گۆڤاری کوردستانیی بۆ لێکۆڵینەوەی ستراتیجیی

EFFECT OF ACETYLATION ON FUNCTIONAL PROPERTIES OF CASEIN, α_s- CASEINAND β- CASEINISOLATED FROM COW AND GOAT MILK AT DIFFERENT pH

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The primary reason for the involvement of the four researchers is the diversity of specializations between chemistry and dairy technology. This interdisciplinary collaboration was crucial for conducting the research comprehensively and interpreting the results with greater accuracy.

Abstract

The effects of acylation on the emulsion activity, solubility, and foaming properties of casein, αs-casein and β-casein isolated from cow and goat milk at different pH were studied. At pH values higher than 6, the highest solubility of casein, αs- casein and β -casein was observed, while at low pH values (less than 5) the solubility of casein and β-casein isolated from goat and cow milk was highly decreased after modification with acylation. The foaming properties of both goat and cow casein were higher in comparison with the foaming properties of αs- casein and β- casein, besides that, modification with acylation decreased the foaming ability of goat and cow milk proteins.

The highest emulsion activity index for both native and acylated goat and cow milk caseins was at pH 10, while for αs-casein, the highest emulsion activity index was observed in native cow αs-casein at pH 8-9. Modification of goat milk β-CN improves emulsion activity index specially at pH 7.

Keywords: Goat milk, Casein, Acylation, Functional properties

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Introduction

Casein is the prevalent class of milk proteins. Caseins are coagulated by rennet mainly or precipitate at a low pH of 4.6 at 20^{o}C from milk. About 80% of the proteins are caseins in cow's milk, or about 26 g / kg is about 80 percent of the milk protein (Wong et al., 1996). The main fractions of casein are α_s1-casein , α_s2-casein ,β-casein and k-casein (Walstra and Jenness, 1984).
At the natural pH of milk, αs1-, αs2-, β- and κ-caseins exist in the form of associated complexes in the presence of calcium phosphate known as "Casein micelles" which have an average diameter of ~150 nm in the native form (Fox and McSweeney, 1998; Haug et al., 2007), These four types of casein differ in their chemical properties. The calcium sensitivity of caseins is in the order of α s1 - casein = αs2 -casein > β-casein > κ-casein, also all included in the pH 4.6 precipitate from milk (Tamime and Robinson, 1999).

Goat milk comes fourth in the world's milk production, behind cow, buffalo, and sheep's milk. It has special economic significance in areas where the climate makes cow production impractical. Goat milk has garnered a lot of attention lately, as has the process of turning it into products with added value (Amigo and Fontecha 2011). The composition of goat milk is different from that of cow milk (Al-Saadi, 2002). It is less allergic, more digestible, alkaline, has a higher buffering capacity, and has some medicinal qualities.
Goat milk has 2.11 g/100 g of casein, or 74% of the total milk proteins, and 0.6 g/100 g of whey protein, or roughly 17% of the goat milk proteins. Goat milk has a total nitrogen concentration of 11% (0.4 g/100g), of which is NPN. Compared to bovine casein micelles, caprine casein micelles have higher levels of non-centrifugal casein, calcium, and inorganic phosphorus (Jenness, 1980; Remeuf and Lenoir, 1986). Caprine milk yogurt and cheese are thought to have a weak texture due to low casein content and other factors such micellar size and αs-casein proportions. In terms of their general classifications as αs-β-, κ-CN, β-lg, and α-la, goat milk proteins and bovine milk proteins are similar; however, there are differences in the genetic polymorphisms and their frequencies in goat populations (Martin, 1993; Grosclaude, 1995; Jordana et al., 1996; Amigo and Fontecha 2011). The main casein components of goat milk differ significantly from those in cow's milk in terms of their relative quantities, according to Remeuf and Lenoir (1986). Less αs-CN is seen in goat milk, which frequently has more αs2 than αs1-cn. According to Mora-Gutierrez et al. (1991), the latter is found in wildly varying levels in different goats. In comparison to cow milk, goat milk has larger proportions of κ-CN, particularly β-CN.
The process of adding an acetyl functional group (-COCH3) to a molecule is known as acetylation, and it can have a profound impact on the functional characteristics of proteins, including casein proteins, which are present in milk from cows and goats. The solubility of casein proteins in various solvents can be impacted by acetylation (Tardy et al., 2021). Acetylation can make casein proteins more soluble in specific solvents or at specific pH ranges because it modifies the hydrophobicity and charge distribution of casein proteins. Additionally, casein proteins' foaming qualities—which are critical for the creation and durability of foams in applications like whipped toppings and meringues—may be impacted by acetylation (Ellis and Lazidis, 2018). Because acetylated casein has different surface activity and interfacial qualities, it can have different foaming behavior, such as changes in foam stability and overrun. The degree, location, and source of the proteins (cow vs. goat milk) are some of the variables that can affect the specific effects of acetylation on the functional characteristics of casein proteins. The functional performance of acetylated casein in food and dairy products will also be influenced by the intended application and the processing conditions.

The current study aimed to investigate the impact of acetylation on functional properties (foaming and solubility and emulsifying activity) of cow and goat milk casein, αs-casein, and β-casein at different pH.

Materials and Methods

Goat Milks Source:

Milk was collected from more than 10 animals. Bulk goat milk were skimmed by centrifugation on 2400 g for 15 min at 4°C. Preparation of goat milk casein: The skim milk of goat was acidified to pH 4.3 with HCL 1M through constant stirring at the heating degree 20°C. The mix was filtered within Whitman number one paper after settling for 20 minutes. The distilled water was used to wash the precipitated casein and it was dissolved by adding (1 M) of NaOH until the pH attained 6.80. The precipitation processes was repeated. Also, the resulting caseinate was re-solubilized and dried by the freeze. Goat and cow milk αs-CN and β-CN were isolated and purified according to (Al-Saadi,2002).

Acetylation:

The method described by Kebary, *et al.* (2003) was used for acetylation of goat and cow milk proteins. 10% suspension of protein into distilled water was instant and, the pH was modified to 8.5, using a 2 M sodium hydroxide. Acetic anhydride (0.9 g/mL protein) was added to the solution and the pH was kept at 8.5 for 60 minutes. Acetylated protein solution was dialyzed versus distilled water for 24h, and freeze-dried.

Functional properties of goat and cow milk protein

Solubility

Stock solutions (0.l% in 0.15 M NaCl, pH 7) of milk proteins coprecipitates were adjusted to the appropriate pH (4–10) with either 0. l N HCl or 0.1 N NaOH and centrifuged at 12 000 g for 15 min at 25°C (or filtered). The protein concentration of the resulting supernatant was determined from the absorbance at 280 nm by spectrophotometer (Labomed/USA). Solubility was expressed as the percentage of protein in solution by the following formula (Al-Saadi and Deeth, 2011):

$$\text{Solubility (\%)} = \frac{\text{amount of protein to dissolve} - \text{the amount of protein filtrated solution}}{\text{mount of protein to dissolve}} \times 100$$

Emulsifying activity

The emulsifying activity was determined using the method of Pearce and Kinsella (1978). Triplicate emulsions of each sample (0.l% in 0.15 M NaCl, adjusted to pH from 4 to 10 with either 0. l N HCl or 0.1 N NaOH) were prepared using 10 mL of sample and 0.6 mL of corn oil. The emulsion was prepared by mixing these ingredients for 1 minute at room temperature using a blinder. A 0.2-mL aliquot of the emulsion was diluted (1⁄250 final dilution) using a 0.1% sodium dodecyl sulfate (SDS) solution. Turbidity of the emulsion was determined spectrophotometrically at 500 nm.

The emulsion activity index (EAI), which measures the area of interface stabilized per unit weight of protein (m2 ⁄ g), was calculated using the following equation:

$$EAI = \frac{(2.303)(2)(A500)(\text{dilution factor})}{(C)(1 - \text{Oil volume})(10000)}$$

Where A500 = absorbance at 500 nm, the dilution factor is 250 and
c = g protein ⁄ mL of the aqueous solution before emulsion.

Foaming

Foaming properties of proteins were studied using Siamand and Al-Saadi (2017) method. Fifteen milliliters of samples (0.1% in 0.15 M NaCl, pH 7) were placed in a column (1.6 cm x 70 cm), containing a sintered glass disc at the bottom. The air was sparged from the bottom of the column for 2 min at a flow rate of 30 mL/min. Foam height was measured (mL) immediately after the gas flow was stopped and at one-minute intervals. Three observations were made of each sample.

Results and Discussion

Milk casein solubility:

The solubility is defined as the quantity of protein in a sample that dissolves in the solution and this characteristic of proteins in food can be used as an indicator for optimizing the influences of adjustments on proteins and also the possibility of disadvantages and advantages of utilizing protein in specific foods. Acylation was done to assess the significance of free amino and carboxyl groups in the solubility of goat and cow milk proteins.

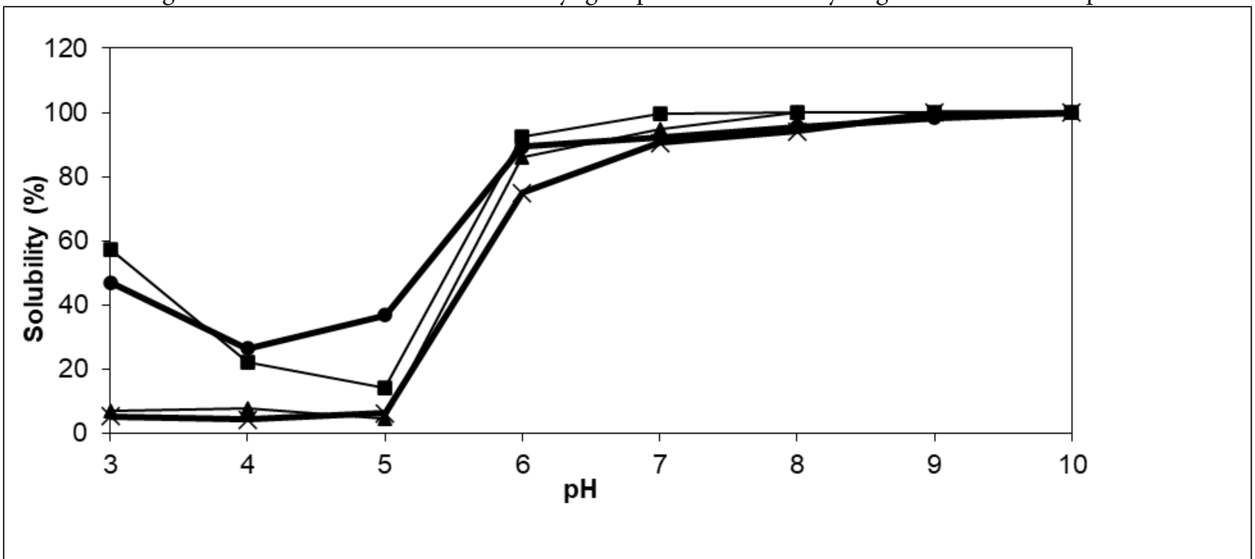
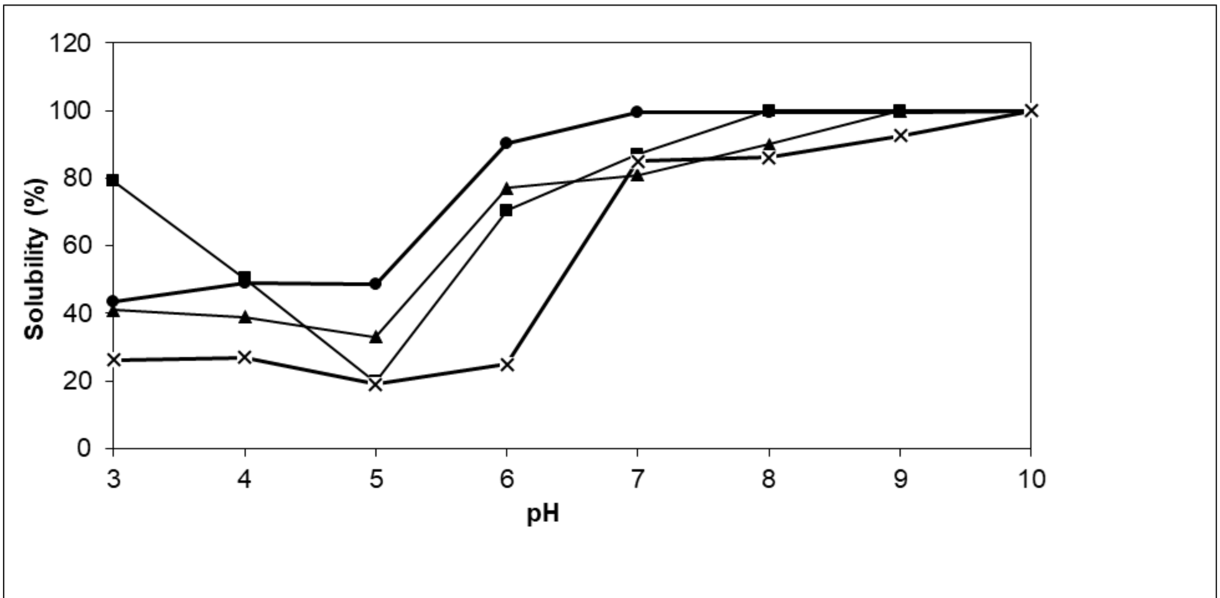


Figure 1: Effect of pH on solubility (%) of cow casein (■), acylated cow casein (▲) , goat casein (●) and acylated goat casein (×) in 0.15 M NaCl .

Acylation decreases protein solubility at low pH (below 6) by adding hydrophobic acyl groups to the protein molecules, altering their surface properties and reducing their overall charge, but this effect diminishes as the pH rises. The values for acylated and non-acylated samples converge at higher pH levels, indicating similar solubility (figure 1). Generally, Casein solubility increases with pH increment for all samples. Similar solubility pattern was obtained by (ALKaisy and Al-Saadi 2019) for camel milk. Around pH 4 to 5 which is close to the isoelectric point of casein, the solubility is at its lowest for both cow and goat milk, which is typical for proteins as they tend to precipitate out of solution at their isoelectric point and the fact that at low pH value the amino groups in proteins play the major role in their solubility and since that the modification in the case of acylated targeted the amino groups in proteins the solubility became less (Fennema 1975). As pH increases beyond 5, solubility rapidly increases for all samples, reaching almost 100% solubility by pH 7 and above because in this condition, the protein's positive and negative net charged molecules interact more with water (Fennema 1975).

Solubility of αs-casein;

The effect of acylation on solubility of αs-casein (Figure 2) and β-casein (Figure 3) of cow and goat milk was also studied. At pH values around 3 to 5, which are acidic conditions, the solubility of αs-casein drops significantly for all samples. The effect of acylation is evident with acylated casein (both cow and goat) showing even lower solubility compared to their non-acylated forms. This is because the introduction of hydrophobic acyl groups to the protein enhances hydrophobic interactions, leading to aggregation and reduced solubility (Fennema 1975).. As the pH increases from 5 to 7, solubility begins to recover. Non-acylated goat αs-casein shows a particularly sharp increase in solubility, reaching complete solubility around pH 7. This suggests that goat αs-casein is less prone to aggregation in this pH range compared to cow αs-casein, potentially due to differences in protein structure or amino acid composition (Muñoz-Salinas et al, 2022). At pH 7-10 solubility reaches a maximum for all casein types, with insignificant differences between acylated and non-acylated samples.



(●) and acylated goat αs-casein (×) in 0.15 M NaCl.

Solubility of β-casein;

The Figure 3 illustrates the solubility of β-casein across a range of pH levels, At low pH values (3-5), β-casein solubility decreases significantly for all samples. The acylated protein (both cow and goat) exhibit lower solubility than their non-acylated counterparts at these pH levels. Whereas, at the pH from 5 to 7, non-acylated form of goat β-casein, shows a sharper increase in solubility around pH 6-7, indicating a less tendency to aggregate compared to cow β-casein.

At higher pH values above 8, solubility for all samples approaches 100%. This is due to the increase in net negative charge on the protein molecules. By this point, the effect of acylation diminishes as the strong electrostatic repulsion overcomes hydrophobic interactions, resulting in similar solubility profiles for both acylated and non-acylated samples (Damodaran, S. 1996).

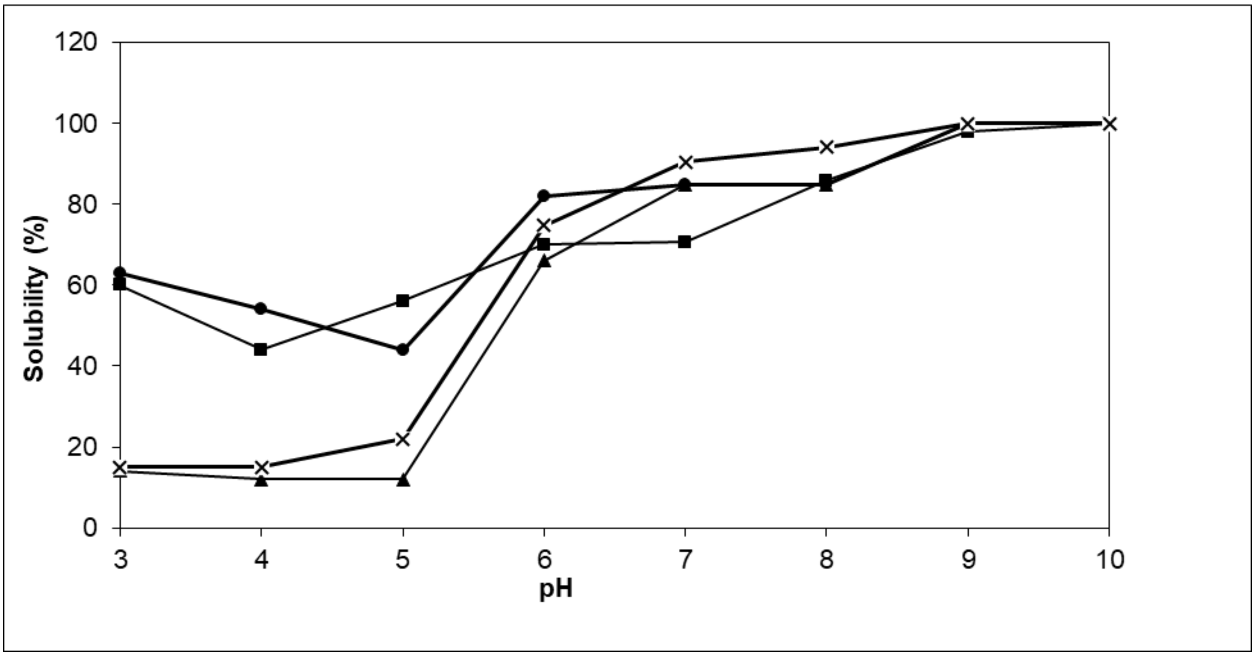


Figure 3: Effect of pH on solubility (%) of cow β-casein (■), acylated cow β-casein (▲) , goat β-casein (●) and acylated goat β-casein (×) in 0.15 M NaCl.

Foaming properties:

Milk casein foam volume and stability;

The Foams which contain gas bubbles dispersed into a liquid. The stability of the air bubbles in the foams is 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). Milk proteins have been identified as good foaming agents as a result of their aggregation state, molecular stability and flexibility, electrostatics, and (surface) exposed hydrophobicity (Hunter et al., 1991; Luey et al., 1991). Chemical modifications have been employed in the past to improve surface activity of less performing proteins. As shown in figure 4 there is little difference in the foam volume between cow and goat casein, as well as between acylated and non-acylated samples.

At the initial time all samples start with a similar foam volume. Compared with non-acylated cow milk caseins, acylated cow milk casein solutions created more foam. This result has a good agreement with the results of ALKaisy and Al-Saadi (2019) who obtained that the acylation enhanced the foam volume for camel milk caseins. This may be correlated to the casein modification, which changed the hydrophobicity surface of proteins (Al-Saadi, 2014). Whereas, the acylated goat milk casein shoes the minimal foam volume, suggesting differences in protein structure or composition between cow and goat milk casein (Yasmin et al. 2020).

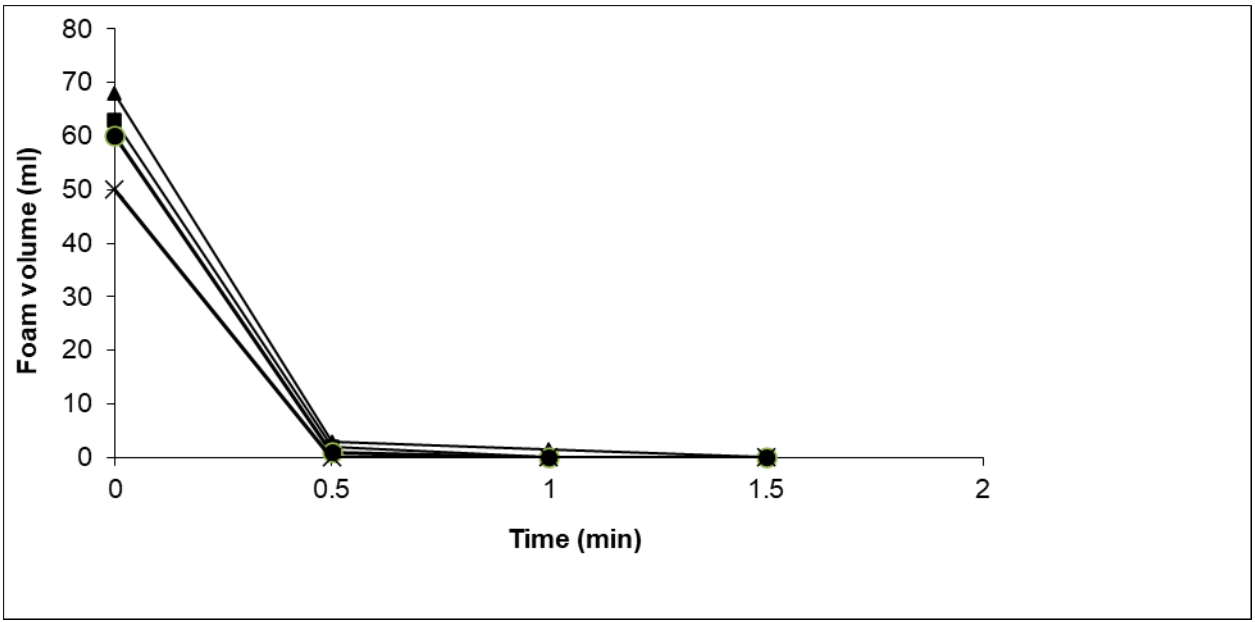


Figure 4: Foam volume (mL) of 0.1% cow casein (■), acylated cow casein (▲) , goat casein (●) and acylated goat casein (×) in 0.15 M NaCl.

Foam volume and stability of milk αs-casein;

The effect of acylation of cow and goat milk αs-casein on foam volume over time was also studied (Figure 5). Non-acylated goat αs-casein shows the highest initial foam volume at around 7 ml, while the αs-casein samples for cow milk starts with a lower initial foam volume (2ml).

Comparable to caseins, acylation has a different effect on foam volume in both acylated cow and goat αs-casein, which begin with lower foam volumes than their non-acylated for goat αs-casein, while for cow milk αs-casein the acylation begin with higher foam volumes than their non-acylated. Foam stability slightly affect by acylation, there are slight differences in the rate of foam collapse between acylated and non-acylated samples. The acylated αs-casein samples seem to show a slightly slower rate of foam collapse compared to the non-acylated samples.

While acylation slightly improves the rate of foam collapse, the overall stability of the foam remains low, with all samples showing a rapid decline in foam volume. This suggests that while acylation may modify the surface properties of αs-casein (Al-Saadi, 2014), it does not significantly enhance foam stability, making it less effective for applications requiring stable foams.

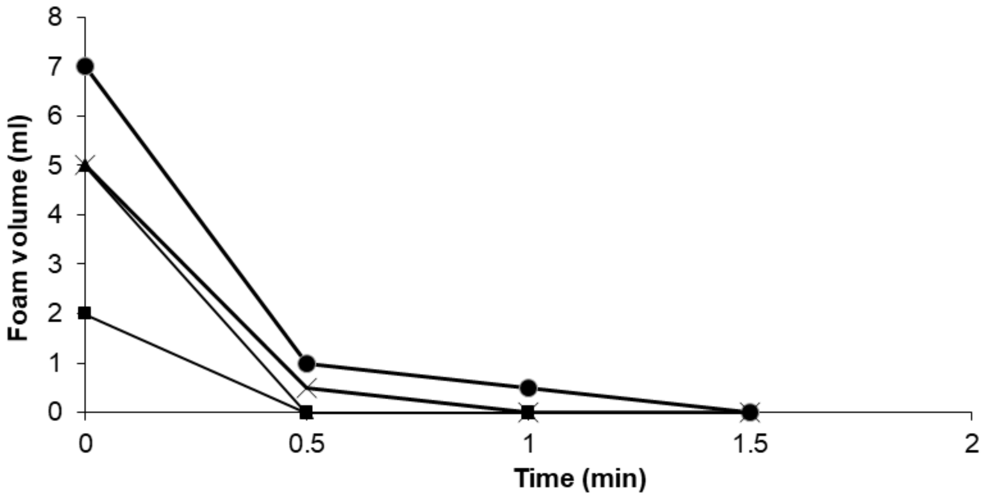


Figure 5: Foam volume (mL) of 0.1% cow αs-casein (■), acylated cow αs-casein (▲) , goat αs-casein (●) and acylated goat αs-casein (×) in 0.15 M NaCl.

Foam volume and stability of milk β-casein;

Acylation of milk β-casein significantly affects the initial foam volume (Figure 6). Both acylated cow and goat β-casein exhibit lower initial foam volumes than their non-acylated form, with a differences between cow and goat β-casein form formation. Acylated goat milk β-casein showing a higher starting foam volume than acylated cow milk β-casein. Acylation decreases the initial foam volume of β-casein, particularly in cow-derived samples, likely due to the modification treatments, which changed hydrophobicity surface of proteins (Kato et al, 1983) and introducing the hydrophobic acyl groups that interfere with the protein's ability to form foam.

Acylation effect on foam stability also seen, it enhance foam stability over time with the foam collapsing more gradually compared to the non-acylated samples. Both non-acylated β-casein show a rapid decrease in foam volume within the first minute. In goat milk β-casein, while acylation reduces foam formation, but it help sustain the foam structure longer and it maintains a slightly higher foam volume than cow milk β-casein over the time. Notably, acylated goat β-casein maintains a relatively stable foam volume over 5 minutes, indicating improved foam stability due to acylation. This characteristic can be beneficial in applications where prolonged foam stability is required.

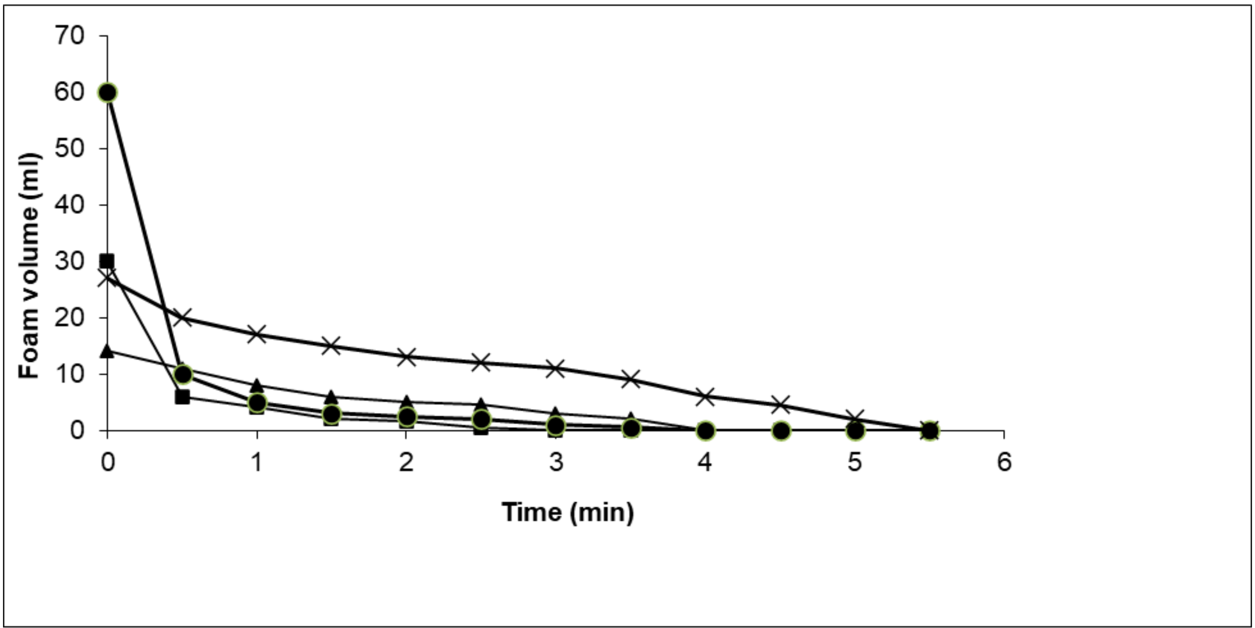


Figure 6: Foam volume (mL) of 0.1% cow β-casein (■), acylated cow β-casein (▲), goat β-casein (●) and acylated goat β-casein (×) in 0.15 M NaCl.

Emulsion activity Index (EAI):

Emulsion activity Index of milk casein;

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. (de Jongh & Broersen, 2012). The characteristics of emulsification rely on the protein ability to spread to the interface of the water-oil, orient, and unfold in the fashion which the hydrophobic sets assistant by the oil. However, groups of hydrophilic were associated with the phase of water (Ali & Al-Saadi,2019). Caseins readily form stable protein films and are used extensively in the food industry, Caseins, because of their flexible structure and many hydrophobic and hydrophilic regions, spread out on the phase interface and interact with all phases to form stable film. Removal of some of most of the negatively and positive charges from casein by chemical modification alters its net protein charge and changes the flexibility of the modified protein structure. Therefore, the ability of modified casein to form stable protein films in an emulsion or foam is a very important functional consideration (Schmidt et al. 1984).

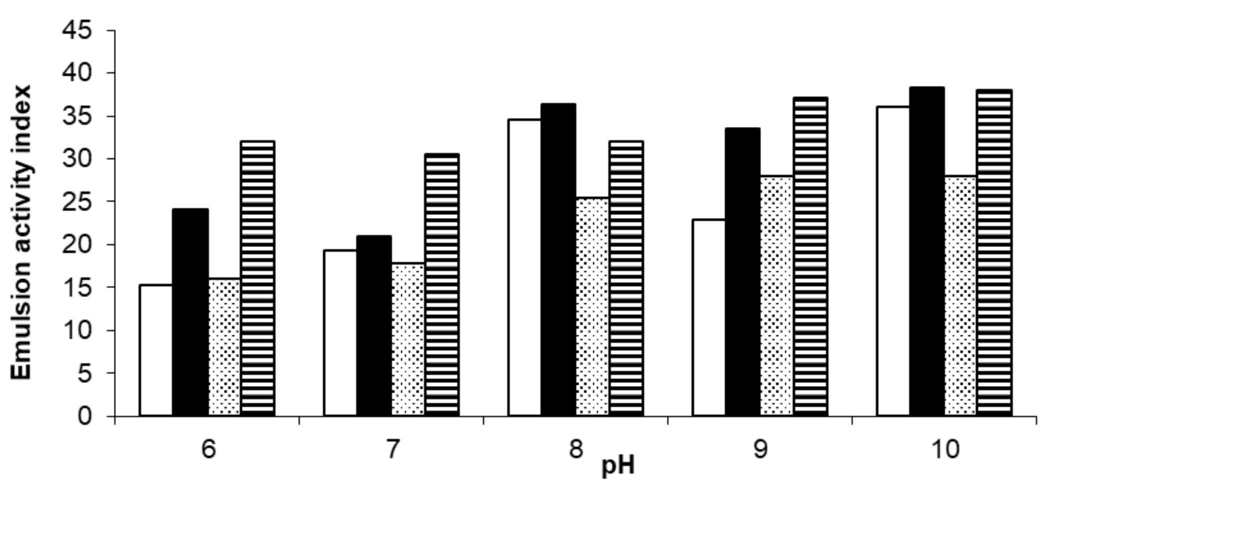


Figure 7: Effect of pH on emulsion activity index of 0.1% cow casein ( ), acylated cow casein (■) , goat casein ( ) and acylated goat casein (◘) in 0.15 M NaCl.

There is a distinct influence of acylation on the emulsifying properties of caseins, the data reveals that the acylated caseins exhibit higher Emulsion Activity Index EAI across all pH levels (6-10) compared to their non-acylated counterparts (figure 7). This increase in emulsifying activity is most pronounced in goat casein at higher pH levels (9 and 10), Conversely with that obtained by ALKaisy and Al-Saadi (2019) for acylated camel milk caseins the highest EAI was at pH 4-5. The findings suggest that acylation enhances the hydrophobic character of casein molecules, thereby improving their ability to adsorb at the oil-water interface and stabilize emulsions effectively. This modification is particularly beneficial for applications requiring strong emulsification under varying pH conditions.

On the other hand, the impact of pH on the emulsifying activity of caseins acylated casein is significant, as it influences the protein's solubility, charge, and conformation, which in turn affects its ability to stabilize emulsions. As pH increases and moves away from the isoelectric point, caseins acquire a higher net charge, enhancing their solubility and ability to adsorb at the oil-water interface (Damodaran, S. 1996) Therefore, at higher pH values (8-10), caseins exhibit better emulsifying properties, as reflected in higher emulsion activity index (EAI) values in such conditions.

Emulsion activity Index of milk β-casein;

Figure 8 showed that there are a significant effect of pH and acylation on the emulsifying activity of β-casein. Acylated caseins tend to show different behavior compared to their non-acylated counterparts. Acylated goat casein shows higher emulsifying activity at pH 7 and 9, suggesting that acylation might enhance the ability of casein molecules to stabilize emulsions under specific pH conditions (ALKaisy and Al-Saadi, 2019) . However, this effect isn't consistent across all pH levels, indicating that the impact of acylation is pH-dependent. The comparison between cow and goat β-caseins, both native and acylated, suggests that goat casein generally exhibits better emulsifying properties at lower pH levels, particularly when acylated. This could be due to differences in the protein structure between cow and goat milk proteins.(Yasmin et al. 2020).

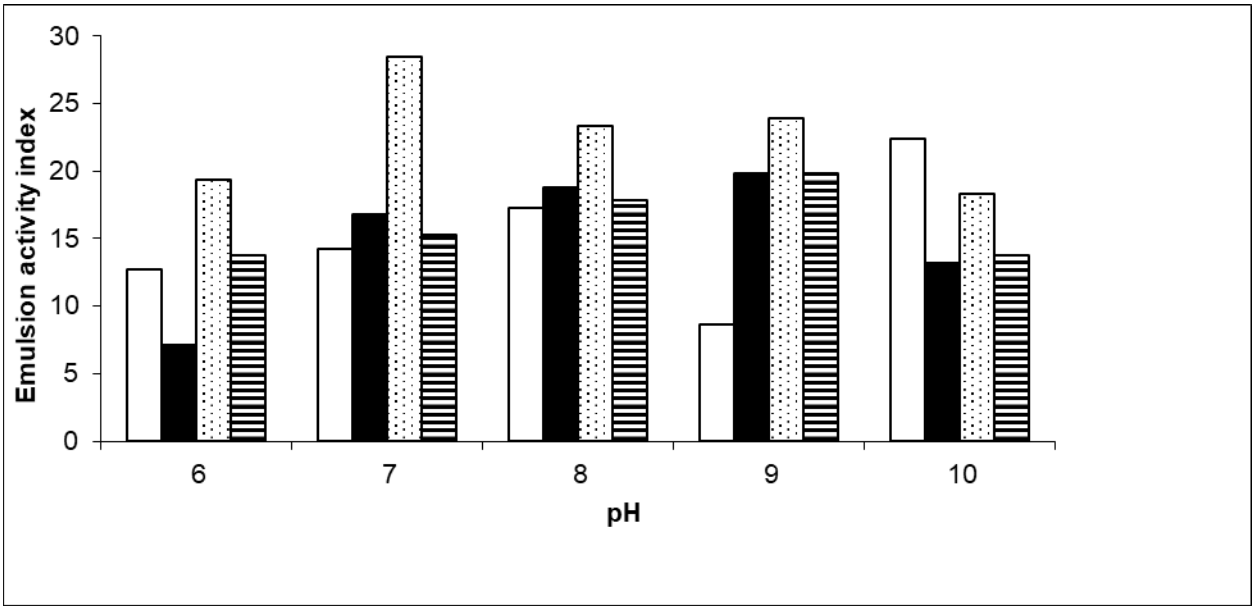
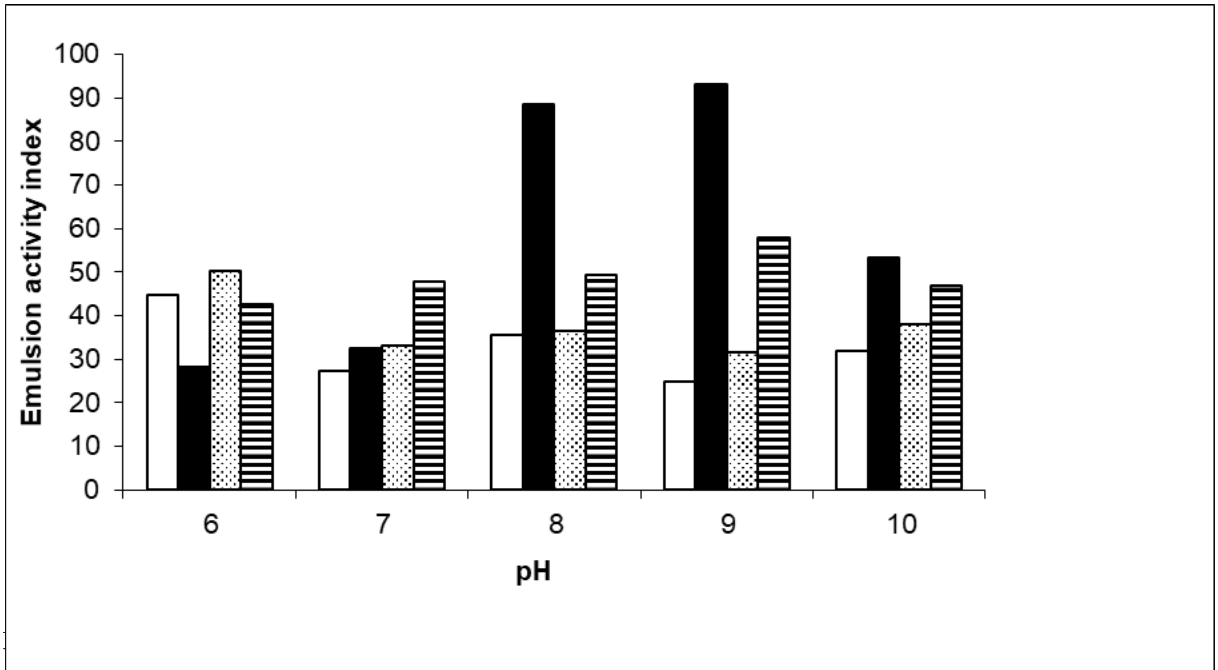


Figure 8: Effect of pH on emulsion activity index of 0.1% cow β-casein (■), acylated cow β-casein (□) , goat β-casein (⬚) and acylated goat β-casein (▤) in 0.15 M NaCl.

Emulsion activity Index of milk αs-casein;

Emulsion Activity Index (EAI) for cow and goat milk αs-casein at various pH levels across different casein types (acylated and and non-acylated) was shown in figure 9. Generally, acylation improves emulsifying activity, as seen in the acylated cow and goat milk αs-casein. Goat milk αs-casein casein (both native and acylated) shows better emulsifying activity at lower pH levels compared to cow casein.

Similarity with β-casein, the EAI increases with pH, particularly from pH 7 to 9, indicating that αs-casein exhibits better emulsifying properties in more alkaline conditions. This trend suggests that the protein's conformation and charge distribution at higher pH levels favor better stabilization of emulsions (ALKaisy and Al-Saadi, 2019).



) , goat αs-casein (⬚) and acylated goat αs-casein (▤) in 0.15 M NaCl.

Conclusion

The study demonstrates that acetylation significantly affects the functional properties of αs-casein and β-casein isolated from cow and goat milk. Acetylation generally reduces solubility at low pH levels by introducing hydrophobic groups, leading to decreased protein solubility and enhanced aggregation. However, solubility improved at higher pH levels. Regarding foaming properties, acetylation increased foam stability and volume for cow milk caseins but had a lesser effect on goat milk caseins, indicating structural differences between them. Acylated proteins exhibited higher Emulsion Activity Index (EAI) values, particularly at higher pH levels, indicating an improved ability to stabilize emulsions. The results suggest that acetylation can be a useful modification for enhancing the functional properties of milk proteins, particularly for applications requiring stable emulsions and foams. However, the specific effects of acetylation depend on the type of casein and pH conditions, highlighting the need for careful consideration of these factors in food processing.

تأثير الأستلة على الخواص الوظيفية للكازين وألفا أس-كازين وبيتا-كازين المعزولة من حليب الأبقار والماعز عند pH مختلف

الملخص

تمت دراسة تأثير الأستلة على نشاط كل من المستحلب، وقابلية الذوبان، وخصائص الرغوة على كل من بروتينات الكازين، وألفاس-كازين والبيتا-كازين المعزولة من حليب الأبقار والماعز. لقد لوحظ عند قيم الأس الهيدروجيني أعلى من ٦، وجود أعلى قابلية للذوبان بروتينات الكازين، وألفاس-كازين وبيتا-كازين، بينما عند قيم الأس الهيدروجيني المنخفضة (أقل من ٥) كان هناك انخفاض في قابلية ذوبان الكازين وبيتا-كازين المعزولة من حليب الماعز والأبقار بشكل كبير بعد التعديل بالأستلة.أما بالنسبة لخصائص الرغوة لكل من كازين الماعز والأبقار فقد كانت القيم أعلى مقارنة بخصائص الرغوة لألفاس-كازين وبيتا-كازين، إلى جانب ذلك، أدت عملية التعديل بالأستلة إلى انخفاض قدرة تكوين الرغوة لكل من بروتينات حليب الماعز والأبقار على التوالي.

كان هناك أعلى مؤشر لنشاط المستحلب لكل من الكازينات الأصيلة والمئستلة في حليب الماعز والأبقار عند قيمة أس هيدروجيني ١٠، أما بالنسبة لكازين الألفأس، فقد لوحظ أعلى مؤشر لنشاط المستحلب في كازين الألفأس البقر الأصلي عند قيمة أس هيدروجيني بين ٨-٩. كذلك فإن تعديل البيتا-كازين في حليب الماعز يحسن مؤشر نشاط المستحلب خاصة عند قيمة أس هيدروجيني ٧.

كلمات مفتاحية: حليب الماعز، الكازين، الأستلة والخصائص الوظيفية

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