Research Article

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Health Science Journal
ISSN 1791-809X

2018
Vol.12 No.5:587

DOI: 10.21767/1791-809X.1000587

Studies on Physiochemical and Functional Properties of Protein Co-Precipitates from Camel's and Goat's Milk

Manal A AlGhsyar[1*], Samir A Salem[2] and Sahar H Ahmad[3]

[1]Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Ministry of Education, Qassim University, Saudi Arabia

[2]Department of Dairy Science and Technology, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

[3]Department of Dairy Science and Food Microbiology, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia

[*]**Corresponding author**: Manal A AlGhsyar, Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Ministry of Education, Qassim University, Saudi Arabia, Tel: 00966542458877; E-mail: manal.alqusayer@gmail.com

Received date: 05 September 2018; **Accepted date:** 19 September 2018; **Published date:** 27 September 2018

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Citation: AlGhsyar MA, Salem SA, Ahmad SH (2018) Studies on Physiochemical and Functional Properties of Protein Co-Precipitates from Camel's and Goat's Milk. Health Sci J Vol.12.No.4:587.

Abstract

Increasing world population, increasing demand for and cost of protein-rich foods, and the continuing need to improve the nutritional and functional properties of protein ingredients have contributed to a greater research into blends or composites as food ingredients. Proteins co-precipitates have a range of biological, physical, chemical, functional, sensory and nutritional properties giving the potential application as product ingredients in the food industry, though relatively little published information is available on this subject.

Camels serve as a major source of milk and meat in the Middle East, where there are great number of camels in Saudi Arabia, which is the second largest country in the production of camel milk after Somalia; Camel milk is considered a highly consumed food in the Arab Gulf states. Also, Proteins from Camels' and Goats' milks are characterized with different properties than cow proteins, and they are an important source for milk in the desert areas.

The aim of this study was to obtain the co-precipitates proteins of camels' and goats' milk using different methods such as heat treatment with or without addition of calcium chloride (CaCl) or hydrochloric acid (HCl) as well as precipitation after the concentration of proteins by ultra-filtration and then study their physical and functional characteristics to recommend their use in the food industry.

Keywords: Milk; Protein; Co-precipitates

Introduction

Milk proteins have long been used as additives for processed food. Their nutritional and functional properties make them one of the best nutritional supplements [1]. Numerous markets are interested in functional proteins and in utilizing them in the food industry which has led to the increasing focus on improving production and separation methods of milk proteins of specific functions [2]. Producing co-precipitates proteins have a number of benefits such as increasing coagulum and functionality as it has higher nutritional value compared to Cazin [3]. Methods for producing milk protein concentrates have widely been developed with differences in structure and characteristics that can be used easily as a food component [4-6].

Several methods for the production and processing of co-precipitates proteins have already been developed and it is possible to discover new ones that allow greater returns for using co-precipitates proteins as commercial products in the food processing sector [7-15].

The industrial future tends to develop new consumer products and find alternatives to cow's milk. In this regard, camel's milk and goat's milk often turns viable candidates [1]. Camel's milk offers a unique advantage for regions where camels are found in large numbers. It urges the usage of nutrition techniques in the production and treatment of camel's milk. Interest for the variety ingoat's milk products has increased as well as researchers' interest in goat's milk commercially as an alternative to cow milk products used in food rations or for children allergic to cow's milk [16-20].

Attention should be given to research by improving marketing of high-quality and of long life milk products. This will generate support from the private sector to advance the development of camels' products in countries where camels are found in large numbers and to satisfy demand by offering camel's milk especially with the growth of the Arab community

[1]. Now is the time for transformational food industries to have come to realize that milk proteins have considerable power to contribute different characteristics in order to enhance a variety of food products [21,22].

Objectives

Preparation of co-precipitates proteins for camels and goat's milk in different technological methods, using heat, CaCl and acid or using ultrafiltration to concentrates the protein before precipitation.

Study the physiochemical and functional properties for the protein to identify and learn about its advantages.

To reach important recommendations for conducting research to manufacture some products that fit these characteristics properties.

Methods

Materials

1. Milk from healthy lactating camels and goats in Al-Qassim area, Saudi Arabia.
2. The chemicals were provided by the Postgraduate Laboratories at College of Design and Home Economics-Qassim University.

Methods

1. **Preparation of milk:** Camels and goats milk were separated at 40°C at pH 6.3
2. **Preparing co-precipitates:** The first method was by heating the milk until it reached 75°C for 5-20 minutes then adding Calcium 0.2% or HCL 0.1 N. The precipitation of the protein was at 85-95°C and pH 5.1. Collecting of proteins was done at room temperature and then filtered through Whatman No.1 paper. Washing the precipitate and removing water was later cared out. The drying process was carried out in a hot air oven at 55 and then finally, grinded the precipitate to a soft powder.

The second method was by using Ultrafiltration (UF). The concentration of milk was determined by Ultrafiltration, using (Ultrafiltration unit Reverse Osmosis–Model: ARMFILD C03962) to Fold-2. The milk was heated to reach 75°C and was hold for about 20-25 minutes. Next, steps were carried out as per the first method.

The gelatin flavor was prevented by adding 0.5-0.1 sodium sulfate and flavor stability was improved by heating the solution to encourage the volatilization of unwanted flavor components that can be removed during drying.

Methods of analysis

Chemical analysis: The chemical analysis for the skim milk was done for both camel and goat milk using (Lactostar (C) 2008 Funke Gerber Firmware 4.0.19#3510-r80403 Model (C) (2008). For (fat, protein, lactose, ash, non-fat solids, freezing point and PH)

Chemical analysis of co-precipitates components: Moisture, protein, fat, carbohydrate, ash and calcium.

Physical properties of co-precipitate: pH in 5% distilled water at 20°C, viscosity at 20°C and using LV spindle No (3) ± RPM=100 and color (b,a,L).

Functional properties of co-precipitate: Solubility, NSI, PDI, WHC, foaming capacity and stability, fat binding capacity, emulsification capacity and stability, proteins electrophoresis separation on the 7.5 alkaline native polyacrylamide gel PAGE with SDS, B-mercaptoethano and discontinuous buffer system.

Statistical analysis: The data were statistically analyzed by ANOVA analysis with (SPSS) 24th Edition and the difference were significant at probability values ($P<0.05$).

Results

1. The highest percentage of the refinement of the inherent sediment for each camels' and goats' milk using HCl was 27.14%, 24.7% respectively. This process was noted by ultrafiltration while adding HCl to goat's milk significantly higher than camel's milk [6].
2. The total percent of solids in co-precipitates of camels' milk that HCl was added is 60.6%, after it comes the one that was done by ultrafiltration with HCl. There were non-significant differences between the percentage of solids in co-precipitates in goats' milk for both $CaCl_2$ and HCl **(Figure 1)**.
3. It has been found while studying the chemical composition of co-precipitates that there is a significant increase in goats' milk moisture content at level 0.05 for that was precipitator with acid and then followed by the one that $CaCl_2$ was used and the lowest is the one that has been done by ultrafiltration with HCl.
4. While in camels' milk the highest significant percentage of co-precipitate moisture was when using $CaCl_2$ and the lowest that was done by ultrafiltration with $CaCl_2$ **(Table 1)**.
5. Protein content in camels' milk co-precipitate which was prepared by ultrafiltration while adding either HCl or $CaCl_2$ was the highest one. Whereas the highest content of goats' milk co-precipitate that was noted while using $CaCl_2$ or HCl.
6. Significant differences have been noticed in the percentage of fat in co-precipitates of camels' milk that was prepared by ultrafiltration with adding either HCl or $CaCl_2$. On the other hand, the highest percentage that was noticed in goats' milk was the sample that was prepared with HCl [9].
7. There was a significant difference in the content of Lactose in the co-precipitates of camels' milk that was higher than goats' milk.

8. The co-precipitates that was prepared of camels' milk using $CaCl_2$ was the highest in ash content. Meanwhile, the highest sample of ash of goats' milk was the one that was prepared by ultrafiltration with adding $CaCl_2$. It's important to say that there were some significant differences of the percentage of $CaCl_2$, and the highest sample was the addition of $CaCl_2$ **(Figure 2)**.
9. There were non-significant differences between the samples of cows' and goats' milk co-precipitates that were prepared using different methods in pH or acid number values [15].
10. While there were some significant differences at 0.05 in the solution's viscosity 10% of co-precipitates between different samples. The highest viscosity value of camels' milk co-precipitates was the sample that used $CaCl_2$ or was done by ultrafiltration with $CaCl_2$. Meanwhile, there were non-significant differences regarding goats' milk, and its highest had HCl **(Figure 3)**.
11. The precipitation substance had a noticeable effect on co-precipitates' color degree. The whitest and the shiniest camels' milk sample was the one that was precipitated with HCl, then the sample that was prepared by ultrafiltration either by using $CaCl_2$ or HCl.
12. While the co-precipitates of goats' milk were significant higher values when prepared by ultrafiltration with $CaCl_2$.
13. As for the degree of greenness (a), there were significant differences between camels' milk co-precipitates samples conversely to goats' milk, except using ultrafiltration with HCl. As for the degree of yellowness, the highest value of camels' milk co-precipitates was the sample that was prepared using ultrafiltration with HCl. It was of a more significant in goats' milk than camels' milk **(Table 2)**.
14. Concerning the solubility of co-precipitates, the highest solubility was at 25°C and on pH=7 for the samples that were done by ultrafiltration with adding either HCl or $CaCl_2$ to camels' milk. The solubility has increased with the increase of protein concentration **(Figure 4)** [19].
15. When studying the solubility on a different pH and on 25°C it was pH=2 the highest parameters while using ultrafiltration with HCl in case of camels' milk, on pH=8 the parameters were by ultrafiltration either by adding $CaCl_2$ or HCl, the best one was on PH=10, at pH=4 there were non-significant differences between the parameters **(Figure 5 and Table 3)**. As for goats' milk, the parameters that were done by ultrafiltration either with adding $CaCl_2$ or HCl on pH=4, 8 and 10 were the best of them. While on pH=2 there were non- significant differences between the parameters [20].
16. The parameters that used ultrafiltration with HCl for both camels' and goats' milk that HCl was added to, and to goats' milk that HCl was added to only, significant differences than the rest of the parameters in both protein distribution and soluble nitrogen Index. This shows the effect that ultrafiltration parameter has on protein spread and on soluble nitrogen Index **(Figures 6 and 7)**.
17. The ability to hold water for co-precipitates in camels' milk parameter that was prepared by ultrafiltration with adding either HCl or $CaCl_2$ is the highest in contrast to goats' milk co-precipitates that was precipitated in $CaCl_2$ or HCl only **(Figure 8)**.
18. When measuring foam formation, it turned out that the highest capacity was found in goats' milk precipitate with $CaCl_2$ and in camels' milk with HCl compared to eggs' albumin. With more condensation, intangibility differences increased and foam size got better. With using 10 g camels' milk co-precipitates has the biggest foam size, using $CaCl_2$ then HCl, as it reached 64.7%-65.7% compared to eggs' albumin. Whereas ultra-filtrated, $CaCl_2$ goats' milk co-precipitates was 61.8% compared to eggs' albumin [1].
19. The highest record of co-precipitates goats' milk foam stability was with using HCl only or $CaCl_2$ only and with using the latter there was little detaching of liquid from foam. While the lowest record was of co-precipitates camels' milk foam that was prepared with ultrafiltration, whether with or without adding $CaCl_2$ or HCl **(Figure 9)**.
20. Using a concentration of 3 g of co-precipitates, the highest foam stability was of goats' milk that was ultra-filtered with added HCl or prepared with added $CaCl_2$. As for camels' milk the highest was the one prepared with ultrafiltration and added $CaCl_2$ [5].
21. When examining fat binding ability of co-precipitate, it was found that goats' co-precipitate milk that was prepared using $CaCl_2$ is more capable of binding with fat at a significant of 0.05 compared to the co-precipitate that was done by ultrafiltration with added HCl, whereas in camels' milk, the co-precipitate that was prepared with ultrafiltration with HCl was the highest in fat binding ability compared to other samples **(Figure 10)**.
22. In examining fat emulsion stability, $CaCl_2$ goats' and camels' milk co-precipitate were the best. Followed by the ones done by ultrafiltration and HCl **(Figure 11)**.
23. An electrophoresis (SDS) separation was done to the co-precipitates proteins of both goat's and camel's milk and there weren't any intangible differences in the casein particles nor in the whey protein whether in regards to electrophoresis rates or the molecular weight for each protein except for case number 1 in which $CaCl_2$ was added to it, as a decrease of the special patterns density has been noted as well as a huge decrease in beta-lactoglobulin. As for goats' milk precipitated, there hasn't been any intangible difference in any of its proteins whether in electrophoresis rates or the molecular weight, the density or the concentration of protein has been noticed to be increasing when treated with ultrafiltration and with added HCl, and it was observed also that the whey proteins disappeared significantly **(Figures 12 and 13)** [16].

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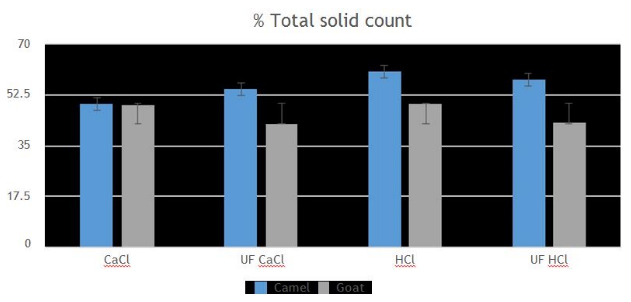
2018
Vol.12 No.5:587



CaCl	co-precipitate Obtained with adding Calcium chloride
UF CaCl	co-precipitate Obtained by using Ultrafiltration with adding Calcium chloride
HCl	co-precipitate Obtained by adding Hydrochloric add
UF HCl	co-precipitate Obtained by using Ultrafiltration with adding Hydrochloric acid

Figure 1 Total solid counts of camel and goat co-precipitates.

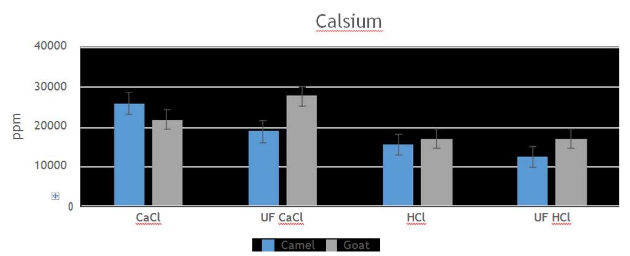


Figure 2 Total calcium count percentage of camel and goat co-precipitates.

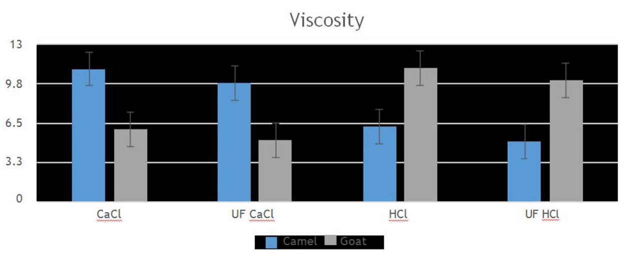


Figure 3 Viscosity values of camel and goat co-precipitates.

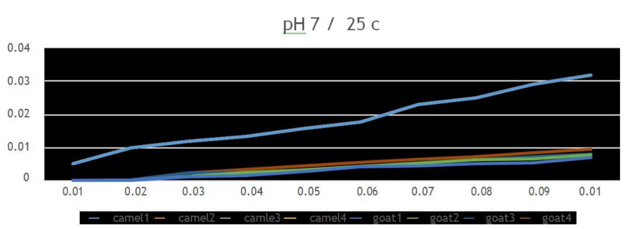


Figure 4 Solubility at 25°C/pH7 of camel and goat co-precipitates.

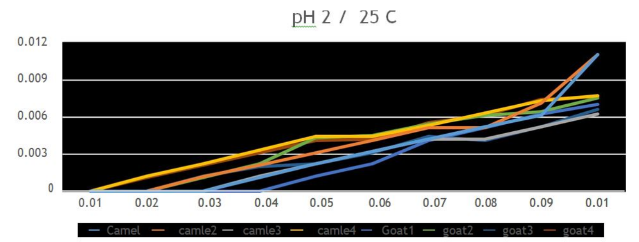


Figure 5 Solubility at pH2/25°C of camel' and goat co-precipitates.

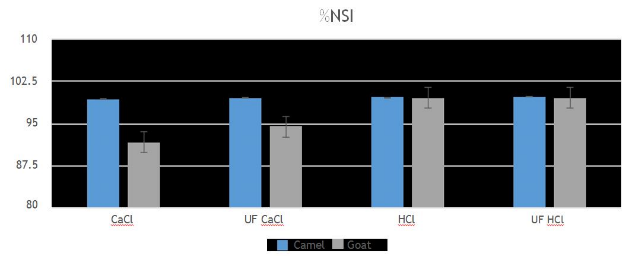


Figure 6 NSI of camel and goat co-precipitate.

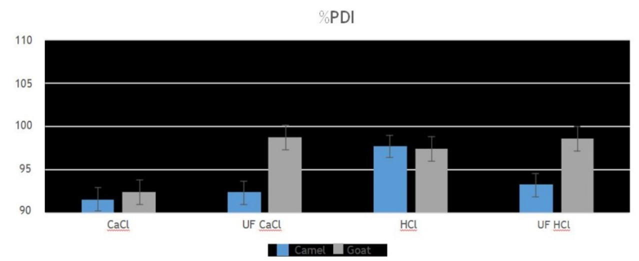


Figure 7 PDI of camel' and goat co-precipitates.

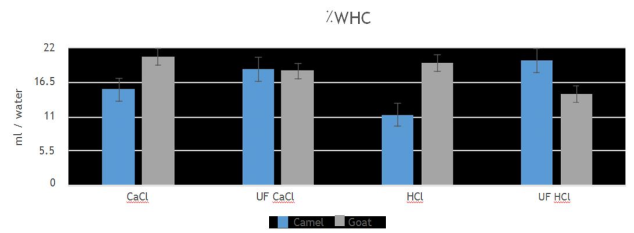


Figure 8 WHC of camel' and goat co-precipitate.

2018
Vol.12 No.5:587

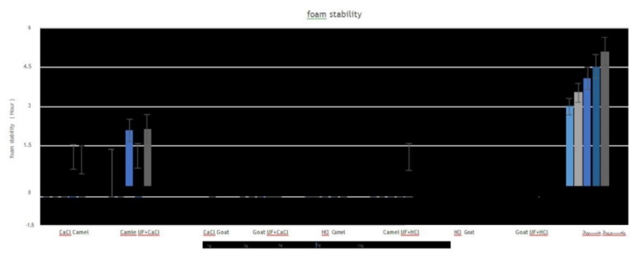


Figure 9 Foaming stability of camel' and goat co-precipitates.

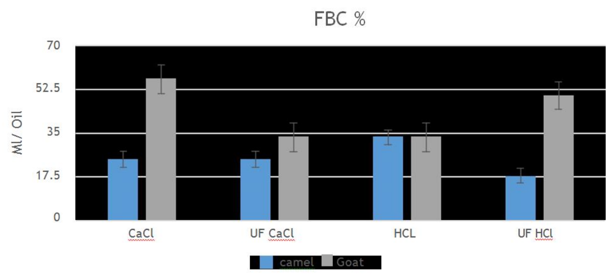


Figure 10 FBC for camel' and goat co-precipitates.

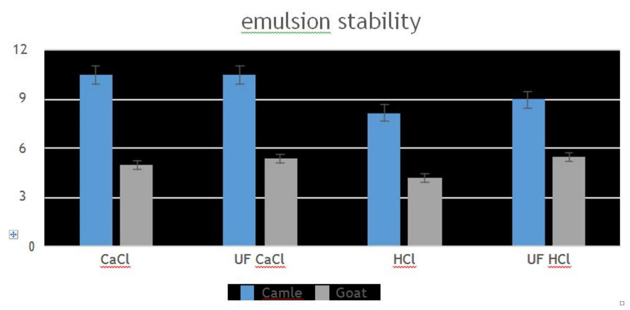


Figure 11 Emulsion stability for camel' and goat co-precipitates.

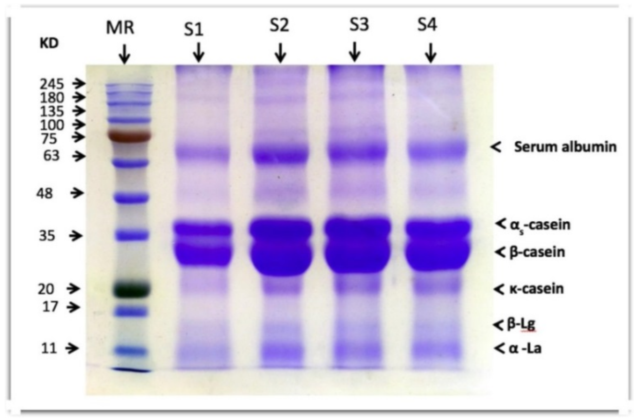


Figure 12 Electrophoreses (SDS-PAGE) for camel co-precipitates.

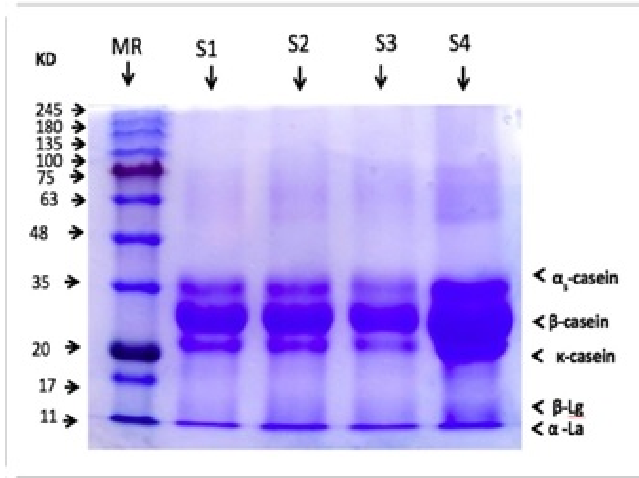


Figure 13 Electrophoreses (SDS-PAGE) for goat co-precipitates, *SDS-PAGE, 12.5% T, was carried out using the discontinuous buffer system described by Laemmli.

Table 1 Chemical composition of camel and goat milk co-precipitates.

Ash		Lactos		Fat		Protein		Mouster		
Goat	Camel	Goat	Camel	Goat	Camel	Goat	Camel	Goat	Camel	
6.03^{b,c} ± 0.06	9.87^{a} ± 0.03	5.85^{c} ± 0.12	25.47^{b} ± 0.11	3.52^{d} ± 0.01	3.61^{c} ± 0.02	82.67^{a} ± 0.07	56.68^{c} ± 0.2	11.58^{b} ± 0.01	10.82^{a} ± 0.03	CaCl
8.06^{a} ± 0.05	9.49^{b} ± 0.03	11.88^{a} ± 0.35	24.68^{c} ± 0.08	5.18^{c} ± 0.01	4.36^{a} ± 0.02	71.69^{d} ± 0.11	57.51^{b} ± 0.1	10.68^{c} ± 0.01	9.32^{b} ± 0.01	UF CaCl
5.21^{c} ± 0.01	8.42^{c} ± 0.02	5.72^{c} ± 0.47	30.03^{a} ± 0.33	7.07^{a} ± 0.05	3.22^{d} ± 0.02	79.16^{c} ± 0.51	54.15^{d} ± 0.33	13.87^{a} ± 0.02	9.08^{c} ± 0.01	HCl
6.21^{b} ± 0.01	8.25^{d} ± 0.02	7.36^{b} ± 0.18	24.76^{b,c} ± 0.29	6.88^{b} ± 0.01	4.06^{b} ± 0.02	77.50^{b} ± 0.29	59.49^{a} ± 0.23	9.06^{d} ± 0.03	8.48^{d} ± 0.05	UF HCl

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aSignificance of co-precipitation, bSignificance of ultrafiltration with $CaCl_2$, cSignificance of both co-precipitation and ultrafiltration, dComposition of $CaCl_2$

Table 2 Color values of camel and goat milk co-precipitates.

Color value						Type of treatment
b		a		L		
Goat	Camel	Goat	Camel	Goat	Camel	
$12.75^a \pm 0.003$	$4.85^d \pm 0.028$	$-2.23^a \pm 0.005$	$-2.2^b \pm 0.028$	$74.10^b \pm 0.057$	$66.23^d \pm 0.057$	CaCl
$6.87^d \pm 0.005$	$7.33^b \pm 0.005$	$-2.10^a \pm 0.17$	$-2.48^c \pm 0.018$	$74.84^a \pm 0.011$	$81.00^c \pm 0.005$	UF CaCl
$8.91^c \pm 0.006$	$6.85^c \pm 0.028$	$-2.26^a \pm 0.005$	$-2.95^d \pm 0.028$	$68.29^c \pm 0.005$	$84.12^a \pm 0.011$	HCl
$10.55^b \pm 0.01$	$8.72^a \pm 0.011$	$-2.55^b \pm 0.02$	$-1.94^a \pm 0.005$	$66.83^d \pm 0.005$	$81.89^b \pm 0.005$	UF HCl
aSignificance of co-precipitation, bSignificance of ultrafiltration with HCl, cSignificance of both co-precipitation and ultrafiltration, dComposition of HCl						

Table 3 Co-precipitation of camel and goat by using CaCl and Hcl.

Camel 1	Goat 1	Camel 2	Goat 2	Camel 3	Goat 3	Camel 4	Goat 4
CaCl		UF CaCl		HCl		UF HCl	
Co-precipitate obtained with adding calcium chloride		Co-precipitate obtained by using ultrafiltration with adding calcium chloride		Co-precipitate obtained by adding Hydrochloric acid		Co-precipitate obtained by using ultrafiltration with adding Hydrochloric acid	

Discussion and Conclusion

The co-precipitate can be used directly to supply or strengthen food sources of low quality or low protein content and can also be used in different foods to raise nutritional value. This requires further Interest to be taken to be used in many goods.

1. Research the minimum protein recovery including extraction and precipitation conditions.
2. Research the preparation of co-precipitate using more than one source of proteins.
3. Evaluation of the effect of supplementation of co-precipitate protein nutritionally and biochemical in both laboratory and experimental animals on chronic diseases such as diabetes, high blood pressure.
4. Evaluation the effect of co-precipitate protein sediment on people who are allergic to bovine s Casin.
5. Research the effect of co-precipitate treatment on food products.
6. Research the effect of co-precipitate protein on the sensory properties of food products.
7. Evaluation of co-precipitate effect on the functional properties in food products.

- **Suggested studies on its use in the following foods are:**
- Ice-cream and web cream using the co-precipitate of goat milk prepared by adding calcium chloride, hydrochloric acid or camel milk using ultra-filtration with both.
- Milk flavored drinks, juices and fermented milk using co-precipitate of camel and goat milk prepared by the ultra-filtration method either by adding hydrochloric acid or calcium chloride.
- Meat products using co-precipitate prepared by ultra-filtration method with the addition of calcium chloride or hydrochloric acid to both camel and goat milk.
- Baked goods using co-precipitate prepared by ultra-filtration method with the addition of hydrochloric acid or calcium chloride to camel milk, calcium chloride or hydrochloric acid for goats' milk.

Recommendation

It's recommend to keep studying the application of these different types of co-precipitates in different kinds of food, especially the ones treated with ultrafiltration with added HCl or $CaCl_2$ like juice or soft dairy products or ice cream and using goats' milk co-precipitates with added HCl or $CaCl_2$ in making baked goods and meat products and its effect on their functional, organoleptic properties. It's also recommended to research the effect of added co-precipitates protein on the nutritional and biological value, whether by laboratory experiments or animal testing (those used for testing chronic diseases like hypertension or diabetes).

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