

**STUDIES ON SOME PHYSICAL
AND CHEMICAL PROPERTIES OF
BUFFALOES MILK WHEY PROTEINS**

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THESIS

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**Dedicated to My parents and My wife
For their love and unlimited
Encouragement they devoted to me.**



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INTRODUCTION

The continuous increase of cheese manufacture has resulted in an increasing loss of highly nutritive proteins in whey. Since many years, studies have been conducted on the utilization of whey proteins in the food industry. The separation of undenatured proteins from cheese whey has become to be an established part of the dairy industry of the world. Several industrial processes, such as ultrafiltration (UF), reverse osmosis, gel filtration, crystallization with alcohol and complexing with metaphosphate or carboxymethylcellulose (CMC) have been developed for recovery whey protein concentrate (WPC) and are presently under evaluation by various researchers.

However, analysis of WPC is of significant interest in relation to their functional characteristics and the properties of individual constituents impart to the bulk material. Also, different processes are observed to occur resulting in structural changes of proteins depending on the procedure by which whey proteins were recovered, such changes influence physico-chemical

and functional properties of WPC which affect the behavior of proteins in food systems during processing, storage, preparation and consumption.

Therefore, information about the chemical and physico chemical properties of whey proteins, especially that of buffalo milk prepared by different methods would be of technological importance and must be given some attention. That was the aim of this study. However, the work was divided to two main parts. In the first part, the chemical properties of WPC prepared by UF and CMC complex processes were studied. The second part was devoted to examine the functional properties of different WPC preparations.

REVIEW OF LITERATURE

1. Preparation of Whey Proteins:

Smith et al (1962) used anionic hydrocolloids and detergents for precipitating the protein of soybean whey and reported nearly complete protein recovery with a corresponding reduction of 8-18% in biological oxidation.

Fox et al (1967) described a simplified procedure for isolation of B-lactoglobulin from acid whey by precipitating with 3% trichloroacetic acid and filtration. The filtrate is concentrated by negative pressure dialysis, dialyzed free of low molecular weight materials and B-lactoglobulin was lyophilized. They reported that their B-lactoglobulin was similar to B-lactoglobulin prepared by salt fractionation.

Cluskey et al (1969) reported the formation of insoluble complexes between casein and carboxy-methyl-cellulose (CMC) in the presence of bivalent cations.

Hidalgo and Hansen (1969) reported that CMC is capable of interacting with B-lactoglobulin (B-L) and α -lactalbumin (α -L) below their isoelectric points. The B L/CMC complex is insoluble and maximum precipitation occurs at pH 4, whereas the α -L/CMC complex is soluble at this pH. This difference in solubility was used to separate the two major proteins from milk whey. They mixed equal volumes of whey and a 0.13% solution of CMC at pH 4.0. The precipitate formed was predominantly B-L/CMC complex and was separated at 5000 x g, the supernatant was adjusted to pH 6.6 and centrifuged. The precipitate formed was a mixture of blood serum albumin and immunoglobulins. α -lactalbumin was precipitated from the supernatant by slow addition of ammonium sulfate to 68% saturation. They concluded that the principle of molecular complex formation between hydrocolloids and whey proteins may be useful in devising new products which possess both stabilizing and fat-emulsifying properties.

Becker et al (1970) studied the recovery of whey proteins by a combination of precipitation with phosphates and column chromatography.

Morr and Lin (1970) used aqueous methanol, ethanol, n-propanol and n-butanol for precipitating whey protein from liquid and dried whey to prepare a whey protein concentrate. They found that 72% ethanol was more satisfactory than the other alcohols, it precipitated 45-60% of the whey proteins and produced a WPC with a fourfold protein enrichment on a solids basis compared to whey. They reported that ethanol extraction of freeze-dried whey was less satisfactory than liquid whey treatments because of substantial coextraction of proteins with the lactose and minerals. Fifty to 70% of the freeze-dried whey protein concentrate was resolubilized at pH 6.6 whereas it was totally soluble at pH 8 to 9. Use of $\geq 72\%$ (W/W) ethanol during preparation improved the solubility of the protein concentrates. Possible mechanisms for ethanol induced whey protein denaturation and precipitation are presented.

Hansen et al (1971) precipitated either a major part of the whey proteins in one single step by varying the conditions of the interactions with CMC. They developed a procedure for recovering over 90% of the protein of whey with CMC and suggested

that this procedure can be used on industrial scale and encourage the utilization of whey. They reported that the product contained approximately 65% protein, 30% CMC and 5% lactose. Later they showed that it was possible to fractionate the whey protein into at least three fractions by selective precipitation with the hydrocolloid under varying conditions of pH and temperature.

Hidalgo and Hansen (1971) described a procedure for obtaining three different protein complexes with CMC by selective conditions of pH, ionic strength, and ratio of CMC to protein. They reported that with a combination of CMC treatment of whey at pH 4.0 and ammonium sulfate precipitation at pH 6.6, α -lactalbumin and immunoglobulines were recovered free of CMC.

O'Sullivan (1971) reported that in recent years, methods such as large-scale GF and UF have been developed which made it possible to produce undenatured WPC.

Hidalgo et al (1972) used interactions between proteins and anionic polyelectrolytes (sodium hexameta-

phosphate) to recover the protein of milk whey. They upgraded the product to contain 88-90% protein either by gel filtration or ion exchange. The upgraded product shows most of the properties of other undenatured whey protein concentrates.

Jones et al (1972) found that virtually all the protein in commercial acid whey is precipitated by ferripolyphosphate at pH 3.2 to 4.0.

Morr et al (1973) showed that functional properties of the various WPC's varied widely with the method of preparation.

Amantea et al (1974) produced an iron fortified WPC ($> 80\%$ protein) by heating concentrated whey (92°C , 15 min) at pH 2.5 to 3.5. They found that the resulting products had good functional properties.

Matthews and Doughty (1977) showed that heating whey produce insoluble product with very high

water holding capacity, a property desirable for some food applications which were being commercially exploited. Marketing of the soluble WPC's produced, e.g. by UF, has been slower than predicted and was currently the main reason hampering their more widespread manufacture, they add that trend toward the industrial production of WPC's especially using UF and IF was evident in countries such as Newzealand and Australia. They concluded that the use of UF for WPC recovery appeared to be advantageous since 98-99% protein separation from the waste stream could be achieved.

Molder and Emmons (1977) prepared a highly soluble WPC by adjusting the pH of sweet whey to 2.5-3.5 and heating at 90°C for 15 min., upon cooling the pH was adjusted to 4.5 and the precipitated protein removed by centrifugation then neutrilized to pH 6.0 and spry dried. They recovered 63-74% of total whey proteins and 35-53% for whey heated in the pH range of 2.5-3.5 with and without add iron.