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# Flavored milk proteins co-precipitate

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# Abstract

This study aimed to develop new dairy product depending on the interactions of calcium with milk proteins during heat treatment.

The ideal conditions for preparation of cow milk proteins co-precipitate were investigated. The optimum concentration of calcium chloride used to prepare milk proteins co-precipitate was 25mM.This concentration of calcium yielded 96.2% of milk proteins.The best pH values to prepare milk proteins co-precipitate were between (5.5-6- 6.5), while the best heat treatments used were between 85-95°C for 20 min.

#### Introduction

Advances in the technology of protein production in the past few decades have made it possible to produce different types of proteins commercially from various sources of raw materials. It is claimed that adding or using as replacement for protein such as soya, sodium caseinet and milk powder improves the characteristics of many food products. Improvement such as appearance, texture, and stability are greatly influenced by physical and chemical properties of the proteins (Al-Saadi & Deeth, 2011).

During the manufacturing of casein only 80% of milk proteins are recovered and about 20% of milk proteins (whey proteins ) are lost .Because of the highly nutritional value and the desired functional properties of whey proteins beside the need to increase the quantity of the result proteins, the manufacturing of coprecipitates was developed (Alu'datt et al., 2013).

Milk protein co-precipitates is a technique to prepare the protein precipitate which is resulted from the precipitation of casein and whey proteins using a combination of heat treatments and addition of acid with or without addition calcium salts .They differ from casein and whey proteins in their functional and nutritional properties (Al-Saadi & Deeth, 2011).

Heat treatment is essential for the production of co-precipitates because it causes denaturation of whey proteins and their interaction with caseins, particularly the interaction of  $\kappa$ - casein and  $\beta$ -lactoglobulin through disulphide bond formation (Guyomarc'h et al ., 2009).

While the function of calcium ions (when added) is to cross-link whey proteins and caseins by the formation of calcium bridges(Al-Saadi & Deeth, 2011).

Compared with casein, whey proteins are more stable in the presence of ionic calcium salts, but sensitive to heat (Chinprahast et al . 2015).

Ju & Kilara (1998) suggested that the heat induced aggregation of proteins in the presence of calcium involved the formation of large aggregates, the size of which is dependent on the calcium ion concentration.

Co-precipitated products can be directly used for supplementing and enrichment of low quantity and poor quality food sources, also co precipitate products can be used in different areas af food and nutrition as beverage (vattula et al. 1979).

## **Literature Review**

## Milk proteins

Milk contains two major groups of proteins, casein and whey proteins.

Casein is the principal protein found in cow milk from which it has been extracted commercially for most of the 20th century. It is responsible for the white, opaque appearance of milk Caseins are phosphoproteins precipitated from raw milk at pH 4.6 at 20 °C. in which it is combined with calcium and phosphorus as clusters of casein molecules, called micelles (Morr, 1989).

They comprise approximately 80% of the total protein content in milk. The principal proteins of this group are classified into  $\alpha$ s1 -,  $\alpha$ s2-,  $\beta$ - and  $\kappa$ -caseins (Wong et al. 1996).

The as1-casein, as2-casein, -casein and k-casein vary in the sequence and number of amino acids and contain 199, 207, 209 and 169 amino acids, respectively (Swaisgood, 1993). The as and B- caseins are extensively phosphorylated and lack to organized secondary structure due to their high proline contents. Hence these proteins are capable of binding large amounts of calcium ions, which can lead to their precipitation (Swaisgood, 1993).

At the natural pH of milk,  $as_1$ ,  $as_2$ , B- and k-caseins exist in the form of associated complexes known as casein micelles which have an average diameter of ~150 nm in the native form and this form are highly stable protein entities (Schmid, 1982).

On the other hand, whey proteins are globular, water-soluble and categorized in to 5 fractions: B-lactoglobulin, a-lactalbumin, bovine serum albumin, immunoglobulin's and proteose peptone fractions (Haug et al. 2007).

The B-lactoglobulin is the major whey protein, about 54% of whey proteins are B-lactoglobulin and it consists of 162 amino acids. It is a globular protein with a molecular weight of 18362 Da for variant A and 18,276 Da for variant B (Wong et al.1996).

The a-lactalbumin is a small globular protein that is relatively stable. It constitutes 21% of whey proteins. Its genetic variant A has a molecular weight of 14,147 Da.Variant B has a molecular weight of 14,175 Da. a-La is composed of 123 amino acid residues (Wong et al. 1996).

#### Milk proteins co-precipitates

Milk co-precipitates are products from whey proteins reacted with caseins and jointly precipitated by heat, calcium addition and pH adjustment.

Milk protein coprecipitates result from the precipitation of casein and whey proteins from skim milk using a combination of heat treatments and addition of acid and/or calcium salts. They differ from casein and whey proteins in their functional and nutritional properties. Heat treatment is essential for the production of coprecipitates because it causes denaturation of whey proteins and their interaction with caseins, particularly the interaction of -casein and B-lactoglobulin through disulphide bond formation (Jang and Swaisgood 1990). Compared with casein, whey proteins are more stable in the presence of

ionic calcium salts, but sensitive to heat (Chinprahast et al . 2015). Vattula et al. (1979) recovered 96% of cow milk proteins by heating skim milk at 85°C and adding 0.2% calcium chloride. Also 0.2% calcium chloride was sufficient to co-precipitate 97% of sheep's milk proteins (Al-Saadi Deeth, 2011).

#### Distribution of calcium in milk

About 34% of the total calcium exists as soluble calcium while 66% exists as insoluble calcium. In the soluble form, calcium exists as salts such as calcium citrate and calcium phosphate or as calcium ions (figure 1) (Gaucheron, 2005). The insoluble form is associated with casein micelles as colloidal calcium phosphate (CCP).



figure 1: The calcium system in milk



figure 2: scheme of work preparation of calcium- milk gel



figure 3 scheme of work preparation of flavored calcium- milk gel

#### **Results and discussion:**

# Effect of calcium chloride on protein recovery in cow milk co-precipitate

protein recovery at 85°C for 20 min. is shown in figure (4) that shows that cow milk protein recovery increased with increasing calcium chloride added. At 0. mM addition of calcium chloride, the recovery of milk proteins was 34.1%, and this increased to 93.2 % at 20mM calcium chloride. The percentage for highest protein recovery was 25mM, which yielded 96.2% of the milk proteins and this result of recovery was nearly the same found by Vattula et al. (1979) who recover 96% of milk proteins using the same concentration of calcium.



figure 4:Effect of the quantity of calcium chloride added to cow milk

#### Effect of pH on protein recovery in cow milk co-precipitate:

The effect of pH on protein recovery is shown in Figure (5). At low pH (5), the recovery of cow milk protein was low( 91.6%) because the pH is very close to the isoelectric point of the caseins, which is 4.6, and this led to the precipitation of casein directly after heating without taking the necessary time to react with whey protein, which resulted in a decrease in protein recovery. Furthermore, lowering the pH significantly increases the denaturation temperature of  $\beta$ -Lg (Boye et al.1997). At higher pH values (5.5-6-6.5), the protein recovery increased because interactions between caseins and whey proteins through disulphide bonds occur (Jang & Swaisgood, 1990).



Figure 5: Effect of pH on protein recovery

#### Effect of heating temperature in cow milk co-precipitate:

The effect of heating temperatures, between 60 and 95°C, on protein recovery as coprecipitate from cow milk is shown in Figure (6).At the lower temperatures (60–65°C), coprecipitate gels was not formed from cow milk because the amount of calcium binding to casein was less than that required to saturate and cross-link the casein at these temperatures (Ramasubramanian, 2012)

At 70°C, casein only was precipitated, heating at this temperature increased the binding of calcium to casein but was not enough to denature whey proteins. At 75°C, whey proteins started to precipitate with casein, and at 85–95°C, the coprecipitate contained the most whey proteins, especially  $\alpha$ -La and  $\beta$ -Lg, owing to complex formation between denatured  $\beta$ -lactoglobulin, and K-casein and  $\alpha$ -lactalbuminand (Reddy & Kinsella, 1990)

after heating at 5°C for 20 min was 96.6% and after heating at 95°C for 20 min was 96.8 %.



Figure 6:Effect of heating temperature on protein recovery

Gel strength of calcium-induced milk gels increased significantly with the amount of calcium chloride . This increase is related to the increase in the number and strength of cross- linked of the milk protein by calcium ions (Lee and marshall, 1984). calcium was not added at levels higher than 20 mM as these cause the gel strength to increase so much that coagulation occurs with expulsion of whey (Ramasubramanian et al, 2012).

#### conclusions

1-Calcium milk coagulum was produced from skim milk using heat treatment and calcium chloride addition.

2-About 96.6 % of skim milk proteins can obtain as co-precipitate by heating pH 6.5 skim milk at 85°C for 20 min. in the presence of 25mM calcium chloride.

3- Calcium-milk gel can be produced by addition of calcium chloride and heat treatment of milk. Adding 13.5 mM calcium chloride gave the gel good texture properties and the best sensory evaluation score.

4-Adding sugar and flavors to milk increased sensory evaluation score of gels.

### Recommendations

1- Study for the mechanism of milk coagulation by calcium chloride and the role of heat treatment on it.

2-Determine the nutritional value of the new dairy product developed in this study (milk proteins co-precipitate, calcium milk gel and calcium-milk coagulum).

3- Study for the effect of milk concentration and other heat treatment such as UHT on the chemical, textural and sensory properties of calcium milk gel and calcium milk coagulum.

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