of 2 to 3. The partition of milk components between the permeate and the retentate was characterised by an overall retention defined as:

$$R_0 = 1 – C_{Pav}/C_0$$

Where:

R_0 is instant retention R of a given component.

C_{Pav} is the concentration of a given component in the permeate collected during the operation.

C_0 is the initial concentration of that component in the milk.

2.5. Preparation of white cheese from natural and nanofilter milk:

Cheese-making trials were performed using 2 kg of milk NF retentate for each trial. The NF milk was heated to 63 °C in a water bath for 30 min and chilled at 37 °C. After addition of microbial rennet (1 mL per 100 kg milk), the NF retentate was kept at 37 °C for 30 min. The curd was gently stirred and separated from whey by means of a cloth. The drained whey and the resulting cheese were weighed. A control cheese was made from whole milk not concentrated by NF using the same technical procedure.

2.6. Determination of cheese yield:

The percentage yield (Y) of cheese is calculated according to **Coggins (1991)** as following:

$$Y = \frac{\text{Kilograms of cheese produced}}{\text{Kilograms of milk used}} \times 100$$

2.7. Determination of chemical composition of white cheese:

Cheese samples were grated to obtain small particles then protein, fat lactose and moisture were determined as described by **Guinee *et al.* (2002)**, while ash was determined according to **Fenelon and Guinee (2000)**.

2.8. Microbiological analyses:

Spore-forming bacteria was determined according to described method by **Herman *et al.* (1997),** yeasts and molds was determined according to **Bradley *et al.* (1992)** while total coliform bacteria was determined according to used method by **Bridson (1990)**.

2.9. Sensory evaluation of white cheese:

Sensory characteristics of white cheese samples were evaluated by the staff of the Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, the evaluation criteria and evaluated treatments were as shown in form A which used by **Piggott (1984)**.

Table 2: Sensory evaluation of white cheese

Sample	Texture	Flavor	Aroma	Overall Acceptability	Scale 1-9
1					Like extremely = 9 Dislike = 5 Dislike extremely = 1
2					

2.9. Statistical analysis:

the data was analyzed according to **Ramadan (2020)** by programe of Statistical Package for the Social Sciences virision 20 (**SPSS V.20**) to study the effect of different treatments on the studied properties according to a complete random design **(CRD)**, and the significant differences between the means were compared by the lowest significant difference **(LSD)**.

3. Results and discussion

3.1. Membrane Morphology:

The influence of polymer concentration on the morphological properties of asymmetric membrane can be observed from the thickness of skin layer formed and the development of macro-voids structures in the porous substructure.Hypothetically, the increase of the polymer concentration in a dope solution will lead to a denser formation of skin layer and lesser and smaller formation of macro-voids structure. The skin thickness of asymmetric polymeric membranes generally increases with increasing polymer concentration in the casting solution, while the mean pore size and surface porosity decrease. As a result, membrane prepared from solutions with high polymer content tend to show relatively high rejections but low permeances. Thus, it indicates that the formation of larger finger-like macrovoids is due to the lower polymer concentration. For PS6 membrane, a thin-skinned layer was also observed similar to PS1 However, a distinct difference of skin layer thickness between the PS1 and PS6 membrane cannot be differentiated clearly due to the limitation in the equipment. Via increasing the initial polymer concentration, higher polymer concentrations are obtained at the polymer/non-solvent interface prior to immersion in the non-solvent coagulation bath. Therefore, diffusion of solvent and non- solvent is slowed down, demixing is being delayed and fewer open membranes are obtained.

Fig. (3-1) illustrated the Filed-Emission Scanning Electron Microscopy (FESEM) cross sectional images of prepared flat sheet membrane. Generally, the membranes show macro void structures. It’s clear that an increasing polymer concentration in the casting solution decreases the number of macro voids and in addition changes the shape of the macro voids from finger-like to tear-like. Also, large pores appear in the macro void walls, especially above PS 20 wt % in the casting solution. Taking kinetics in to account, the increase in viscosity that coincides with the increase in polymer concentration in the casting solution, slows down the demixing **(Cano-Odena *et al.,* 2009** and **Darvishmanesh *et al.,* 2011)**. With this more delayed demixing, the membrane morphology transitions from macro voids to amore spongy structure, even though for the 27 wt.% of polymers in the casting solution, the macro voids still appear. In addition, finger-like macro voids observed in the porous sublayer has decreased in length as most of the macro-voids observed are shorter compared to the PS1 membrane. However, few large fingerlike macro voids are still present. This suggests that, an increase in polymer concentration in the dope solution led to a reduction in the macro-voids structure. The demixing is thus slowed down but not enough to be able to call it ‘delayed demixing’ in the generally accepted nomenclature. Considering thermodynamics, a bigger miscibility gap in the phase diagram has been linked to more macro voids, which is indeed the case for the studied system **Chung *et al.* (1997)** has reported in their work that chain entanglement in a polymeric-solvent mixture plays an important role in tailoring the characteristics of the membrane. From their work, they suggested that the significant increase in the degree of chain entanglement occurs at a concentration more than PS 25 wt.% (defined as the critical concentration) for PS/NMP.

Within the parameter currently set, it is thus impossible to create a macrovoid-free membrane, which is sometimes said to be desired for the mechanical strength of the membrane (**Li *et al.,* 2011)**. Thermodynamic (bigger miscibility gap) and kinetic aspects (viscosity) are found to be in line to explain the observed membrane morphologies. On the other hand, the formation of spongy-like structure of the PS6 membrane is also mainly due to the higher viscosity of the dope solution. Figures (3-1) PS4 and PS6 show the cross-sectional SEM micrographs of PVP-containing PS membranes. Characteristic morphology of asymmetric membranes comprising of a skin top-layer and porous sub-layer was observed for the current membranes. The micrographs indicate that, when PVP is added to the casting solution, the size and number of channel and finger-like voids are changed.

The presence of PVP in the cast solution also facilitates water diffusion to the polymer cast film, causing faster solvent and non-solvent (water) exchange rate during phase inversion process and leading to formation of long finger-like voids **(Sirinupong *et al.,* 2018)**. For membranes prepared from 25 and 27 wt. % of PS with addition of PVP (Figure 3-1 PS 4 and 6), the size and number of channel-like voids increased compare to the membrane without PVP (Fig. 3-1 PS 3 and 5). Also, the size and number of finger-like voids increased compared to the same PS concentration without PVP. Overall, the effect of polymer concentration on the morphological properties of the membrane can be observed on the changes occurred in the thickness of the skin layer a well as bottom layer and on the formation of the macrovoids structure. As the polymer concentration increased, the thickness of skin and bottom layer also increased whereas the macro-void's structure changes from a large finger-like structure to a teardrop structure.

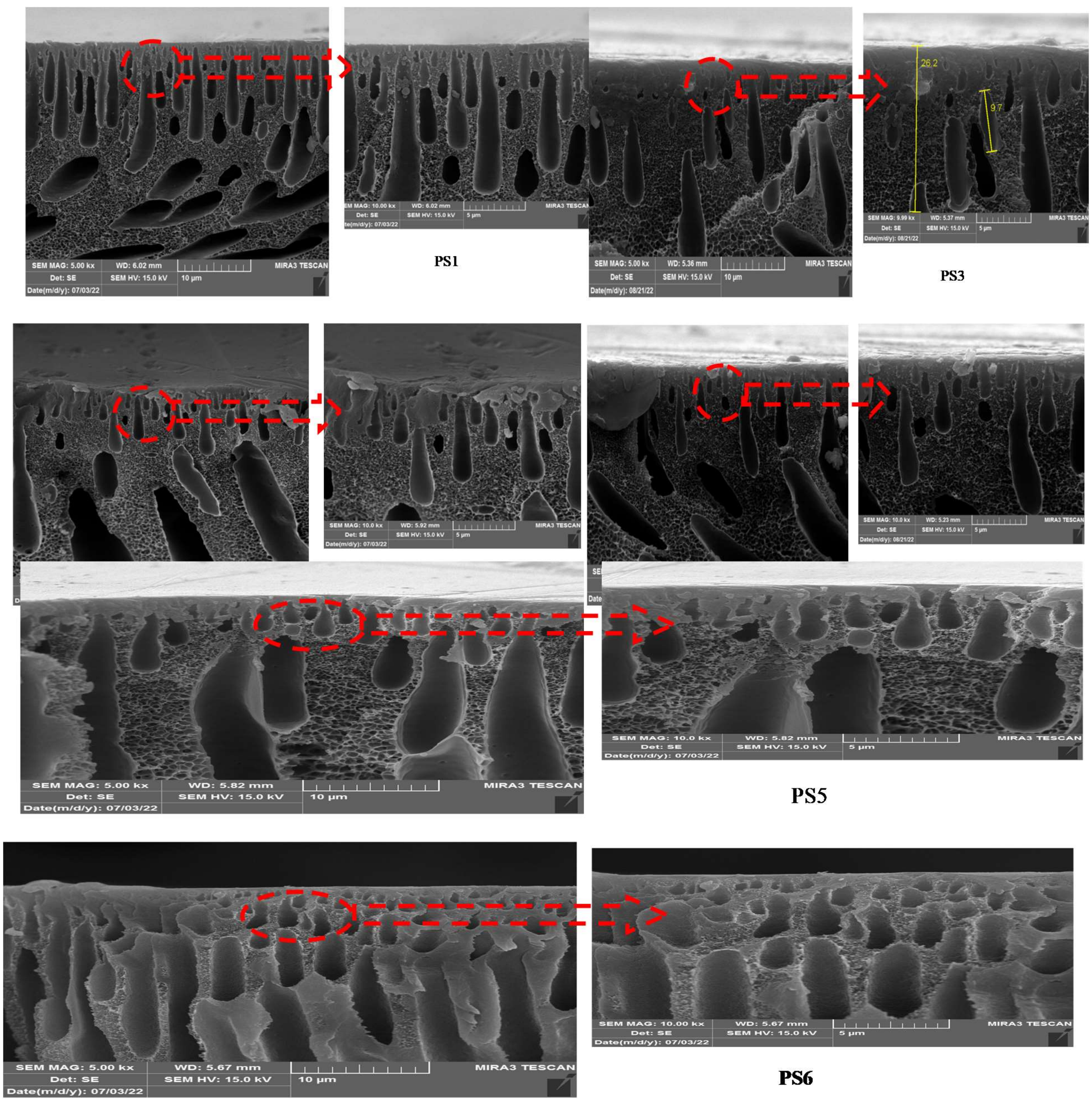
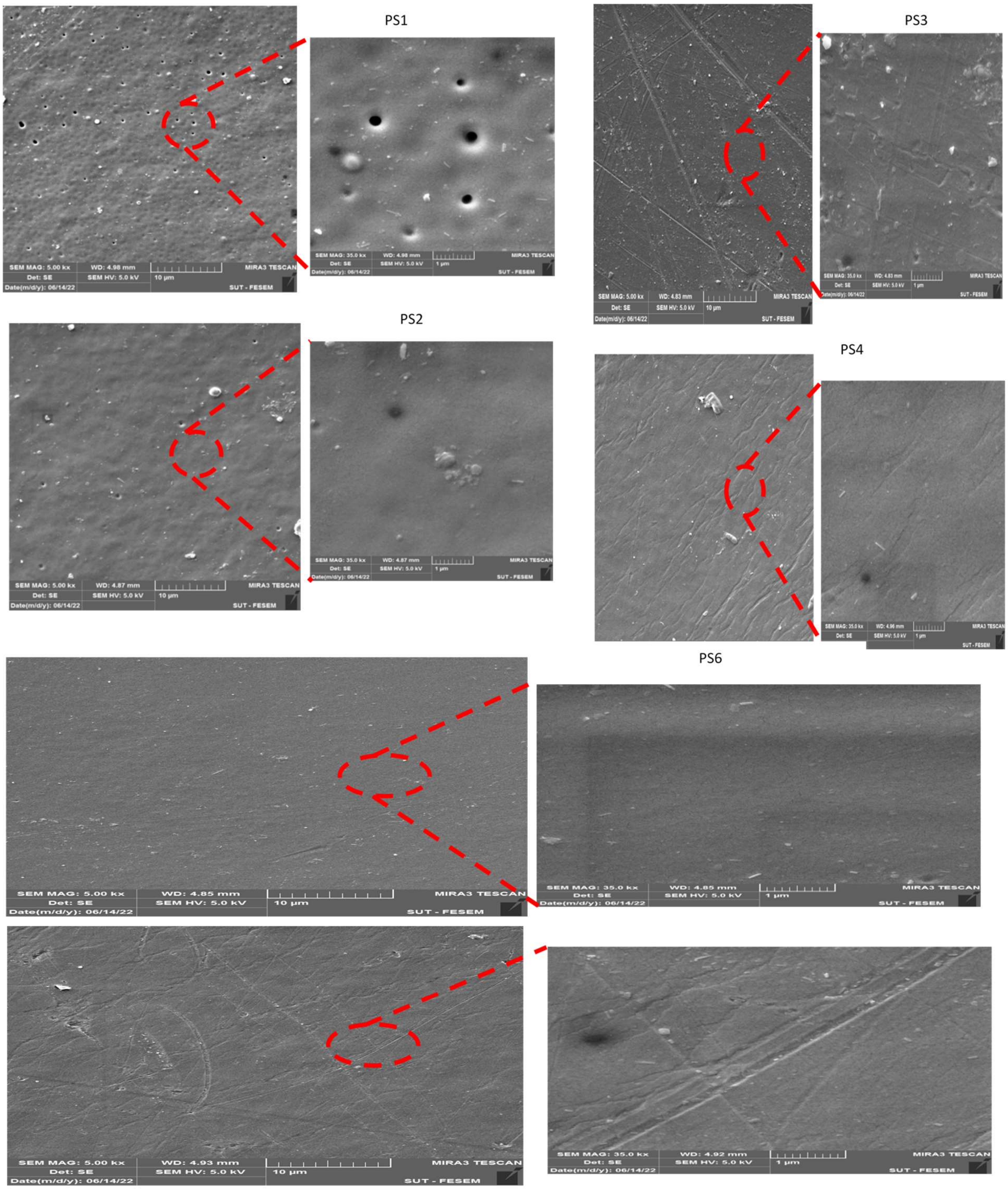


Figure (3-1): Cross-sectional SEM micrographs of membranes.



PS5

Figure (3-2): Filed-Emission Scanning Electron Microscopy (FESEM) of membranes.

Filed-Emission Scanning Electron Microscopy (FESEM) pictures of the membrane's top surface fabricated using PS with different concentrations in the dope solution are illustrated in

Figure (3-2). In membranes prepared with PS variation, a marked difference between the structures was observed. The PS1 membrane was found to be more porous than PS2 and PS3 formulated membranes. The increase of PS concentration also caused a decrease of pore number and size at the membranes top layer, which is more evident for membranes coagulated in pure water. These observations are in agreement with what is observed in literature **Alvi *et al.* (2019)**, polymer concentration is one of the major factors affecting membrane morphology, and its effect overcomes that of additives or other parameters.

By addition of PVP the viscosity of the polymer solution was increased and slow the mutual diffusion between the water in the external solidification bath and the NMP in the casting film this is may be due to the fact that addition PVP to the dope solution that will leads to hindered the counter diffusion through the phase inversion causes a delay in the separation process. PVP flowed into external solidification bath and created pores on the membrane surface, this will lead to increase the pores density.

3.2. Membrane hydrophobicity:

The contact angle measurements were used to study the hydrophilicity of the surfaces of the prepared membrane. Figure (3-3) presents the contact angle results for PS1, 2, 3 and 5 wt.% PS with and without PVP containing. It was observed that with the membranes prepared by changing the concentration of PS polymer, the surface became more hydrophobic as the concentration of polymer increased. This can be attributed to the fact that the hydrophilicity of the formulated membrane depends on the pore size and porosity. The flux results clearly indicate the trend of hydrophilicity in the formulated membranes, whereas the contact angle was observed to decrease when PVP added to the dope solution. It was found that the neat 20 wt.% PS membrane had a contact angle of 58°, whereas 1% PVP containing PS membrane showed remarkable decrease down to 54° and also for PS 27% was 61.8° with a decrease to 56.8° with the PVP added.

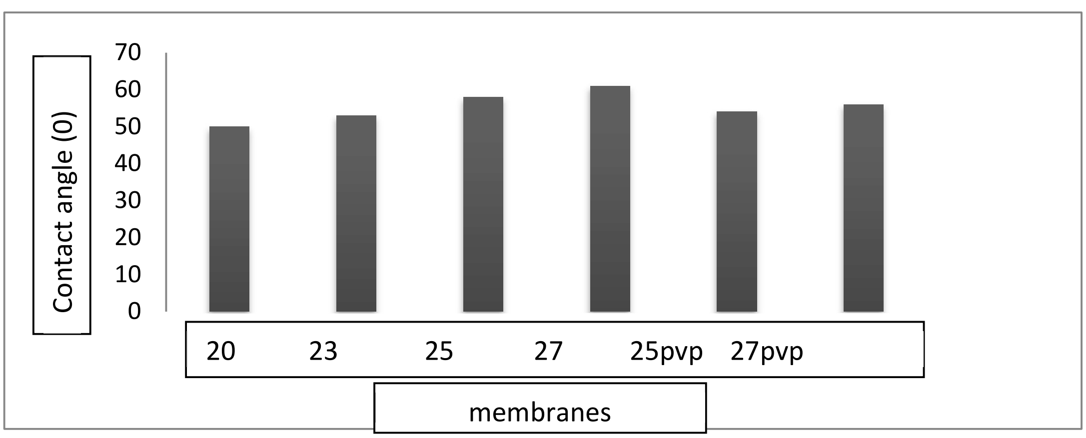


Figure (3-3): Contact angle of membranes

3.3. Membrane Porosity and Pore Size:

As it is clear in Figure (3-4) that by increasing the polymer concentration in the dope solution from 20 wt.% to 27 wt.%, the porosity and mean pore size of the membrane decreased which is also confirmed by the data represented in Table (3-1). The reduction in the pore size upon rising in the PS polymer concentration can be attributed to the growth rate and viscosity of polymer solutions. Basically, high polymer concentration contributes to high viscosity of the polymer solution, hence reducing the droplet growth rate of pore membrane. It's clear that the effect of PVP a pore former additives content in the PS polymer dope solution on the porosity of the prepared membrane. Upon adding 1 wt.% PVP into the PS polymer casting solution, the porosity of the membrane improved from 42.34 % to 47.6%. The membrane porosity following the addition of hydrophilic additives (PVP), so that adding hydrophilic particles in the polymer dope solution produced an accelerated solvent/nonsolvent exchange rate, which in turn led to the enhancement of a highly porous membrane structure. Adding PVP led to growing thermodynamic instability in coagulation bath, thus eliminating the dense top layer and enhancing the porosity of the membrane surface.

Table (3-1): Porosity and Pore Size of membrane.

Membrane code	Porosity (%)	Mean Pore size (nm)	Root Mean Square (nm)
PS1	63.50	60.00	23.5382
PS2	46.19	56.00	14.3604
PS3	42.34	45.80	4.49116
PS4	47.60	47.40	6.67194
PS5	35.11	32.00	2.64678
PS6	45.51	36.00	6.33412

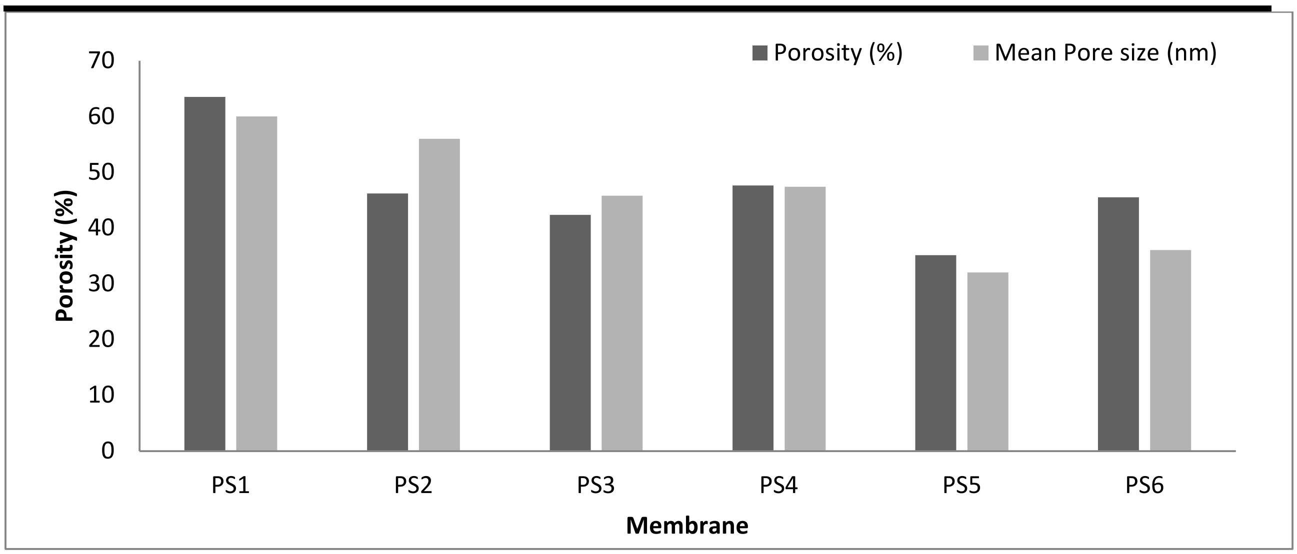


Figure (3-4): Porosity and Pore Size of membrane.

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3.4. Membrane Surface Morphology:

Roughness is a direct metric of surface topography which can be measured with AFM. The 3D AFM figures (3-5) of PS (1 to 6) wt.% flat sheet membranes were displayed in Fig. In these pictures, the dark sections represent the depressions (membrane pores) and the bright ones are representatives of nodules. On the other side, the roughness of the substrate is assigned to the height of the surface's lumps. It is obvious that there was a change in surface morphology of the membranes and the surface roughness of PS1 membrane was higher than that of PS2 membrane.Which in turn is higher in roughness than PS3 and PS5. The decrease in the membrane surface roughness can be attributing to the increase in the concentration of the polymer that led to the increase of the membrane contact angle and thus resulted in the decrease in the membrane surface roughness. According to the Wenzel equation, a membrane that is slightly hydrophilic becomes more hydrophilic as the surface roughness increases. There is a slight increase in the membrane surface roughness for the PS 4 and PS 6, this may be due to the addition of PVP to the PS membrane dope solution.When adding PVP in the PS/PVP dope solution an increment in membrane roughness was obtained. This fact is due to the PVP content in the casting solution, amount of PVP particles initially existed near the interface layer between the membrane surface and coagulation bath (water). This PVP leaked out into the water bath and formed the porous outer surface of the membrane, thereby increasing the surface roughness (increase the pore density) **(Matsuyama *et al.*, 2003)**.

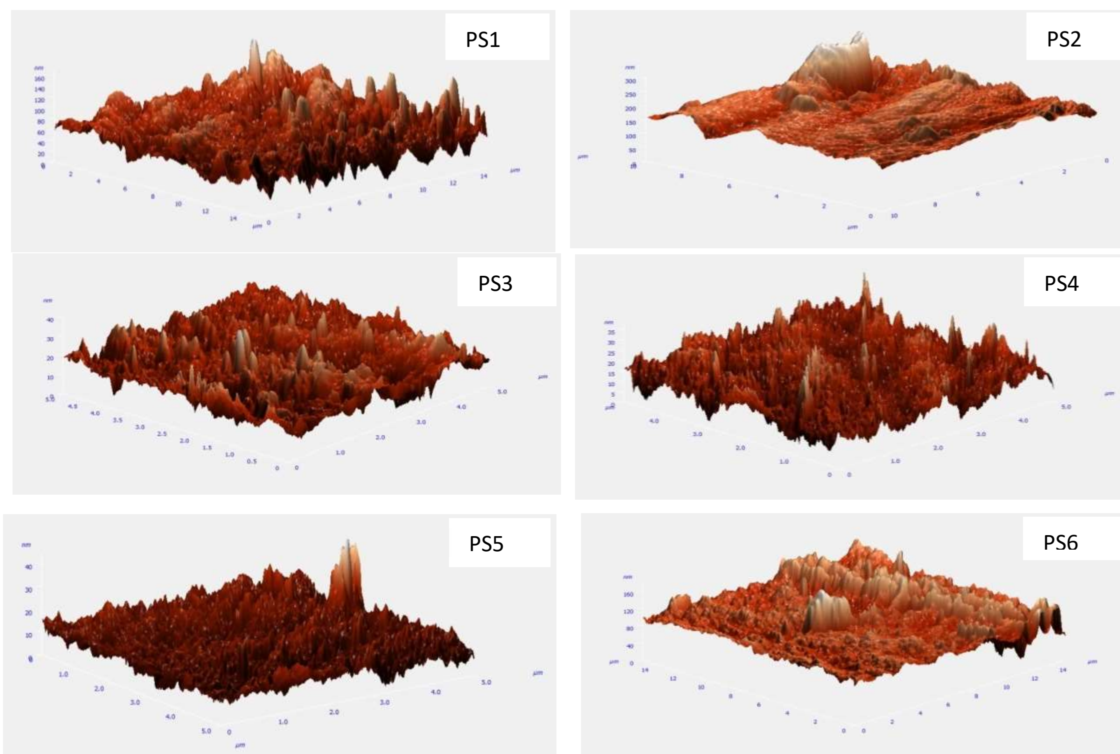

Figure (3-5): Surface morphology of membranes.

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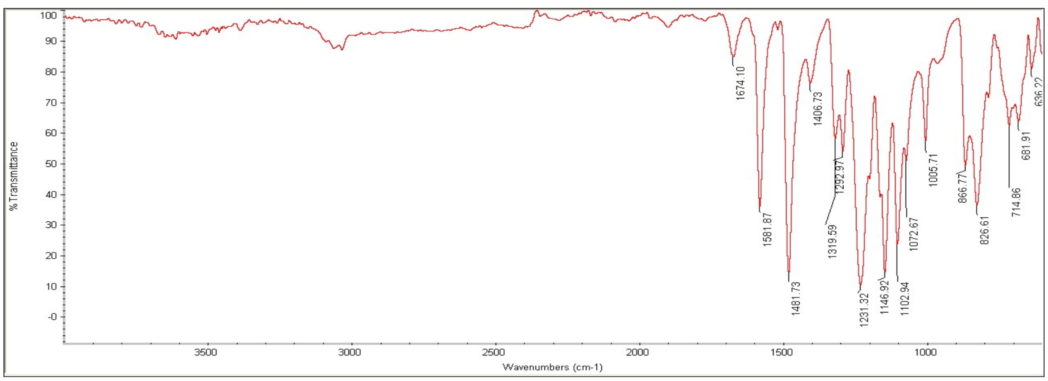
3.5. Structural observations:

ATR-FTIR was employed to elucidate the membrane structural changes when PVP was used as an additive. Figure (3-6) shows the infra-red spectra (ATR) of the neat PS and its surface changes as PVP was added. New absorption peak in the infrared spectra at 1675 cm^{-1} was more prominent in PVP-containing PS membranes while the rest of the spectrum remained similar. This new band is assigned to the carbonyl of PVP. This indicates that PVP is trapped in the PS network and form an integral part of the polymeric structure, providing the polymer surface with a more hydrophilic nature than the surface of neat PS. In addition, the literature findings proposed that the interactions between PS and PVP could be either between pyrrolidone groups in PVP and sulfone groups in PS or between side cyclic groups of PVP and aromatic ring of PS through investigation of viscoelastic behavior of their polymer mixtures **(Sun *et al.,* 2009).** This interaction, in addition to entrapment of PVP molecules, ensures that residual PVP remains in the PS matrix and membrane surface even after washing the PS membane with copious amount of water.



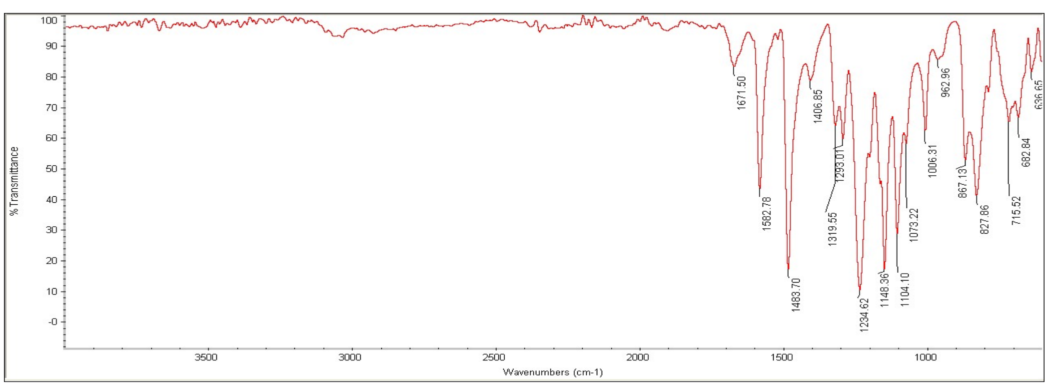


Figure (3-6): Infra-red spectra of membranes.

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3.6. Membrane Performance:

The permeate flux and rejection of all the membranes were measured to determine their performance. Figure (3-7) represents the permeate flux of the synthesized membranes. It is clear from figure that the PS 1 membrane has high values of permeate flux. Results were supported by FESEM analysis as seen in Figure (3-2). The sublayer channels were smaller than the membrane with highest level of PS (6 wt.%). Furthermore, the permeate flux reduced with increase in concentration of PS polymer in the casting solution of prepared membranes. It can be attributed to increase in thickness and formation of dense skin layer with increase in polymer concentration. Similarly, the productivity of the formulated membrane for liquid separation also decreases. On the other hand, the dilute polymer solution formed a thin and porous skin layer with increased values of flux **(Mustaffar *et al.,* 2013)**.

By increasing the concentration of polymer, the viscosity of the polymer solution increased, and the coagulation was slowed down due to strong interaction of solvent and polymer. Moreover, it also resulted in molecules aggregation of polymer because the interaction of polymer and water as nonsolvent was higher. As a consequence, it decreased the dissolving capacity of polymer for solvent **(Kimmerle and Strathmann, 1990)**. The membrane with high polymer concentration and high viscosity can slow down the diffusional exchange rate of NMP and water in the sublayer structure of the membrane. This resulted in the slowdown of the precipitation rate at the sublayer level **(Kim and Lee, 1998)**.

It is worth noting that an increase in the flux of the PS4 membrane can be observed despite the increase in the concentration of the polymer and this can be attributed to the addition of PVP to the membrane. This can be explained by the increased number of pores (pore density) and size of pores due to the presence of PVP, because PVP acted as a pore-forming agent during the phase inversion. It is also clear to note that the increase in the concentration of PS polymer in the dope solution leads to an increase in the membrane rejection, this is due to the decrease in the porosity of the prepared membranes (and as indicated previously) until PS3 membrane, which gives the highest rejection after that a decline in the membrane rejection will take place when increasing the PS concentration more than 25 wt. %.

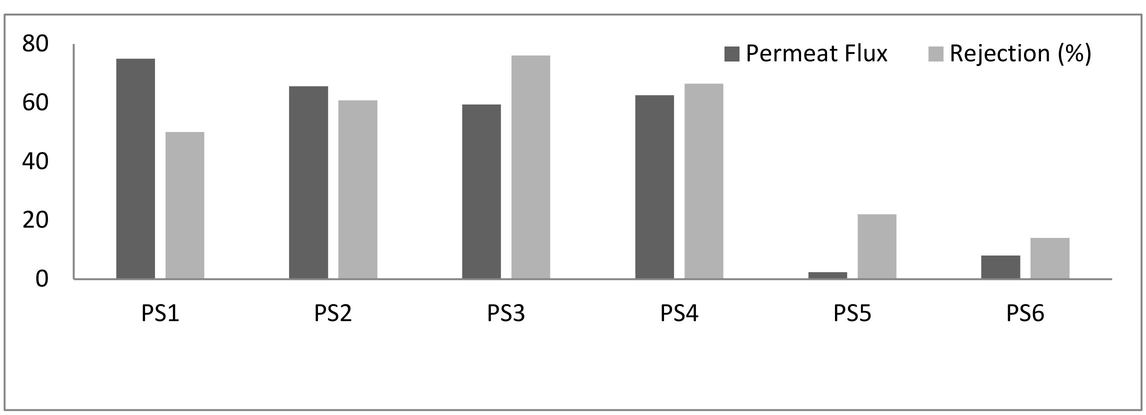

Figure (3-7): Permeate and rejection of membranes.

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3.7. Effect of rennet ratio on curd formation of natural milk and concentrated milk

Time required for cheese formation and the proportion of cheese yield to natural milk and concentrated milk at different percentages of rennet were estimated. The results are shown in the following table (Table 3-2). From this table, it can be seen that when rennet is added in the same proportion (100%) to the same weight of (NM) and (CM) (260 gm) there was a significant difference at the clotting time, where (NM) recorded a longer time than (CM), the values were 35 and 8 min for (NM) and (CM) respectively.

In the same context, also it can be noted that the yield of cheese produced from (NM) is significant less than that of (CM) despite the addition of rennet in the same proportion. This is due to that despite the addition of the same percentage of rennet to the same weight, the same weight of concentrated milk increases in its total solids content, because most of the water was removed during filtration **(Hasan *et al.*, 2021)**. moreover, **Ozturk *et al.* (2022)** found that the yield of cheese produced from (CM) increases around 30-35% over (NM) and **Carter *et al.* (2021) and Aleksic *et al.* (2022)** attributed this to that the concentrated milk containing casein beside whey proteins that were significant lost with whey using traditional methods.

From the same data, it can be observed that there is an opposite correlation between the percentage of rennet added and the time of cheese formation, the time of cured was showed a gradual decrease with increase of rennet ratio, the time was 30 min at 25% of rennet then it gradually decreased to 18, 12 and reached to 8 min at 100% of rennet, and this is in agreement with what found by **Talbot and Selomulya (2021)**.

Table (3-2): Effect of rennet ratio on curd formation of natural milk and concentrated milk.

Treatment	Milk Weight (gm)	Rennet ratio (%)	Curd time (min)	Cheese yield (%)
NM	260	100 a	35 a	12.11 b
CM	260	25 d	30 a	26.63 a
CM	260	50 c	18 b	26.49 a
CM	260	75 b	12 bc	26.57 a
CM	260	100 a	8 c	26.51 a
LSD value	---	12.37 *	6.07 *	4.51 *

3.8. Chemical composition of cheese made from natural and concentrated milk:

Chemical composition of cheese made from natural milk (**NC**) and cheese made from concentrated milk (**CC)** was determined and the obtained results were tabulated in Table (3-3). From this table it is noticed that moisture for (NC) represented the significant highest percentage

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(P<0.05) among the components 63.12%, followed by fat 18.25%, then protein 13.30%, ash was scored 3.16% while Lactose was the significant lowest value among the components, where it was scored 2.17%, these results were similar to what found by **Salman *et al.* (2022)** and **Soltani *et al.* (2022)**.

Likewise, The same behavior can be observed in the case of (CC) there was an significant increase in the proportion of protein, fat and ash of (CC) than (CM), the values of (CM) were 6.3, 9.7, 0.96% respectively, then increased to 21.41, 26, 4.52% respectively for (CC), this is due to the decrease in moisture percentage from 87.52% to 63.12% for (NC) and from 74.84% to 43.91% for (CC) as a result of the separation of water due to the addition of salt which reduces the moisture content and also when the curd formation **(Juan *et al.,* 2022).** Also, the high percentage of ash is attributed to the addition of salt (NaCl) to give white cheese the desired taste **(Nasr *et al.,* (2022)**.

On the contrary, the data show a significant higher percentage at (P≤0.05) of all chemical component (protein, fat, lactose and ash) for (CC) compared to (NC), this is due to that (CM) contains a higher percentage of total solids than natural milk **(Lambrini *et al.,* 2021)**. Moreover, it contains less whey because most of the water was eliminated during filtration **(Pires *et al.,* 2021)**. In the same context, **Salunke and Metzger (2022)** mentioned that cheese made from concentrated milk contains casein and whey proteins, which were lost with whey using traditional methods, which is attributed to the superior ability of membrane filtration to separate water and preserve other components.

Table (3-3): Chemical composition of cheese made from natural and concentrated milk.

Treatment	Chemical Components (%)				
	Protein	Fat	Lactose	Ash	Moisture
RM	3.34 d	3.77 d	4.7 d	0.67 d	87.52 c
CM	6.3 c	9.7 c	8.2 c	0.96 c	74.84 d
NC	13.30 b	18.25 b	2.17 b	3.16 b	63.12 a
CC	21.41 a	26.00 a	4.16 a	4.52 a	43.91 b
LSD value	3.16 *	3.91 *	1.07 *	0.887 *	6.293 *

3.9. Microbiological quality of cheese made from natural and concentrated milk

Microbial quality of the studied cheese was one of the most important dimensions that had to be carefully considered in this study, where the microbial status of cheese made from natural and concentrated milk was assessed and the results were shown in Table (3-4). From this Table, it could be observed that the (NC) was recorded the highest value of total microbial count 0.23×10^2

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CFU/gm while this value was 0.11x10^2 CFU/gm for (CC) but within the acceptable range, These results were in agreement with **(Chen *et al.,* 2019** and **Reig *et al.,* 2021**), and this is due to that the total microbial count of (RM) was higher than that of (CM). Also, the heat that was used to heat the milk during the preparation of cheese was insufficient to eliminate microbes with high efficiency, whereas, membrane filtration was more effective in isolating milk microbes because of its reliance on complete cell separation due to its small pore diameter **(Reig *et al.,* 2021)**. Also, **Blais *et al.* (2022)** confirmed that cheese made by traditional methods contains more microorganisms than cheese made from concentrated milk by membrane filtration.

From the same data, It can be concluded that both of (NC) and (CC) were contained a very low number of molds and yeasts, as well as spore-forming bacteria, where both of them were marked with (±) either at molds and yeasts or spore-forming bacteria. Also, the data showed that a coliform bacterium test of both samples was negative (-). These results were consistent with **(Cruzado-Bravo *et al.,* 2020** and **Kamona *et al.,* 2021)**, and this is due to the good microbial status of both the natural and concentrated milk used. Not only this, The amount of salt was not high, but the nano-membranes prevent the passage of microorganism cells, change in pH and change in osmosis in general **(Bansal and Mishra, 2020** and **Prabawati *et al.,* 2022)**, and especially, coliform bacteria are more sensitive to harsh conditions than molds, yeasts and spore forming bacteria **(Simões *et al.,* 2021)**.

Table (3-4): Microbiological quality of cheese made from natural and concentrated milk.

Treatments	Microbiological quality			
	Total viable count (CFU/mg)	Yeast and mold(CFU/gm)	Spore forming bacteria(CFU/gm)	Coliform bacteria(CFU/gm)
RM (CFU/mL)	8.21x10^6	3.2 x10^2	0.55 x10^2	98 x10^2
Control	0.66x10^3	0.25 x10^3	0.41 x10^3	-
NC	0.23 x10^2	0.19 x10^2	0.31 x10^2	-
CC	0.11x10^2	0.15 x10^2	0.23 x10^2	-

Values calculated as means of triplicates

3.10. Sensory evaluation of cheese made from natural and concentrated milk:

Sensory properties (texture, flavor, aroma and overall acceptability) of cheese made from natural and concentrated milk were evaluated and scores were showed in Table (3-5). From the obtained data, the statistically analyzed results showed that (CC) was obtained the significant highest scores ($P<0.05$) at all parameters compared to (NC), Where was scored the highest degree of texture 8.3 while (NC) was scored 6.8. This is results were in agreement with **Salunke and**

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Metzger (2022) who attributed this to that concentrated milk contains casein in a higher proportion than natural milk, because casein is the main determinant of the ratio of converting milk to cheese, as it serves as the structure for cheese, which holds the components of other milk and thus gives cheese a cohesive texture. Also, In addition, **Deshwal *et al.* (2021)** was reached the same conclusion and confirmed that the fat also plays an important role in giving the cheese the preferred texture, because it gives the cheese a special elasticity and softness without weakening its texture, and also this was consistent with Table (3-2) where the of fat value of (NC) and (CC) were 18.25% and 26% respectively.

Likewise and in the same manner, the data were showed that (CC) was obtained the significant highest scores ($P<0.05$) at both of flavor and aroma compared to (NC), the scores of (CC) were 8.2 and 8.4, while were 6.8 and 7 respectively. This is because (CC) contains proteins, fats and lactose in a higher proportion than (NC), as enzymes break down proteins into peptides, amino acids and aromatic compounds, and the great diversity of these compounds is what gives cheese its unique taste **(Li *et al.*, 2022)**. Also, the fat gives the cheese the desired creamy taste by the consumer, as it decomposes into free fatty acids that give the cheese its special taste and aroma **(Deshwal *et al.*, 2021)**, and actually this results are in agreement with the scheduled data in Table (3-2), where the value of protein and fat were 21.41 and 26% for (CM), while it was 13.30 and 18.25% for (RM) respectively.

Finally, the significant highest overall acceptability ($P<0.05$) in terms of the panelist was observed at (CC), the scores of (CC) and (NC) were 8.2 and 7 respectively. This are in agreement with **Achaw and Danso-Boateng (2021)** and **Lameloise (2021)** who reported that production of cheese from concentrated milk with membrane filtration technology preserves the sensory quality of the cheese and also improves it.

Table (3-5): Sensory evaluation of cheese made from natural and concentrated milk.

Treatments	Sensory evaluation			
	Texture	Flavor	Aroma	Overall acceptability
NC	6.8 b	6.8 b	7.0 b	7.0 b
CC	8.3 a	8.2 a	8.4 a	8.2 a
LSD value	1.17 *	0.934 *	0.781	0.795 *

4. Conclusion

This work was crowned with success in proving its theory of the possibility of using concentrated milk by nano-filtration technology instead of natural milk in the production of soft cheese. Where, economically, the production of cheese using nano-filtration technology was more feasible whether it reduced the time required for curd formation or increased cheese yield. Technologically, the cheese produced using nano-filtration technology had the advantage in terms of chemical, microbial as well as sensory properties. Therefore, use of nanofiltration technology in the production of soft cheese is an economical, effective, well-established and reliable method, and it may open new horizons to improve other dairy products.

References

Achaw, O. W., and Danso-Boateng, E. (2021). Milk and Dairy Products Manufacture. In Chemical and Process Industries (pp. 293-374). Springer, Cham.

Al Musa, R. S., and Al Garory, N. H. (2022). Study of the chemical content and antimicrobial activity of Iraqi butter milk of cows and buffaloes. Texas Journal of Agriculture and Biological Sciences, 9, 22-23.

Aleksic, B., Djekic, I., Miocinovic, J., Miloradovic, Z., Memisi, N., and Smigic, N. (2022). The application of Failure Mode Effects Analysis in the long supply chain–A case study of ultra filtrated milk cheese. Food Control, 138, 109057.

Alvi, Muhammad Azeem UR, Muhammad Waqas Khalid, Nasir M. Ahmad, Muhammad Bilal K. Niazi, Muhammad Nabeel Anwar, Mehwish Batool, Waqas Cheema, and Sikandar Rafiq (2019). "Polymer concentration and solvent variation correlation with the morphology and water filtration analysis of polyether sulfone microfiltration membrane." Advances in Polymer Technology.

Bansal, V., and Mishra, S. K. (2020). Reduced-sodium cheeses: Implications of reducing sodium chloride on cheese quality and safety. Comprehensive Reviews in Food Science and Food Safety, 19(2), 733-758.

Blais, H. N., Schroën, K., and Tobin, J. T. (2022). A review of multistage membrane filtration approaches for enhanced efficiency during concentration and fractionation of milk and whey. International Journal of Dairy Technology.

Bradley, R. L., Arnold, E., Barbano, D. M., Semerad, R. G., Smith, D. E., and Vines, B. K. (1992). Chemical and physical methods. Standard methods for the examination of dairy products, 16, 433-531.

Bridson, E.Y. (1990). The Oxoid Manual. 6th Ed. Unipath Ltd, Wade Roade, Basingstoke RG24 OPN.

Cano-Odena, Angels, Pieter Vandezande, Katrien Hendrix, Rolph Zaman, Khaled Mostafa, Werner Egger, Peter Sperr, Jérémie De Baerdemaeker, and Ivo FJ Vankelecom (2009). "Probing the molecular level of polyimide-based solvent resistant nanofiltration membranes with positron annihilation spectroscopy." The Journal of Physical Chemistry B 113, no. 30: 10170-10176.

Carter, B. G., Cheng, N., Kapoor, R., Meletharayil, G. H., and Drake, M. A. (2021). Invited review: Microfiltration-derived casein and whey proteins from milk. Journal of Dairy Science, 104(3), 2465-2479.

ANNALS OF FOREST RESEARCH
DOI: 10.5281/zenodo.7410153

Chen, G. Q., Leong, T. S., Kentish, S. E., Ashokkumar, M., and Martin, G. J. (2019). Membrane separations in the dairy industry. In Separation of functional molecules in food by membrane technology (pp. 267-304). Academic Press.

Chung, Tai Shung, Soo Khean Teoh, and Xudong Hu (1997). "Formation of ultrathin high-performance polyethersulfone hollow-fiber membranes." Journal of membrane science 133, no. 2: 161-175.

Coggins, J. S. (1991). Predicting Cheddar cheese yield in an individual plant: Van Slyke revisited ,Journal of Dairy Science Volume, 74: 359-368.

Cruzado-Bravo, M. L., Barancelli, G. V., Andreote, A. P. D., Saldana, E., Vidal-Veuthey, B., Collado, L., and Contreras-Castillo, C. J. (2020). Occurrence of Arcobacter spp. in Brazilian Minas frescal cheese and raw cow milk and its association with microbiological and physicochemical parameters. Food Control, 109, 106904.

Darvishmanesh, Siavash, Johannes C. Jansen, Franco Tasselli, Elena Tocci, Patricia Luis, Jan Degrève, Enrico Drioli, and Bart Van der Bruggen (2011). "Novel polyphenylsulfone membrane for potential use in solvent nanofiltration." Journal of Membrane Science 379, no. 1-2: 60-68.

Deshwal, G. K., Kadyan, S., Sharma, H., Singh, A. K., Panjagari, N. R., and Meena, G. S. (2021). Applications of reverse osmosis in dairy processing: an Indian perspective. Journal of Food Science and Technology, 58(10), 3676-3688.

Fenelon M.A. and Guinee T.P. (2000). The Effect of Fat Content on the Microbiology and Proteolysis in Cheddar Cheese During Ripening. J. Dairy Sci. 83(10), 2173-2183.

Gamlath, C. J., Lo, K. Y., Leong, T. S., Ashokkumar, M., and Martin, G. J. (2022). Protein fortification of model cheese matrices using whey protein-enriched double emulsions. Food Hydrocolloids, 108209.

Gao, M., Liu, G., Gao, Y., Chen, G., Huang, X., Xu, X., ... and Xu, D. (2021). Recent advances in metal-organic frameworks/membranes for adsorption and removal of metal ions. TrAC Trends in Analytical Chemistry, 137, 116226.

Guinee, T. P., Feeney, E. P. , Auty, M. A. E. and Fox. P. F. (2002). Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. J. Dairy Sci. 85:1655–1669.

Hasan, S. M., Ullah, S., Umer, M., Shah, M. K., Zaman, A., Hussain, I., ... and Khan, M. S. (2021). Mini Review An-overview on Buffalo Condensed Milk and its Products with Emphasis of Their Biochemical Properties: Mini Review: Biochemical Properties of Buffalo Codensed Milk. Biological Sciences-PJSIR, 64(3), 301-306.

Herman L.M.F., Vaerewijck M.J.M., Moermans R.J.B., Waes G.M.A.V.J.(1997). Identification and detection of Bacillus sporothermodurans spores in 1, 10 and 100 milliliters of raw milk by PCR, Appl. Environ. Microbiol. 63 3139–3143.

Jayalakshmi, A., Rajesh, S., Senthilkumar, S., and Mohan, D. (2012). Epoxy functionalized poly (ether-sulfone) incorporated cellulose acetate ultrafiltration membrane for the removal of chromium ions. Separation and purification technology, 90, 120-132**.**

Juan, B., Trujillo, A. J., and Ferragut, V. (2022). The Effect of Salt Reduction and Partial Substitution of NaCl by KCl on Physicochemical, Microbiological, and Sensorial Characteristics and Consumers' Acceptability of Semi-Hard and Hard Lactose-Free Cow's Milk Cheeses. Frontiers in Nutrition, 9.

Kajak, K., Zielińska, D., Łepecka, A., Jaworska, D., Okoń, A., Neffe-Skocińska, K., ... and Kołożyn-Krajewska, D. (2022). Effect of Lactic Acid Bacteria on Nutritional and Sensory Quality of Goat Organic Acid-Rennet Cheeses. Applied Sciences, 12(17), 8855.

Kamona, Z. K., Daher, A. A. A., and Al Shamary, E. I. (2021). The use of Vitally Active Cellulose Membranes for the Reduction of Pathogenic Bacterial Count in White Cheese. Iraqi Journal of Science, 1121-1127.

Kanagaraj, P., Nagendran, A., Rana, D., Matsuura, T., Neelakandan, S., Karthikkumar, T., and Muthumeenal, A. (2015). Influence of N-phthaloyl chitosan on poly (ether imide) ultrafiltration membranes and its application in biomolecules and toxic heavy metal ion separation and their antifouling properties. Applied Surface Science, 329, 165-173.

Kim, J. H. and Lee, K. H. (1998). "Effect of PEG additive on membrane formation by phase inversion," Journal of Membrane Science, vol.138,no.2,pp.153–163,.

Kimmerle K. and Strathmann H., (1990). "Analysis of the structure determining process of phase inversion membranes, "Desalination,vol.79,no.2,pp.283–302,.

Kujawski, W., Li, G., Van der Bruggen, B., Pedišius, N., Tonkonogij, J., Tonkonogovas, A., ... and Kujawa, J. (2020). Preparation and Characterization of Polyphenylsulfone (PPSU) Membranes for Biogas Upgrading. Materials, 13(12), 2847.

Lambrini, K., Aikaterini, F., Konstantinos, K., Christos, I., Ioanna, P. V., and Areti, T. (2021). Milk nutritional composition and its role in human health. Journal of Pharmacy and Pharmacology, 9, 8-13.

Lameloise, M. L. (2021). Filtration Membranes for Food Processing and Fractionation. In Handbook of Molecular Gastronomy (pp. 335-341). CRC Press.

Li, Norman N., Anthony G., Fane, W.S., Winston, H.O., and Takeshi Matsuura, eds (2011). Advanced membrane technology and applications. John Wiley and Sons,.

Li, S., Zhang, Y., Li, X., Yin, P., Wang, T., Li, Y., ... and Li, B. (2022). The Effect of the Ratio of Gamma Aminobutyric Acid-Producing Saccharomyces cerevisiae DL6–20 and Kluyveromyces marxianus B13–5 Addition on Cheese Quality. Frontiers in microbiology, 13.

Matsuyama, H., Maki, T., Teramoto, M., and Kobayashi, K. (2003). Effect of PVP additive on porous polysulfone membrane formation by immersion precipitation method. Separation science and technology, 38(14), 3449-3458.

Mollahosseini, A., Lee, K. M., Abdelrasoul, A., Doan, H., and Zhu, N. (2021). Innovative in situ investigations using synchrotron-based micro tomography and molecular dynamics simulation for fouling assessment in ceramic membranes for dairy and food industry. International Journal of Applied Ceramic Technology, 18(6), 2143-2157.

Mustaffar, Ismail, M. I., A. F., and Illias, R. M., (2013). "Study on the effect of polymer concentration on hollow fiber ultrafiltration membrane performance and morphology, p.12.

Nasr M, N., MH Hassaan, H., AS Khalifa, E., and A Metry, W. (2022). Enhancing the Vitality of Free Fat UF-Soft Cheese Supplemented with Some Nutritious Additives by Adding Probiotic Bacteria. International Journal of Family Studies, Food Science and Nutrition Health, 3(1), 124-144.

Ouyang, H., Kilcawley, K. N., Miao, S., Fenelon, M., Kelly, A., and Sheehan, J. J. (2022). Exploring the potential of polysaccharides or plant proteins as structuring agents to design cheeses with sensory properties focused toward consumers in East and Southeast Asia: a review. Critical reviews in food science and nutrition, 62(16), 4342-4355.

Ozturk, G., Liang, N., Bhattacharya, M., Robinson, R. C., Shankar, S., Huang, Y. P., ... and Barile, D. (2022). Glycoproteomic and Lipidomic Characterization of Industrially Produced Whey Protein Phospholipid Concentrate with Emphasis on Antimicrobial Xanthine Oxidase, Oxylipins and Small Milk Fat Globules. Dairy, 3(2), 277-302.

Piggott, J.R. (ed.) (1984). Sensory Analysis of Foods, Elsevier Science Publishers, London.

Pires, A. F., Marnotes, N. G., Rubio, O. D., Garcia, A. C., and Pereira, C. D. (2021). Dairy by-products: A review on the valorization of whey and second cheese whey. Foods, 10(5), 1067.

Prabawati, E. K., Turner, M. S., and Bansal, N. (2022). Lactiplantibacillus plantarum as an adjunct culture exhibits antifungal activity in shredded Cheddar cheese. Food Control, 109330.

Ramadan, A. M. E. G. (2020). Chemical and technological studies on quality and validity of edible olive oils (doctoral dissertation, al-azhar university).

Reig, M., Vecino, X., and Cortina, J. L. (2021). Use of membrane technologies in dairy industry: An overview. Foods, 10(11), 2768.

Salman, K. H., Ali, F. A. Z., and Elhanbaly, R. (2022). Effect of cultured white soft cheese on the histopathological changes in the kidneys and liver of albino rats. Scientific Reports, 12(1), 1-17.

Salunke, P., and Metzger, L. E. (2022). Functional characteristics of process cheese product as affected by milk protein concentrate and micellar casein concentrate at different usage levels. International Dairy Journal, 128, 105324.

Simões, J., Yang, Z., and Dong, T. (2021). An ultrasensitive fluorimetric sensor for pre-screening of water microbial contamination risk. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 258, 119805.

Sirinupong, T., Youravong, W., Tirawat, D., Lau, W. J., Lai, G. S., and Ismail A. F. (2018). "Synthesis and characterization of thin film composite membranes made of PSF-

ANNALS OF FOREST RESEARCH
DOI: 10.5281/zenodo.7410153

TiO2/GO nanocomposite substrate for forward osmosis applications." Arabian Journal of Chemistry 11, no. 7: 1144-1153.

Soltani, M., Saremnezhad, S., Faraji, A. R., and Hayaloglu, A. A. (2022). Perspectives and recent innovations on white cheese produced by conventional methods or ultrafiltration technique. International Dairy Journal, 125, 105232.

S**un, M., Su, Y., et al., (2009).** Improved antifouling property of PS ultrafiltration membranes using additive of silica/PVP nanocomposite. Industrial and Engineering Chemistry Research 49 (2), 790–796.

Talbot-Walsh, G., and Selomulya, C. (2021). The effect of rennet casein hydration on gel strength and matrix stability of block-type processed cheese. Food Structure, 28, 100174.

Xiang, J., Liu, F., Wang, B., Chen, L., Liu, W., and Tan, S. (2021). A literature review on maillard reaction based on milk proteins and carbohydrates in food and pharmaceutical products: advantages, disadvantages, and avoidance strategies. Foods, 10(9), 1998.

Xiao, S., Yu, S., Yan, L., Liu, Y., and Tan, X. (2017). Preparation and properties of PPSU/GO mixed matrix membrane. Chinese Journal of Chemical Engineering, 25(4), 408-414.