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FUNCTIONAL PROPERTIES OF CHEMICALLY MODIFIED CAMEL MILK WHEY PROTEINS

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ABSTRACT : The effect of acylation, esterification and deamination on solubility, foaming and emulsifying properties whey proteins of camel milk investigated. In pH values greater than8, the solubility, of control, acylation and deamination camel milk whey proteins was high. In pH values under than 5 the solubility of control whey proteins of camel milk were greater than the solubility of acylation and deamination camel milk whey proteins. Compared control whey proteins of camel milk, acylation whey proteins of camel milk solutions produce more foam instantly subsequent sparing,while esterification and deamination reduce whey protein ability to for foam. The highest EAI for control and acylation camel milk whey proteins was at pH 10. Modification of camel milk whey proteins with esterification and deamination change their highest EAI to 9.

Key words : Camel milk whey proteins, functional properties, chemical modification of proteins.

INTRODUCTION

Whey protein is a term often used as a synonym for milk-serum, proteins that rest soluble following the isoelectric precipitation of casein at pH 4.6 at 20 ^{0}C or after the coagulation of casein by incomplete proteolysis with rennet, (O'Mahony *et al*, 2013). Whey protein part shows about (18-20%) of whole milk protiens.

Whey is a complex mixture of various proteins. In generally, the major ingredients cover β-LG (55%), α-LA (24%), immunoglobulins (15%) and serum albumin (SA, 5%) (Wong, *et al,* 1988).

Camel whey is recorded to have great content of antimicrobial factors such as immunoglobulin and lactoferrin (Elagamy, 2000). Another main distinction like between bovine whey and camel is the lack of β-LG found in various live cattle ruminants' milk. The major ingredients of camel whey contain SA, lactoferrin, α-LA, immunoglobulins, and, peptidoglycan recognition proteins (Farah, 1993; Kappeler, 1998). Additionally, camel milk is much difference of bovine milk in its physical characteristics. It is whiter and less viscous, however it include all of the main nutrients in bovine milk (Elagamy, 2006). At mediocre, camel milk includes further whey protein than bovine milk (Farah, 1993).

Functional features like as foaming and emulsifying of bovine milk whey proteins have been totally studied and recorded. In recent years, it has been recoded that there is an improvement in functional features of proteins following adjustment of some amino acids. As a case, acylation of wheat proteins (Grant, 1973), cottonseed flour protein, (Childs and Park, 197)), and sunflower proteins isolate (Kabirullah and Willis, 1982) improved the functional features of proteins for particular food applications.

While, there is little notification about these features of camel whey proteins. Understanding these functional features is fundamental to foretell the perception of consumers and how they will react to certain food product because of its textural properties. Taste and perception of food products are very influenced by the structures that are formed by the ingredients used to devise it.

Improvements of functional properties of whey proteins are favorite. In this research, the impacts of acylation, esterification and deamination on the functional features of camel milk whey protein were examined.

MATERIALS AND METHODS

Camel milk source

Bulk camel milk was collected from thirteen animal from Al-Najef desert –Iraq .Milk was skimmed by centrifugation on 2400g for 15 min at 4R"C.

Preparation of camel milk whey proteins

Whey resulting in isoelectric precipitations of casein at was obtained and confused with TCA (24%) 10:10 for 30 minutes. The precipitate was attained utilizing ûltration and flusfed twice with 12% TCA, thawed in distilled water with attaching, NaOH (2 M) to pH 7, dialyzed upon tap water for 48 hours and then freeze-dried.

Modification of camel milk whey proteins

Acetylation

The method defined by Kebary et al (2003), was used to acetylation of camel milk whey proteins. A 10% suspension of whey proteins in distilled water was prepared and pH was readjusted to 8.5, utilizing a (2 M) sodium hydroxide. Acetic anhydride (0.9 g / mL protein) was annexed to the solution and the pH was kept at 8.5 for 60 minutes. Acetylated casein solution was dialyzed upon distilled water for 24 hours, and freeze-dried.

Esterification.

Esters of camel milk whey proteins were prepared by utilizing an adjustment of the procedure defined by Fraenkel-Conrate and Olcott (1945). Camel acid casein was suspended in the cold methanol to yield a 1 % suspension. While the protein-alcohol suspension was stirred, concentrated HC1 was gradually added to obtain the suspension (0.07 M) in HC1. The mixture was stirred on 4 °C for 24 hours and was hen diluted 10:l0 by cold deionized water, dialyzed upon (0.001 M) HC1, and freeze-dried.

Deamination

Deamination of the camel milk whey proteins were implemented utilizing a modiûed version of the procedure defined by Mimouni, *et al*(1994)., A diffusion with casein : HCl (0.5 N) ratio of (1:2) was prepared and hydrolyzed by heating at 70 °C for 2 hour in a water bath. The reaction was stopped by cooling the specimen instantly in ice bath, accompanied by readjusting to pH (4.6) and centrifuging at 3000g for 15. The pellet was neutralized pH (6.7–7) by NaOH 1 (N) and freeze- dried.

Electrophoresis

Electrophoresis in decreasing conditions for samples were done using Laemmli method,(1970). EL-Agamy *et.al* (2009) was report bands identification.

Functional properties of milk protein

Solubility

At 12 000 g, the stock solution 0.l% in (0.15 M) NaCl, pH 7 of whey protein was modified to hither fitting pH (3–10) by either (0.l0 N) NaOH or (0.10 N) HCl and centrifuged for fifteen (m.) to 25 °C., The protein density of the outcoming supernatant is specified of a absorbance at (280 nm). Solubility is known as the rate of protein into solution, .Averages obtained counted of at least three tests (Al-Saadi & Deeth, 2011).

Foaming

Foaming properties of whey protein was, examined by employed gas-sparing proceduer of Waniska & Kinsella (1979). (15) Milliliters of specimens (0.l% in 0.15 M NaCl, pH 7) were put in the column (1.6 cm ×70 cm). The Nitrogen gas was spared from the undermost of the column for two (m.) at a flow percentage of 30mLD min. Foam elevation was calculated soon after the gas influx was prevented. Three observations were made of each specimen.

The activity emulsifying

The procedure of Pearce & Kinsella (1978) was defined the Emulsifying activity. Triplicate emulsions of each specimen contained the following :(a) 0.l0 % in 0.150 M NaCl, and (b)modified pH to be in the range from 3.00 to 10.00 utilizing (0.10 N) HCl or 0.10 N) NaOH. It was arranged by utilizing 0.60mL from corn oil, and 10 mL of specimen. The emulsion preparation was finalized by mixing these ingredients to 1.0 minute at room heat using blinder., A 0.2-mL aliquot of the emulsion was reduced (1/250 final dilution) utilizing (01%) sodium dodecyl sulphate (SDS) solution. At 500 nm, the turbidity of emulsion is specific spectrophotometrically. The emulsion effectiveness directory (EAI), that calculates the domain of the interface stabilized per unit weight of protein (m^2D g), is measured via utilizing the next equating:

$$EAI = \frac{(2.303)(2)(A_{500})(\text{dilution factor})}{(c)(1-\text{oil volume})(10000)}$$

A500 =,absorbance upon 500 nm,

The Dilution egent is 250 and

c = g protein D mL of aqueous solution before the emulsion.

Outputs are the mean of 3 replicates,..

RESULTS AND DISCUSSION

The effect of chemical modification on whey proteins of the camel milk

Electrophoretic patterns of acylated, esterificated, delaminated and control camel milk whey proteins are presented in figure 1. α-La and BSA are shown as major dark staining components in the control gel(1), while there was no band for β-Lg in the gel . This result agree with A study by, Kappeler (1998) shown the camel milk does not contain of β-lg,

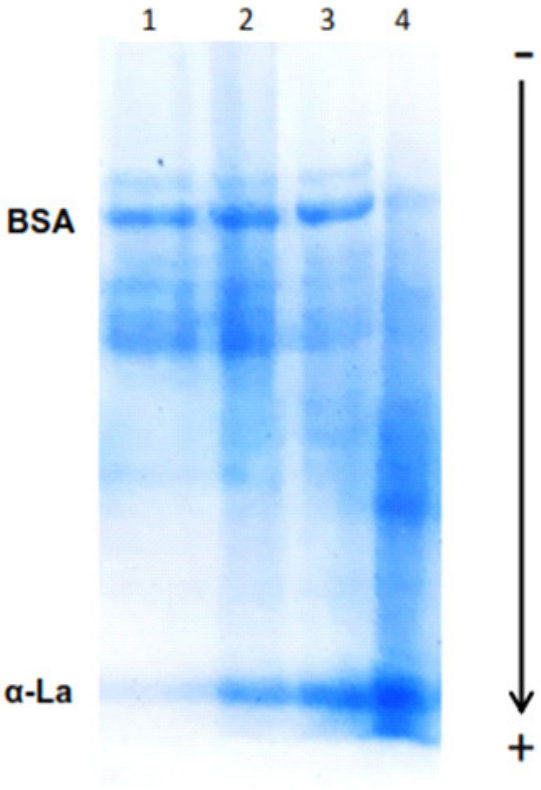

Figure 1 : THE SDS-PAGE of control (1), esterified (2), acylated (3) and deamided (4) camel milk whey proteins.

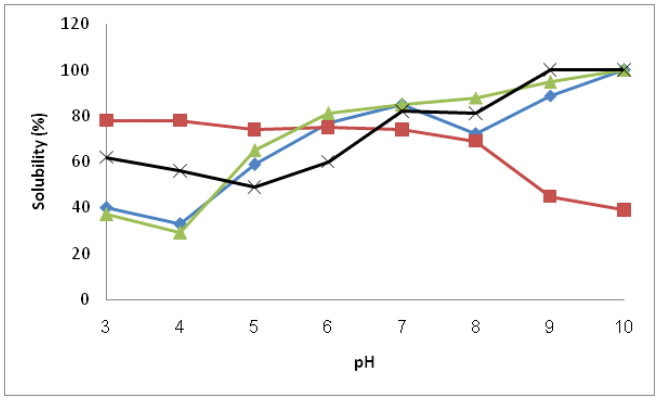

Figure 2 : The effect pH of on the solubility of 0.1% control, (x), esterified ( ) , acylated ( ) and deamided ( ) camel milk whey proteins in 0.15 M NaCl.

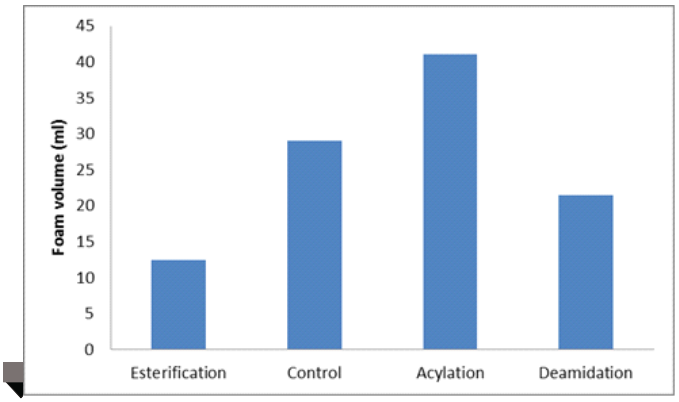

Figure 3 : Foam volume (mL) of of 0.1% control, esterified, acylated and deamided camel milk whey proteins in 0.15 M NaCl.

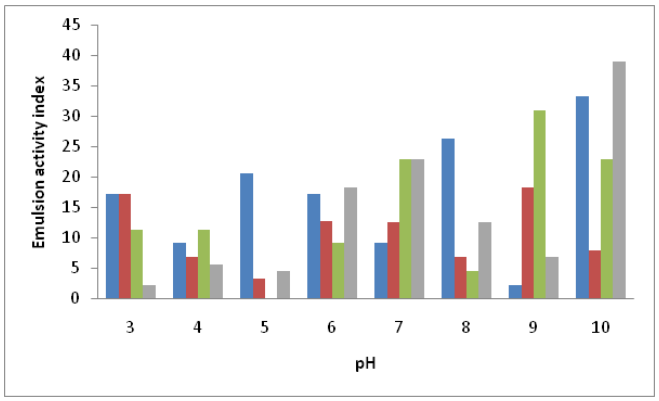

Figure 4 : The effect of pH on emulsion activity directory of 0.1%, control ( ) , esterified ( ) , acylated ( ) and deamided ( ) camel milk whey proteins in 0.15 M NaCl.

These components did not change substantially upon treatment with esterification acylation, and deamination but the density of bands was changed .The higher changed in BSA band density was noticed upon treatment with deamination, while treatment with esterification and acylation cause less decrease in BSA band density. However, in SDS-PAGE there were increase in the density of low molecular weight bands below α-La band especially upon treatment with deamination which indicate that this treatment cause hydrolysis whey proteins camel.

Solubility of camel milk whey proteins

The solubility of protein is regarded as the proportion of the nitrogen in a protein output whose is into this soluble status beneath particular circumstances. Solubility is the quantity of protein in a specimen that dissolve in the solution. Proteins prescribed as food additives can be partially or wholly soluble or perfectly insoluble in water (Zayas, 2012). In order to evaluate the importance of free amino and carboxyl groups in solubility of camel milk whey proteins, acylation, esterification and deamination were conducted.

To pH values greater than 8, the solubility of control, acylated and demanded camel milk whey proteins was high (figure 2) greatest protein solubility was observed at high pH values because in this condition, the protein's positive and negative net charged molecules interact more with water (Fennema 1975). At pH values lower than 5 the solubility of control camel milk whey proteins was higher than the solubility of acylated and demanded whey

proteins of camel milk and that may be related to the facts that at low pH values the amino groups in proteins play the major role in their solubility and since that the modification in the case of acylated and demanded targeted the amino groups in proteins the solubility became less (Fennema, 1975) . In pH values between 5-7 the solubility of demanded whey protein was higher than the solubility of control and acylated whey proteins and this related to the fact that deamination increase the electrostatic repulsions between whey proteins (Miwa *et al*, 2010). Also shown in figure 2 the solubility of esterified camel caseins was different. The solubility was high in acidic pH values and decreased with the increment of pH until it reached to 39% at pH 10 and this can explained by the role of esterification in modification of carboxyl group responsible of protein solubility at high pH values. Esterification can neutralize negative charges from carboxylate moieties in proteins by addition ester collection. The nucleophilic assault by the alcoholic collection to the free carboxyl collection of a protein precipitates important variations in the protein net charge, hence in its conformation, and therefore in its particular characteristics (Sitohy *et al,* 2001).

Foaming characteristics of camel milk whey protein

The Foams which contain gas bubbles dispersed into a liquid. The stability of the air bubbles in the foams defined by, the foaming agent which forms a layer of adsorbed molecules separating the air bubbles from the continuous liquid phase,

(de Jongh & Broersen, 2012). Compared with control whey proteins of camil milk, acylated camel milk, solutions of whey protein produced more foam immediately after sparging (Figure 3), while esterification and deamination reduce whey protein ability to for foam.

Control camel milk whey proteins foam volume immediately after sparging was 29 ml, and this volume changed to 12.5, 41 and 21.5 ml after esterification, acylation and deamination respectively. This variation may be related to the chemical modification treatments, which changed surface hydrophobicity of proteins (Kato, *et al* 1983). The ability of acylation in increasing foaming ability of whey proteins can explained by its role in reduce the polarity of these proteins and made them able to adsorb at an interface and to interact with higher concentration of hydrophobic compounds (Wierenga *et al,* 2005).

This result agrees with the results of,Childs and Par, (1976) who found that acylation enhances the water-holding, oil-holding, and foam properties of glandless cottonseed flour .

Emulsion activity of camel milk whey proteins

Emulsions consist of two immiscible liquids phase, oil and water, in which the droplets are termed as dispersed phase and the liquid surrounding the droplets is called continuous phase. According to the concentrations of each liquid and the environmental conditions, oil-in water emulsions or water-in-oil emulsions can be instituted. These consist of oil droplets in a continuous water phase, and water droplets in a continuous oil phase, respectively, (de Jongh & Broersen, 2012).

Figure 4, shows the effect of pH on (EAI) whey proteins of camel milk. The highest EAI for control and acylated camel milk whey proteins was at pH 10. Modification of camel milk whey proteins with esterification and deamination changed their highest EAI to 9. The changes in emulsifying properties of modified whey protein of the camel milk at different pH values can be ascribed to the modification process utilized to the produce, resulting in exposure of beforehand disappeared hydrophobic domains on the protein determination (Jahaniaval *et al,* 2000). Lawal & Adebowale (2004) found that acetylation of proteins eliminates the positive lysyl residues with noncharged side groups. Acylation with succinic or other dicarboxylic anhydrides replaces lysyl residues with negatively charged carboxyl groups. Both of these modifications cause a rise of protein solubility and electrostatic repulsion between droplets at the proteins and thus lead to enhance the emulsifying activity .

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