



The Physico-Chemical Properties of Bovine and Buffalo Whey Proteins Milk by Using Ultrafiltration Membrane Technology

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Abstract: The current study aims to prepare whey from bovine and buffalo fresh milk to make three types of cheese, namely: thermal, acidic and enzymatic. Afterward, whey proteins have been separated, then the concentration process of whey proteins has been conducted by using ultrafiltration membrane technology. Through the previous step, two products have been obtained; first, concentrated whey proteins which is called (Retentate), while the other is called (Permeate). Applying rotary evaporator, whey proteins are concentrated and then drying in two methods: spray-drying and freeze-drying in a form of white and soft powder. The chemical composition has been studied at each phase. The results show the separation, purification, and concentration of bovine and buffalo whey proteins by using ultrafiltration membrane technology. The results show that buffalo whey proteins produced by the method of enzymatic and dried with spray-drying are better than bovine whey protein. Finally, the results show a low ratio of lactose, salts and moisture content at the stages of filtration and concentration. The results present a high proportion of protein to 80 .and low ratio lactose and salt.

Keywords: Whey, Type of Whey Proteins, Cheese making, Ultrafiltration Membrane Technology, Concentrated Whey Proteins.

Introduction

Milk, with its different sources, is considered one of the most important nutrition. It consists of a complex formula that includes many nutrition components and compounds of different quantities, qualities, and specifications which make it naturally homogeneous. This substance gives its natural and chemical characteristics and the nutritional value as well (Dissanayake, 2011; Santos & Lies, 2015). The milk proteins are

characterized by its high nutritional value and functional properties especially whey proteins

that are regarded as a part of milk protein. Milk proteins are widely used as ingredients in processed food since they are by-products and economical. Milk proteins are classified within generally recognized as safe and high nutritional value due to the high content of amino acids that contains sulfur. They contain vital effectiveness for digestibility. In addition, they can be work as antimicrobial, antiviral drugs and anti-cancer drugs for

enhancing the immune system as well as the chemical and physical properties represented by its ability to water holding capacity and form a gel, foam stability and emulsifying (Saha & Bhattacharya, 2015; Henriques *et al.*, 2016; Królczyk *et al.*, 2016).

Moreover, its applications are widely used in the food industry such as dairy products, pastry, meat, juices and infant food as well. They are also used in packing and wrapping food for the purpose of extending products shelf-life, preserving and maintaining vital compounds. There are many studies about using a material like natural protein that capable to work as antioxidant and anti-natural factors for inhibiting the growth of microorganisms as an alternative to chemical material. However, there are many doubts about their safety in terms of health aspects for the reason that some of them are carcinogenic or toxic for long term-use (Shang *et al.*, 2018).

Ultrafiltration membrane technology is widely developed in the field of the dairy industry. It is used as an integral part of dairy technology for the purpose of separation and concentration of milk, proteins, peptides, sugar, fat and other ingredients of foodstuffs. Furthermore, there are many features in this technology. Firstly, this filtration technology is considered one of the highly selective technologies and doesn't require energy to process with necessary time-reduction for the separation process. Secondly, it is an easy and simple method to be conducted with time-reduction in preparing the sample and extending the shelf-life. Besides that, the separation process leads to a highly concentrated product and the foods manufactured by this method contain high quality, healthy and safe with a high nutritional value. Consequently, it will be lead

to a diversity in its products as well as it is considered as one of the methods of food preserving and minimizing pollution risks (Mohammad *et al.*, 2012).

Dairy factories in the world are considered to develop and improve the process of purification and separation of proteins by using modern technologies such as membrane filtration that aims to concentrate the proteins of milk for the purpose of improving the nutritional value and the diversity of milk ingredients by the concentration and separation of certain components. Therefore, the ultrafiltration membrane has the ability to separate the ingredients of milk on the basis of different sizes or weight of molecules of separated proteins. The used membranes have variedly related to the wanted product type (Hurt & Barbano, 2010; Macwan *et al.*, 2016).

The separated and concentrated whey proteins can be produced by using this technology in which the separation process depends on molecular weight and the type of product. Ultrafiltration (UF) was initially used in the dairy industry to pre-concentrate milk before cheese making. It has also been successfully used to recover proteins from whey and concentrate milk proteins from smaller compounds such as lactose, vitamins and minerals. Ultrafiltration of skim milk produces milk protein concentrate (MPC), while ultrafiltration of cheese whey produces whey protein concentrate (WPC), It is estimated that more than 90% of whey protein concentrates (WPCs). Microfiltration membrane has attracted the attention of many scholars in an attempt of increasing the purification, separation, concentration and raising the proportion of solid material. Membrane filtration can be divided into ultrafiltration membrane technology that

represented by applying the number of alternate physical or chemical steps in separating the components of both caseinate proteins and whey proteins from water, sugar, and minerals. Proteins were preserved by the membrane and the reserved ultrafiltration product is called retentate. But lactose, mineral, and water are permeated together with membranes which are called permeate (Hurt *et al.*, 2010). The membrane separation process can be classified within groups depending on driving motive force through the membrane. When the pressure is the motive force through the membrane, there will be four well-known filtration techniques Mukherjee (2019), namely: 1-Filtration by using reverse osmosis (RO), 2-Nanofiltration (NF), 3-Ultrafiltration (UF) and 4-Microfiltration (MF).

Materials & Methods

Milk Collection

Bovine milk was obtained from the Agricultural Research Centre, College of the Agriculture, University of Basrah and buffalo milk from breeders in Al Midaina district.

Whey Proteins Production

The whey has been produced in the acid method that is followed by Mohamed *et al.* (2013). In order to produce the sweet whey from bovine and buffalo milk according to the method set out in Yazdanpanah *et al.* (2015) with conducting minor modification. The method is very similar to the whole steps for the same manufacturing and the only difference is the replacement acid with rennet taking into account the temperature and the rennet in 37° C. Thermal -acidic whey production from bovine and buffalo milk in accordance with the method described in Yazdanpanah *et al.* (2015) with conducting some modification by using a temperature and

adding acid which is completely similar in each and every step of the same manufacturing. The difference is only occurred by adding the acid taking into consideration the temperature.

The Concentration of Whey

Jayaprakasha (1992) has been adopted for concentrating whey proteins by using ultrafiltration device type (Haddad), Iranian origin and filter papers were used type (Ceramic) with porosity type cut-off 10 KDa at 5 bar, temperature 50-55 °C. The capacity of the device is 50 litre, then heating to 50° C. The membrane filtration took about 30 minutes that has been separated into two-part; first (Permeate) 37 kg and the other protein concentrates (Retentate) 11 kg. After opening and cleaning the device, the fat layer was removed and then the concentration of the whey protein step has been conducted after using the ultrafiltration membrane. The samples have been concentrated by using evacuated rotary evaporator as in fig. (1).

The Drying of Whey Proteins

For studying the chemical composition of concentrated bovine and buffalo whey proteins, spray drying device has been used type, Anozzale supplied by CORSA (Iranian Company) with minor modification and taking into consideration the work conditions (a type of sample) upon running and operating the device. The method in which described by Lorenzen & Schrader (2006) has been adopted.as in the table (1). The choose the best drying method.

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Fig. (1): The Ultrafiltration plant (Experimental Dairy, Dairy Science College, Tehran)

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Fat Ratio

The ratio of fat has been estimated by using Gerber method according to the method mentioned in James (1995).

Table (1): The working conditions of the machine.

Working conditions of the machine of Spray-drying(modification)		
160°C	temperature	Inlet
80 °C	temperature	Outlet
0.2	Spray close	
8.0	Spray Open	
15.0 par	Pump rate	
50%	Aspiration Ratio	

Determination of Protein

The total ration of nitrogen has been estimated for studied samples of bovine and buffalo freeze and dried whey proteins by using Automatic Nitrogen Analyzer as perSemi-Micro-Kjeldahl showed by Egan *et al.* (1988) by multiplying the output by the

general conversion factor of milk 6.38 to get the percentage of protein.

Determination of Moisture Content

Moisture has been estimated based on the said method in Nielsen (2010) by using an electric dry- oven at 105 °C for a period of hours until the weight is stabled.

Determination of Ash Ratio

Ash has been estimated as per the method mentioned in Nielsen (2010) at 550 °C by igniting the samples, then transferred to Muffle Furnace and the samples are left until the whitish-gray has happened.

Determination of Lactose

Carbohydrates ratio has been calculated by the difference among the components previously mentioned as illustrated by Egan *et al.* (1988).

Determination of Titratable Acidity

The titratable acidity of milk and whey has been measured by calibration with standard sodium hydroxide NaOH 0.1 N by using phenolphthalein as mentioned by Marshall (1992).

Determination of pH

pH has been estimated by using pH-Meter type, Crison (German Origin) as per the method described by Egan *et al.* (1988).

Statistical analysis

Statistical analysis was performed by SPSS (2018). The analysis variance (ANOVA) Design Randomized complete (DRC) and difference were considered significant. L.S.D if $P < 0.05$. Parson's correlation was used to determine any significant differences among the whey protein types before and after using Ultrafiltration (UF) as well as differences between the drying methods.

Results & Discussion

Whey is produced from bovine and buffalo milk by three methods: Acidic, enzymatic, and thermal-acidic. The results show that there are differences in the three whey proteins result from the components and physical properties by comparison between them. This may attribute to the reason for the

source of milk, the type of cheese and used thermal treatment. These links with high and low pH and total acidity as shown in table (2).

The concentration of ultrafiltration whey proteins

Table (2) and fig. (2) showed the physicochemical properties of the bovine milk before and after ultrafiltration membrane. Upon close examination in the below table, the statistical analysis results showed no significant differences ($P \leq 0.05$) in physicochemical properties and the physicochemical characteristics among types of bovine whey before the ultrafiltration membrane except for the total acidity ratio.

The results are also showed significant differences ($P \leq 0.05$) in the physicochemical properties and physicochemical characteristics among types of bovine whey after ultrafiltration membrane except for ash, lactose and fat ratio. This may attribute to the difference of whey protein composition to the difference in the method of curdled.

Therefore, the results showed that before whey protein types filtration, there is a low in protein ratio and the water content in acidic whey and thermal-acidic whey. This may attribute to the heating treatment of about 80° C for 15 minutes that lead to evaporate the water ratio and protein denaturation about 20-50 %. This ratio depends on the time and temperature where it plays an important and crucial role in the capability of linking water. For instance, the denaturated proteins are increased by its capability to bear water and it will work to form a three-dimension net composed of a protein complex which is called whey protein-casein complex.

Table (2): The properties physic-chemical of bovine of whey protein before and after using Ultrafiltration.

Type whey Properties Physic-chemical	before of Ultrafiltration			after of Ultrafiltration		
	Whey acid	Whey Enzymatic	Whey theromal acid	Whey acid	Whey Enzymatic	Whey theromal acid
pH	4.50	6.33	5.11	4.45	6.21	4.86
Titratable Acidity	0.69 ^a	0.16 ^b	0.32 ^b	0.75 ^a	0.21 ^c	0.58 ^b
Protein Ratio	0.84	1.35	0.85	62 ^b	67 ^a	63 ^b
Determination of lactose	4.85	6.24	5.79	4.6	3.86	4.7
Fat Ratio	0.58 ^b	0.68 ^a	0.56 ^c	3.9	3.6	3.8
Moisture Content	93	91	92	29 ^a	25 ^b	28 ^a
Ash Ratio	0.73 ^b	0.73 ^b	0.8 ^a	0.5 ^b	0.54 ^a	0.5 ^a

before of Ultrafiltration	R.LSD :0.1565 Titratable Acidity R.LSD:N.S:for other properties	after of Ultrafiltration	R.LSD:0.1565 Titratable Acidity R.LSD:2.6842 Protein Ratio R.LSD:2.6842 Moisture Content R.LSD:N.S for other properties :
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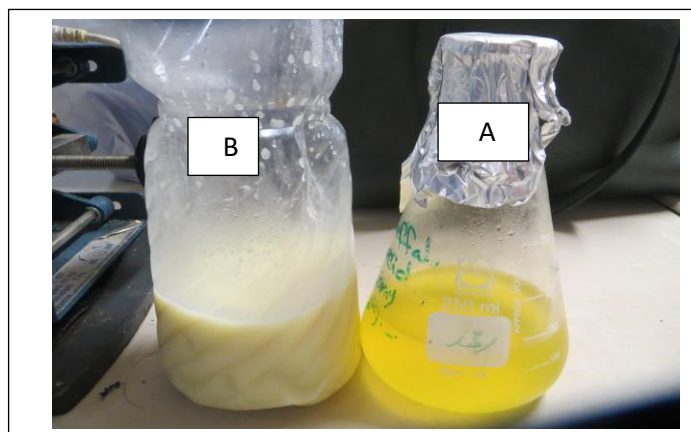


Fig. (2): A: whey proteins befor and B: after using Ultrafiltration.

The whey proteins form 10 % and casein protein 50 % in the formation of firm gel (cheese) prevents leakage with remaining whey. Therefore, its protein and water ration are low in both two types of whey (Arora. & Khetra (2017)).

It can also be seen from the table (2) there is a difference in physicochemical properties of bovine whey protein after filtration. So, the results showed that there is an inconsistency between whey proteins and their types. This may attribute to the reason of low total solid

material in bovine milk that forms 12.8 %, which contributes to extend the period of curdled. While pH is low ranging between (4.52-6.21). Such values indicated the closeness of whey proteins to the isoelectric point that enhancing our results by increasing the capability of protein to bear water and low water content in both types of acidic and thermal-acidic whey.

The data presented at the table (3) and fig. (3) showed physicochemical tests of buffalo whey before and after ultrafiltration

membrane. There are no significant differences ($P \leq 0.05$) in physicochemical properties and physicochemical characteristics among the types of buffalo whey before the ultrafiltration membrane except for total pH and ash. There are significant differences ($P \leq 0.05$) in physicochemical properties and physico-

chemical characteristics among the types of buffalo whey after the ultrafiltration membrane except for lactose and fat ration. The difference in the ratio of protein among the types of whey is related to the type of protein which is considered as the most important factor that contributes to the milk formula.

Table (3): The properties physic-chemical of buffalo of whey protein before and after using Ultrafiltration.

Type whey	Properties Physic-chemical	before of Ultrafiltration				after of Ultrafiltration	
		Whey acid	Whey enzymatic	Whey theromal acid	Whey acid	Whey enzymatic	Whey theromal acid
	pH	4.90	6.45	5.53	4.75b	6.32a	5.33ab
	Titratable Acidity	0.44a	0.18c	0.33b	0.50a	0.16c	0.36b
	Protein Ratio	1.45	1.9	1.50	66b	68a	65b
	Determination of lactose	5.02	6.62	5.83	3.4	3.66	3.89
	Fat Ratio	0.74b	0.72c	0.77a	4.0	4.8	4.61
	Moisture Content	91	90	91	26a	23b	26a
	Ash Ratio	0.76b	0.76b	0.8a	0.60a	0.54b	0.5c

Before of Ultrafiltration	R.LSD:0.0268 Titratable Acidity R.LSD:0.0268: Ash Ratio R.LSD:N.S N.S for other properties	After Ultrafiltration	R.LSD:2.36725 pH R.LSD:0.0219 Titratable Acidity R.LSD: 0.0219 Ash Ratio R.LSD:2.6842 Protein Ratio R.LSD:2.6842 Moisture Content R.LSD:0.0115 Ash Ratio R.LSD :N.S S for other properties
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Fig. (3): Whey proteins concentrate (Retentate) and permeate.

This difference in the ratio of protein may attribute to the concentration of the proteins in

milk based on its source, the method of curdled, the protein stability towards

temperature, and the used temperature. It has been noticed that the high ratio of protein in enzymic whey of buffalo in comparison with acidic and thermal whey. This may attribute to the low temperature used that preserves the natural protein and buffer capacity as well as the quality of Glycomacropetides (Božanić *et al.*, 2014).

It can be concluded that the high ratio of buffalo whey protein belongs to the interspecies variability in protein content of milk or could be due to the variation in animal genetic factors such as animals body size which required more feed intake which can enrich protein quantity and quality, feeding type can also enhance the protein ratio (Salman *et al.*, 2014).

The studies have proved that there is a high ratio in whey protein and a low ratio in lactose and mineral. This could attribute to lactose is dissolved solids which membrane cannot be retained. Hence, the average of existing in concentrate depends on the ratio of water which is constantly decreased by the filtration process several times which is called Diafiltration. Then, adding water to concentrate for the purpose of disposal of lactose and mineral, after that the process of diafiltration that is consistent with Bonnaillie *et al.* (2014).

The results are close to the concluding marks of Vyas & Tong (2003) who reported that the ash ratio is decreased after concentration by using ultrafiltration techniques. The average whey produced before filtration is 0.75 while the concentrate is about 0.65. Depending on these numbers and in comparison with the ash ratio infiltration, we have found that the retention ratio is 65 % of mineral salts (ash) in the final from white to brown. It has been noticed that the ratio of lactose and the ratio of free amino

concentrate. The ash ratio could attribute to the ratio of calcium and phosphorus that attained the highest ratio of retention among mineral components. The reason behind this relates to 50 % of calcium linked with protein. Finally, samples are concentrated by using an evacuated rotary evaporator and the result has been matched with the conclusion of Jayaprakasha & Yoon (2005).

The drying of whey proteins

The table (4) and fig. (4) illustrated the methods of drying concentrated whey proteins by spray-drying and freeze-drying. Upon close examination of the study of chemical content once again for each acidic and enzymatic whey proteins, it has been found that the statistical analysis showed that there is a significant difference ($P \leq 0.05$) in the ratio of protein as in the source of protein and decomposition method. While the statistical analysis showed that there is no significant difference ($P \leq 0.05$) in pH and the ratio of fat in the protein source and decomposition method. The results indicate that there is a high ratio in whey proteins in the form of spray-drying and this could be attributable to denaturated proteins which are called polypeptides. In the table, it has been noticed that there is a low ratio in water content, sugar, and mineral.

The least result has been noticed in the freeze method, where concentrated bovine and buffalo whey proteins have been freeze. Whey proteins are characterized with soft white color powder while the dried whey protein in the freeze method is characterized with hard creamy yellow powder with a low ratio of protein. In respect of whey produced by the thermal method, it has been exposed to colorful changes during the period of storage acids that contain amino acid (lysine) that promoting to get early Maillard reactions

(Bernard *et al.*, 2011). So that, such acidic-thermal whey proteins have been removed. Therefore, the whey proteins produced by the method of enzymic or dried with spray-drying give the best results in comparison with the remaining proteins produced by the two methods of drying.

The results showed that buffalo whey proteins produced by the method of

enzymatic and dried with spray-drying are better than bovine whey protein. The spray-drying technique requires good adjustment for work conditions and also appropriate for solutions contain active components. The most important factors of succeeding in the drying process are the log on/off temperature, the ratio of spray, pressure average and the concentration of solid material (Gallo *et al.*, 2011; Santana *et al.*, 2018).



Fig. (4): Spray drier and Freeze-drying (Experimental Dairy, Dairy Science College, Tehran).

Table (4): The comparison of two methods of spray drying and freeze-drying.

Properties physic- chemical	Spray-drying				Freeze-drying			
	Whey bovine		Whey buffalo		Whey bovine		Whey buffalo	
	Acid	Enzymatic	Acid	Enzymatic	Acid	Enzymatic	Acid	Enzymatic
pH	4.11 ^b	5.79 ^a	4.34 ^b	5.88 ^a	4.42 ^b	6.05 ^a	4.26 ^b	6.28 ^a
Protein Ratio	78 ^b	80 ^a	79.30 ^b	83.50 ^a	75	76	77 ^b	79 ^a
Fat Ratio	4.72	5.77	4.71	5.95	3.92	4.17	4.10	4.20
Moisture Content	6.81	6.79	6.84	6.96	14.75 ^a	13.50 ^b	13.87 ^a	12.50 ^b

R.LSD:1.331pH between the Spray-drying	R.LSD:1.331	PH	Freeze-drying
sedimentation methods			
R.LSD:1.331 type proteins	R.LSD: 1.331	Protein	

	between deposition methods and protein type.
R.LSD:1.307 Moisture Content deposition methods	R.LSD:1.153 ratio between proteins and sedimentation methods
R.LSD: N.S Fat, acidic, Moisture Content and protein ratio between type proteins.	R.LSD: 1.153 Fat and the acidic ratio of proteins

The spray-drying is characterized to remove water at limits of 96 % and it works for α -helixes unfolding that leads to form random coils. Thus, it will promote β -sheet to form crosslinking with controlling each acidic function and pH of protein where it is considered as one of the external factors that affect producing gel (Nishanthi *et al.*, 2017).

It has been found that there is no vital or active protein entered informing the three-dimension net. In this case, this could be attributable to the difference in the sequence, the rates of amino acids and the type of peptides of protein after heat treating in both two protein. Generally, protein contains more than 31.5 % of hydrophilic polar amino acids and hydrophobic polar amino acids. They play an important and crucial role in linking water (Damodaran *et al.*, 2017).

Conclusions

This study has shown that physicochemical characteristics were determined before and after the using of the ultrafiltration technique. The most important results can be summarized by using ultrafiltration technology. The chemical composition and the difference between the two drying methods of spray- drying and freeze-drying were used in this study. The results show that buffalo whey proteins produced by the

methods of enzymic and dried with spray-drying are better than bovine whey protein.

The spray-drying technique requires good adjustment for work conditions to produce height protein percentage. Thus, the differences in protein ratio among the types of whey are attributable to the type of protein in which it considered as one of the most important factors that contribute to milk formula. The difference in the formula and composition of whey protein may attribute to the difference in protein deposition method. Finally, the results show a low ratio of lactose, salts, and moisture content at the stages of filtration and concentration.

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Conflicts of interest

The authors-declare-that they-have-no-conflict of interests.

Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

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الخصائص الفيزيوية- كيميائية لبروتينات شرش حليب الأبقار والجاموس باستعمال تقنية الترشيح الفائق بالأغشية

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المستخلص: هدفت الدراسة تحضير الشرش من الحليب الابقار والجاموس الطازج وصدع الجبن بالأنواع الثلاثة حراري – حامضي والحامضي وانزيمي ثم فصل بروتينات الشرش وبعدها أجريت عملية تركيز بروتينات الشرش بتقنية الترشيح الفائق بالأغشية Ultrafiltration ومن ثم ركزت بروتينات الشرش بتقنية الترشيح الفائق بالأغشية و بهذه الخطوة تم الحصول ناتجين أحدهما بروتينات الشرش المركزة ويطلق عليه (Retentate)، أما الراشح يطلق عليه (Permeate) ثم ركزت البروتينات الشرش بالاستعانة بالمبخر الدوار ومن تم تجفيف بطريقتين هما طريقة التجفيف بالرداذ Spray-drying و طريقة التجفيد Freeze-drying بهيئة مسحوق ابيض وناعم وتم دراسة تركيب الكيميائي في كل خطوة وقد أظهرت النتائج المستحصل عليها فصل وتنقية وتركيز بروتينات الشرش الابقار والجاموس بتقنية الترشيح الفائق بالأغشية Ultrafiltration أن بروتينات شرش حليب الجاموس المنتج بالطريقة الانزيمية والمجفف بطريقة التجفيف بالرداذ افضل مقارنة ببروتينات شرش حليب الجاموس أظهرت النتائج النهائية وارتفاع نسبة البروتين الى 80% وانخفاض نسبة اللاكتوز والملح والرطوبة.

الكلمات المفتاحية: الشرش، انواع بروتينات الشرش، صناعة الجبن، تقنية الترشيح الفائق بالأغشية، بروتينات الشرش المركزة.