CASEIN-WHEY PROTEIN INTERACTION IN HEAT TREATED BUFFALOE'S MILK

B Y

AHMED ISMAIL METWALLY

B.Sc. (Agric.) 1980

T H E S I S

Submitted in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

IN

DAIRYING

Food Science Department

Faculty of Agriculture

Ain Shams University

1985

APPROVAL SHEET

Title of Thesis: Casein-Whey Protein Interaction In Heat Treated Buffaloe's Milk.

Name of Student: Ahmed Ismail Metwally.

This Thesis for M.Sc. Degree has been approved by:

Dr. M. M. [illegible]

Dr. S. A. El-Shabrawy

Dr. G. A. Mahran

(Committee in Charge)

Dat 25 /12/1985.

بسم الله الرحمن الرحيم

TO MY PARENTS

FOR THEIR LOVE AND UNLIMITED

ENCOURAGEMENT.

ACKNOWLEDGEMENT

This work has been carried out at the Food Science Department, Faculty of Agriculture, Ain-Shams University under the supervision of Prof. Dr. G. A. Mahran, Prof. Dr. Laila B. Abd El-Hamid and Dr. H.F. Haggag to whom I have the pleasure of expressing my great gratitude for their kind advice, constructive criticism and unremitting assistance right through the course of investigation.

TABLE OF CONTENTS

INTRODUCTION

The properties of dairy products are influenced by the heat treatment to which the milk has been subjected during their preparation. This treatment is widely employed in the dairy industry to improve the keeping quality of milk and milk products. The changes which are caused in milk by heat treatment have been the subject of intensive investigations over the past 40 years.

It has been well established that when milk is heated the whey proteins denatured and that an interaction between them and casein occurs. Since that time many investigators have suggested a complex between: 1) K-casein and B-lactoglobulin (Zittle, et al.,1962); 2) Crude K-casein and B-lactoglobulin (Long, et al., 1963); 3) Whey proteins and k-casein (Hartman and Swanson, 1965); 4) Alpha Lactalbumin and milk protein(Baer, et al., 1976); 5) B-Lactoglobulin and casein micelles, (Smits and Van Brouwershaven 1980) and 6) The effect of carbohydrate moiety of k-casein on the complex formation with B-lactoglobulin, (Doi, et al., 1981).

In contrast, only limited number of studies have been devoted to heat induced complex formation between whey proteins and other milk proteins in respect of buffaloe's milk. However, the effect of heat on serum proteins in particular is of industrial importance because of its role in rennin action, heat stability and the production of cooked flavour in milk (Wilson and Wheelock, 1972 and Fax and Morrissey, 1977).

Considering this important points, the object of this work is to determine precisely the role of the interaction between whey protein and casein when Egyptian buffaloe's milk subjected to different heat treatments between 60°C and 95°C. The study was carried out under two main parts. The first part was devoted to find out the effect of heat treatment, hydrogen peroxide and iodo acetic acid on buffaloe's milk proteins. The second part was concerned with the factors affecting the interaction of milk proteins in a model system.

REVIEW OF LITERATURE

In this literature survey an attempt was made to provide an adequate background knowledge to the general features of milk proteins, with particular emphasis on the chemical changes which occur as a result of heat treatment.

1. Casein:

Mulder (1838) was the first person who precipitated casein from bovine milk by the addition of acid. For many years, casein was believed to be a single protein. However, Linderstrom-Lang and Kodama (1925) showed that casein was in fact heterogeneous. The ultracentrifugation studies by Pedersen (1936) demonstrated that casein sediments as a polydisperse. When investigating the electrophoretic behaviour of casein in borate buffer at pH 8.6, Mellander (1939) obtained three bands. These were designated α -, B-, and γ-casein in order of decreasing electrophoretic mobility.

After the discovery of the heterogeneity of the casein several attempts were made to isolate the various components with reasonable purity. Present day methods of preparation of each of these fractions are given by

McKenzie (1967), Mackinlay and Wake (1971), and Fox (1982).

Waugh and von Hippel (1956) were first discovered that α -casein consist of α_s- and k-casein. After the discovery of the heterogeneity of the α -casein, Ribadeau-Dumas (1961) reported that α_s-casein was homogeneous. But using ionexchange chromatography Waugh, et al. (1962) obtained two main fractions (α_{s1} and α_{s2}) and a few minor fractions. Moreover, Thompson et al., (1962) were able to demonstrate three variants for α_{s1}-casein, which they named as α_{s1}-A, B, and C casein.

The B-casein was assumed to be homogeneous until Aschaffenburg (1961, 1963) had shown that B-casein exhibits genetic polymorphism (A, B, and C). Variant A was found to be the most common and variant C the least common. So far five genetic variants A^1, A^2, A^3, B and C had been reported by Thompson, et al. (1964).

The most complex casein component in its properties and functions is k-casein. Kirschenbaum, (1973) reported that k-casein appears to be the simplest in genetic terms having only two genetic variants (A and B). Unlike the other caseins, k-casein contains a significant amount of half cystine (1.4%) and carbohydrate.

2. <u>Whey proteins</u>:

The solution which remains after the precipitation of casein from milk at pH 4.6 contains the whey protein. The isolation and properties of the individual whey proteins have been reviewed by McKenzie(1971).

Beta-lactoglobulin makes up about 50% of the whey proteins. Larson and Jenness, (1951) reported that B-lactoglobulin is the only milk protein which contains the amino acid cysteine and therefore has free-SH groups. Aschaffenburg (1965), recognized three genetic variants of B-lactoglobulin (A, B and C).

The second largest whey protein is α -lactalbumin, Gordon (1971) reported that α -lactalbumin contains 4 disulphide bonds, has a high content of tryptophan, and also contains a high ratio of polar to nonpolar groups.

3. <u>The effect of heat on milk proteins</u>:

The effect of heat on B-lactoglobulin was first investigated by Briggs and Hull (1945) at pH 7.0 using moving boundary electrophoresis technique. The identified two distinct reactions. The first occurred at temperatures above 65°C and resulted in an increase in the particle weight. The second reaction occurred after the first one if the temperature was then lowered. The second reaction caused a further increase in the particle size. If the temperature increased above 75°C the second reaction became progressively less important and at 99°C was negligible.

The various proteins in whey were shown by Larson and Rolleri (1955) to be heat denatured at different rates; the immunoglobulin fraction was denatured first followed by the serum albumin which, although not a milk protein per se. The B-lactoglobulin was less rapidly affected under the same heating conditions while α-lactalbumin was the most resistant.

Della Monica,et al. (1958) had shown that electrophoretic studies alone did not provide conclusive evidence as to whether B-lactoglobulin and α-casein form a complex when heated, because the heating of B-lactoglobulin solutions increases electrophoretic mobility of the protein until it was close to the mobility of α-casein. However, by using precipitation studies, they found that in the mixture B-lactoglobulin was not precipitated by a concentration of calcium chloride that will precipitate it when heated alone. It was concluded that the reduction in the precipitation of B-lactoglobulin by calcium chloride in the presence of α-casein, might be due to formation of a complex with the casein.

Trautman and Swanson (1958 and 1959) had shown that the presence of sulfhydryl blocking agents during heating of skimmilk prevented the transformation of B-lactoglobulin into a complex which moved electrophoretically with α-casein at pH 6.8, u = 0.1, and the well known stabilization of evaporated milk by fore-warming.

A comparison had been made on the heat denaturation of B-lactoglobulin A and B by Gough and Jenness (1962). They found that B-lactoglobulin "B" was more rapidly denatured than B-lactoglobulin "A" by heat treatment of skimmilk or solutions of the isolated proteins at pH 6.7 in the temperature range from 67° to 75°C. Three criteria of denaturation, solubility at pH 5.0, -SH groups activity, and specific optical rotation, all demonstrated the difference in denaturability of the two proteins.

Zittle, et al., (1962) demonstrated by means of moving-boundary electrophoresis, ultra-centrifugation, stabilization by calcium chloride, and clotting by rennin, that mixtures of k-casein and B-lactoglobulin formed a complex upon heating. The complex had an electrophoretic mobility at pH 2.1 intermediate between those of the two proteins heated singly. They suggested that the reaction may involve exposure of the -SH groups in the B-lactoglobulin. They also reported that it was unnecessary to heat the two proteins together to optain a complex but when B-lactoglobulin was heated alone and then mixed with unheated k-casein, a complex was also formed.

Aurand, et al., (1963) used gel and immunoelectrophoresis to provide that a complex formed on heating between B-lactoglobulin and casein and between casein and serum albumin. The sulfhydryl blocking agents used