ISOLATION, CHARACTERIZATION AND BIOACTIVITY OF CASEIN AND ALBUMIN FROM FRESH AND PACKED COW MILK SAMPLES

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Abstract

The aim of the study is to isolate and characterize the casein and albumin from selected fresh and packed cow milk samples as well as to determine the minerals and antioxidant activities. Preliminary milk quality tests of both milk samples showed that pH (6.8-6.5) were within normal range in the presence of carbohydrate and reducing sugar gave good quality with alcohol test. The main component of casein I and II (9.11 % and 7.36 %) from both samples were isolated by adjustment of isoelectric point (pH 4.6) with 10 % acetic acid. After removing of casein, 2.2 g of $CaCO_3$ was added to the filtrate (whey solution) to precipitate albumin I and II (2.18 % and 0.21 %). The isolated casein and albumin were characterized by FT IR spectroscopy, amino acid tests (such as Millon's test, Biuret test, Ninhydrin test, Xanthoproteic test) and protein precipitation tests (salt test, organic solvent test, acidic agent test, heat and acid test and heavy metal ions test) respectively. FT IR spectra of isolated casein I and II illustrate better fit with the reported spectra than those of albumin I and II. Atomic absorption spectroscopy (AAS) of isolated casein and albumin showed sufficient amount of minerals. The isolated casein possessed mild antioxidant activity whereas albumin possessed practically inactive by DPPH assay. Therefore, it may be better to use the fresh cow milk for the production of more nutritious dairy food.

Keywords: Fresh and packed cow milk, quality tests, casein, albumin, FT IR, protein tests, AAS, antioxidant activity

Introduction

Milk is the most nutritionally complete food found in nature. All kinds of milk, human or animal, contain vitamins, minerals, proteins (most casein), carbohydrates (principally lactose), and lipids (fats).The amounts of these nutrients present in different types of milk differ greatly. However, cow's milk and goat's milk are almost identical in every respect (Seyhan Ege's Homepage, 1997). Milk contains three kinds of protein: caseins, lactalbumins and lactoglobulins, all of which are globular protein (Spurlock, 2014). Casein is a combination of phosphoproteins presenting in milk and cheese. It is the amount of 3 % in milk along with 4-5 % of lactose and 3-4 % of fats and the rest is water (Ahluwalia and Dhingra, 2005). Caseins exist in micelles which are composed of submicelles linked by the characteristic of hydrocolloid which are freely suspended in the aqueous phase of milk (Tarte, 2019). Casein can be electrophoretically fractioned into four major components: alpha-, beta-, gamma- and kappa- casein. Casein develops precipitation from milk at pH 4.6, which has negative charge by comparing the pH of the milk. Therefore, it can be precipitated as salt by adding acids (Miller, Jarvis and Mcbean, 2006). Casein can be used in glues, the coating of paper, and the binding of colours in paints and wallpaper. It is also used as a coating for fine leather, and is cured with rennet to produce a plastic material used for buttons. Casein is also employed in the manufacture of pharmaceutical and nutritional product (Seyhan Ege's Homepage, 1997). The aim of the study is to isolate and characterize the casein and albumin from fresh and packed cow milk samples as well as to determine the minerals and antioxidant activities.

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Materials and Methods

Sample Collection

Packed cow milk was collected from Myoma Market, Taunggyi Township. Fresh cow milk was freshly collected from the cow farm at Panthakwar Village, Taunggyi Township, Southern Shan State and immediately operated after collection.

Measuring of Milk Quality Test

Alcohol Test

The test was done by mixing equal amount of each milk sample (5 mL) and 68 % of ethanol solution (5 mL) in a test tube. If the tested milk is good quality, there will be no coagulation, clotting or precipitation. Presence of flakes or clots indicated poor quality milk.

Determination of pH Value

Milk sample (50 mL) was taken in a beaker and pH meter was put in a beaker for 5 min to determine milk quality. The result of pH value was obtained.

Test for Carbohydrates

Cow milk sample (2 mL) and 2 drops of 10 % α-naphthol solution were added to the test tube. Concentrated H_2SO_4 (1 mL) was carefully poured into dropwise using a dropper on the inner wall of the test tube. A red ring colour appeared at the junction between the two layers.

Test for Reducing Sugars

Cow milk sample (5 drops) and 2 mL of Benedict's solution were added to the test tube. The mixture was boiled into water bath for 5 min. The formation of green precipitation within 3 min indicated the presence of reducing sugars.

Isolation of Casein and Albumin from Fresh and Packed Cow Milk Sample

Milk (100 mL) was warmed to 40 ºC in water bath. After warming, 10 % acetic acid was added in a drop wise manner to adjust the pH to the isoelectric point of casein. At isoelectric point casein precipitated out along with butter fat leaving a liquid component called whey. The milky whey liquid becomes clear when casein separates out completely. Casein was separated from the whey by straining the precipitate through four layers of cheese cloth. The precipitated casein was washed with 20 mL 0f 95 % ethanol with vigorous stirring for 5 min. The suspension was then filtered and washed with 20 mL of ethanol: ether (1:1) mixture. Finally the precipitate was washed with 30 mL of ether. After the final wash the precipitate was transferred into porcelain basin and dried at 40-50 ºC in an oven. After drying, the weight of casein I and II were recorded as 9.11 % and 7. 36 %. All the milk samples were similarly treated.

After the casein filtration, 2.2 g of powdered calcium carbonate was added to the filtrate and the solution was mixed thoroughly. The mixture was boiled for about 10 min and stirred continuously. (Pamarthy, Bhat and Sukumaran, 2016). And then, the mixture was filtered on a filter paper to obtain precipitated albumin. The albumin was dried and weighed. The yield of albumin I and II from different milk samples were recorded as 2.18 % and 0.21 %

Identification of Isolated Casein and Albumin

The FT IR spectra of isolated two caseins (I and II) and two albumins (I and II) from fresh and packed milk were recorded on FT IR- 8400, SHIMADZU, Japan, at the Department of Chemistry, University of Yangon.

Characterization of Isolated Casein and Albumin

Amino Acid Tests for Casein

Millon's test

Casein (1 g) was placed in a test tube. Millon's reagent (5 drops) was added and the tube was immersed in a boiling water bath for 5 min. Yellow precipitate indicated the presence of tyrosine residue which occurred in nearly all proteins.

Ninhydrin test

Three drops of 1 % solution of ninhydrin reagent was added to 1 g of Casein. The solution was heated for 5 min in a boiling water bath. They gave characteristic deep blue colour. It indicated the presence of α-amino acid and proteins containing free amino groups.

Biuret test

Casein (1 g) and 3 M NaOH (2 mL) were mixed thoroughly in a test tube. Then, 0.5 % $CuSO_4.5H_2O$ solution was added drop by drop to the above mixture. The result was obtained as blue colour.

Xanthoproteic test

Casein (1 g) was added to conc: HNO_3 (1 mL) in a test tube. A white precipitate was formed and then heated in water bath. They turned yellow with tyrosine and orange with the essential amino acid "tryptophan" indicating a high nutritive value.

Protein Precipitation Tests for Albumin

Precipitation by Salt

Albumin (3 mL) was taken into a test tube. Equal volume of ammonium sulphate was added to it. The mixture was allowed to stand for about 5 min and filtered by using filter paper. The filtrate (3 mL) was taken into another test tube and the same volume of NaOH was added to it, then $CuSO_4$ solution was added drop by drop to the filtrate. The white precipitate was obtained.

Precipitation by Organic Solvents

Albumin (1 mL) was added to 4 mL of EtOH in test tube. The solutions were mixed well and were allowed to stand until the white precipitate was obtained.

Precipitation by Acid Agents

Albumin (1 mL) was added to an equal volume of picric acid solution. The yellow precipitate was observed.

Precipitation by Heavy Metal Ions

Albumin (1 mL) was added to 10 drops of lead acetate solution in test tube. The formation of white precipitate was noted.

Precipitation by Heat and Acid

Albumin (10 mL) was taken into a test tube and then a few drops of 1 % acetic acid were added to it. Coagulation was taken place and albumin was precipitated (Essay UK, 2018; Chemistry. mcmaster, 2014).

Results and Discussion

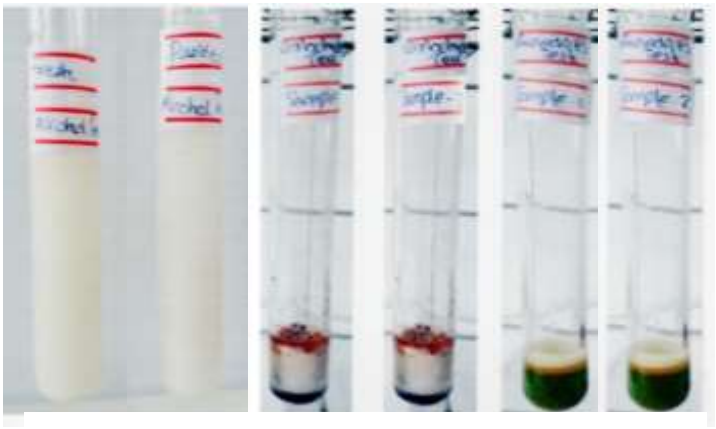
Preliminary Milk Test

Fresh and packed cow milk samples were collected from Taunggyi area. The alcohol test and pH measurement of milk are important to determine milk quality. Alcohol tests of fresh and packed cow milk samples showed the good quality from the absence of coagulation, clotting or precipitation. The measurement of pH of milk is important in testing for impurities, spoilage, and signs of mastitis infection. Fresh and packed cow milk samples indicated the pH 6.8 and 6.6 which are within the normal range (6.8). Red ring and green precipitate indicate the presence of carbohydrates and trace of reducing sugars. These data are shown in Table 1 and Figure 1.

Table 1 Milk Quality of Fresh and Packed Cow Milk Samples

Test	Chemical Reagent	Observation	Results	
			I	II
Alcohol test	65 % ethanol	no coagulation	good quality	good quality
pH	pH meter	6.8-6.6	6.8	6.6
Carbohydrates	10 % α-naphthol, conc. H_2SO_4	red ring	+	+
Reducing sugars	Benedict's solution	green ppt.	+	+

(+) = positive test, I = fresh cow milk, II = packed cow milk, (ppt.) = precipitate



(a) I II (b) I II (c) I II

Figure 1 Milk quality test of (a) alcohol (b) carbohydrates and (c) reducing sugars tests from fresh (I) and packed (II) cow milk samples

Identification of Isolated Compounds

Caseins I and II (9.11 % and 7.36 %) and albumins I and II (2.18 % and 0.21 %) were obtained by precipitation with acid and $CaCO_3$ (Figure 2). Yield percentage of casein I and albumin I of fresh cow milk are higher than casein II and albumin II of packed cow milk. Therefore, only

casein I and albumin I have been chosen for further study of mineral content by AAS and antioxidant activity by DPPH Assay.

The FTIR spectra of the isolated caseins I and II are very similar to the reported casein as described in Table 2 and Figure 3.

The bands around 1600 cm^{-1} and 1500 cm^{-1} assignable to amide I and II are prominent. The broad band about 3500-2500 cm^{-1} shows O-H stretching band, the weak band around 3100 cm^{-1} shows olefinic C-H stretching. Saturated sp^3 C-H stretching bands also appear at just below 3000 cm^{-1}. The comparison of the FT IR spectra of the isolated albumins I and II with the reported one for albumin in Figure 4 are not fitted as the case with the casein samples. Amide I and II bands appear to be very weak, so also is the band around 3000 cm^{-1} for N-H and O-H stretching. The aliphatic sp^3 C-H stretching bands below 3000 cm^{-1} remains, but it appears to be very weak in albumin II.

In this experiment, chemical tests on isolated casein from both milk samples were done in order to determine the presence of specific amino acids in this type of protein. Millon's test is given by any compound containing phenolic hydroxy group. Consequently, any proteins containing tyrosine gives a positive test of a yellow precipitate. Ninhydrin test is used to detect the presence of α - amino acid and proteins containing free amino groups. They gave characteristic deep blue colour. Biuret test is a chemical test used to determine the presence of peptide bond in a substance. A positive test indicated a deep blue colour due to copper ion complex with the amide group of the protein. Xanthoproteic test was used for the detection of aromatic amino acids. Appearance of yellow colour obtained was due to the nitration of aromatic ring as shown in Table 3 and Figure 5.

The results of precipitation tests such as salt test (white ppt.), organic solvent test (white ppt.), acidic agent test (yellow ppt.), heavy metal ion test (white ppt.) and heat and acid test (white ppt.) indicate the presence of protein in isolated albumin I and II as shown in Table 4 and Figure 6 (Essays UK, 2008).

Figure 2 Isolation of casein I, II, albumin I and II from fresh and packed cow milk samples

Table 2 Comparison of FT IR Spectra of Isolated Caseins I and II with Reported Casein*

Wave number (cm^{-1})			Assignment
Casein I	**Casein II**	**Reported Casein***	
3279	3280	3500-2500	O-H and N-H stretching
2917, 2850	2919, 2851	2950- 2830	ν_{as} and ν_s C-H stretching of CH_3 , CH_2
1714	1742	1730	C=O stretching
1629, 1537	1639, 1533	1600-1500	amide I and amide II
1172, 1095	1172, 1094	1200-1000	C-O-C stretching
719	720	750	secondary amide N-H wagging

(*Hewavitharana and Bram van Brakel, 1997)

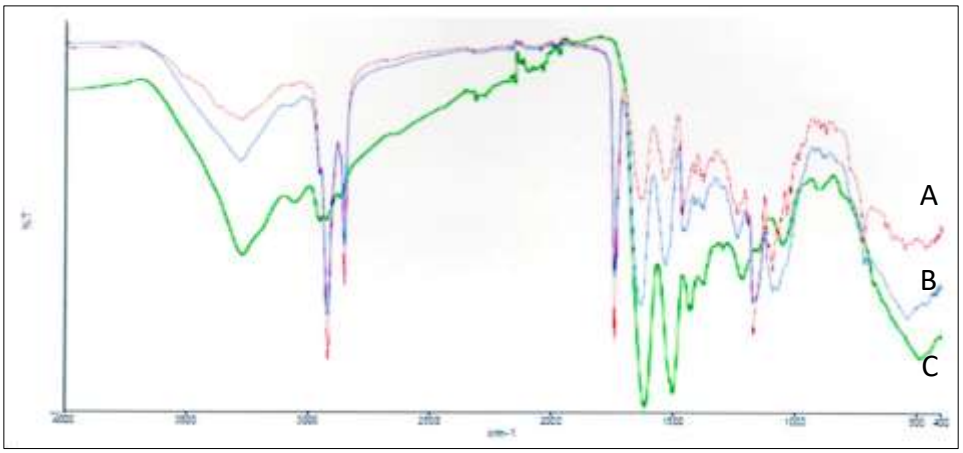


Figure 3 Comparison of the FT IR spectra of the isolated casein I (A) and casein II (B) with that reported casein *(C)

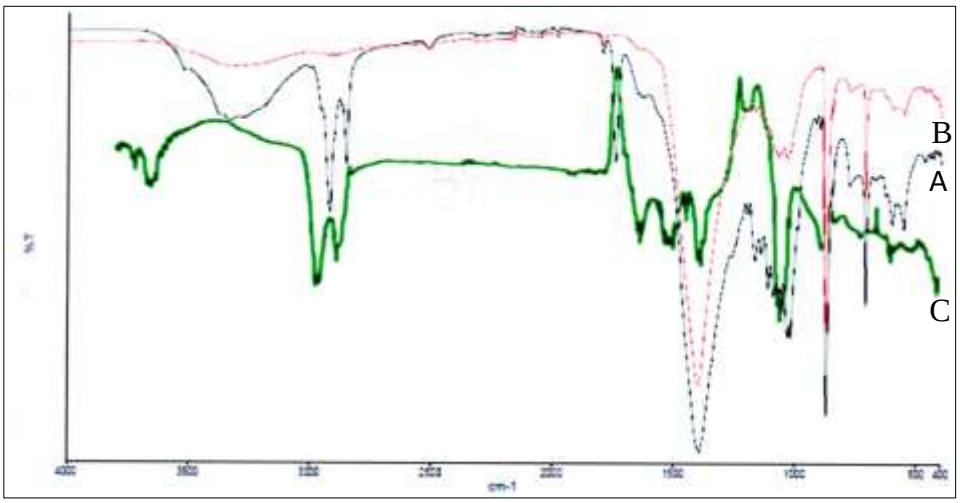
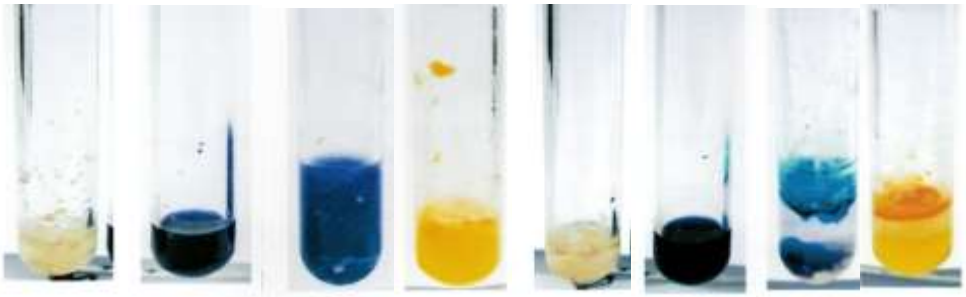


Figure 4 Comparison of the FT IR spectra of the isolated albumin I (A) and albumin II (B) with that reported albumin *(C)

Table 3 Results of Some Chemical Tests for Isolated Caseins I and II

Test	Chemical Reagent	Observation	Results	
			Casein I	Casein II
Millon's test	$HgSO_4$, 15 % H_2SO_4	yellow ppt.	+	+
Ninhydrin test	Ninhydrin, ethanol, acetic acid	blue colour	+	+
Biuret test	3 M NaOH, 0.5 % $CuSO_4.5H_2O$	deep blue colour	+	+
Xanthoproteic test	Conc. HNO_3, NH_4OH	yellow colour	+	+

Millon Ninhydrin Biuret Xanthoproteic Millon Ninhydrin Biuret Xanthoproteic



Casein I Casein II

Figure 5 Some chemical tests of isolated caseins I and II

Table 4 Results of Some Chemical Tests of Isolated Albumins I and II

Test	Chemical Reagent	Observation	Results	
			Albumin I	Albumin II
Salt test	$(NH_4)_2SO_4$ solution, NaOH, $CuSO_4.5H_2O$	white ppt.	+	+
Organic Solvent test	ethanol	white ppt.	+	+
Acidic Agent test	picric acid solution	yellow ppt.	+	+
Heavy Metal Ion test	1 % lead acetate	white ppt.	+	+
Heat and Acid test	1 % CH_3COOH	white ppt.	+	+

Figure 6 Some chemical tests of isolated albumins I and II

Some Heavy Metals Present in Isolated Casein I and Albumin I

Atomic absorption spectroscopy (Perkin Elmer Analysis – 300 AAS, USA) was used for determination of some heavy metals present in the isolated casein I and albumin I. The isolated casein I and albumin I were found to contain, Fe (6.4616 and 6.5969 ppm), Mn (0.4721 and 0.5193 ppm), Zn (1.3661 and 0.8366 ppm) and Cu (0.3828 and 0.3723 ppm), respectively which indicate the presence of sufficient amounts of minerals. The results are shown in Table 4.

Table 4 Elemental Analysis of Isolated Casein I and Albumin I

Elements	Casein I (ppm)	Albumin I (ppm)
Fe	6.4616	6.5969
Mn	0.4721	0.5193
Zn	1.3661	0.8366
Cu	0.3828	0.3723

Antioxidant Activity of Isolated Casein I and Albumin I

The value of percent inhibition in isolated casein I was very low and that in albumin I was not found. As a consequence, the isolated casein I gave low antioxidant activity and albumin I was no antioxidant activity (Table 5, Figure7).

Table 5 ***In Vitro*** **Antioxidant Activity of Casein I and Albumin I Against DPPH Radical Scavenging**

Sample	Concentration (µg/mL)	% Inhibition	Method
Casein I	1000	5.99 ± 1.4	DPPH Radical
Albumin I	1000	0	DPPH Radical
Ascorbic acid (standard)	500	84.78 ± 0.39	DPPH Radical

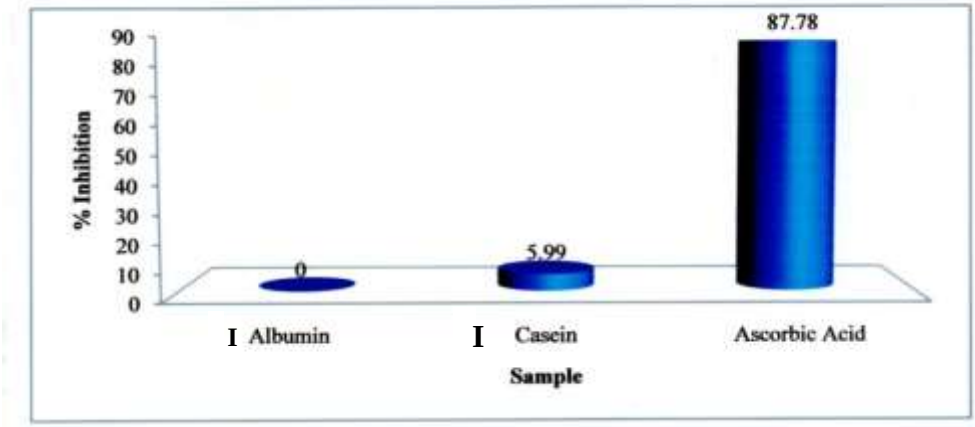


Figure 7 Bar graph of isolated albumin I, casein I and standard ascorbic acid

Conclusion

The alcohol test (no coagulation), pH (6.8- 6.5), carbohydrates test (red ring) and reducing sugars test (green precipitate) showed good quality for the fresh and packed cow milk samples. The yield of caseins I and II (9.11 % and 7.36 %) and albumins I and II (2.18 % and 0.21 %) by precipitation method indicated that the fresh cow milk was richer in these compounds than the packed milk. The FT IR spectral data showed that the isolated caseins I and II which were more agreeable with the reported spectra than those of the isolated albumins I and II. The isolated casein and albumin were also observed to give positive by amino acid test and protein precipitation tests. Furthermore, the isolated casein I and albumin I were also found to contain Fe (6.4616 and 6.5969 ppm), Zn (1.3661 and 0.8366 ppm), Mn (0.4721 and 0.5193 ppm), and Cu (0.3828 and 0.3723 ppm) by AAS. However, Albumin I exhibited no antioxidant property and casein I was very low antioxidant activity by DPPH method. Above all, it may be concluded that, fresh cow milk is a more nutritious food dairy product than packed cow milk.

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