Rheological Properties of Gels Produced from Citric Acid Modified Whey Proteins

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ABSTRACT

The impact of citric acid modification on the calcium-induced gelation behavior of whey protein concentrate (WPC) was examined in this work. After heating at 90°C for 30 minutes, gels with a 7% protein concentration and different calcium chloride concentrations (20–90 mM) were prepared using both native and citric acid-modified WPC. The gels' viscosity, hardness, cohesiveness , springiness , water-holding capacity, and spontaneous whey separation were assessed. According to the findings, native WPC gels showed gradual increases in gel characteristics as the calcium concentration increased up to 60 mM, and at higher concentrations gel properties slightly decreased. On the other hand, at calcium concentrations between 40–60 mM, citric acid-modified WPC gels showed better functional characteristics, including noticeably greater water holding capacity , viscosity , hardness, and cohesiveness .These results imply that whey protein gels treated with citric acid have potential as functional additives with improved textural qualities for use in the food sector, especially where regulated gel strength and water retention are needed.

Keywords Whey protein, citric acid, cross-linking, gelation, rheological properties

1 INTRODUCTION

Whey proteins may be chemically altered and form covalent connections using enzymes, chemical agents, or physical techniques [3] .Whey protein gels are being employed to deliver bioactive ingredients and improve food texture [4]. Calcium-induced gelation is one of the most popular gelation processes, with calcium ions playing an important role in cross-linking protein molecules and producing a three-dimensional gel network [5].

Whey proteins, which make up around 20% of all milk proteins, are the proteins that are left in whey after caseins are separated. These proteins are sensitive to heat treatments, do not precipitate with acids, and are unaffected by the chymosin enzyme [1]. Because of their unique functional qualities, application in the production of food products, and high nutritional value which piques consumer interest, whey proteins are extremely important [2]

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evaluate the effect of citric acid modification on calcium-induced gelation behavior.

2 Materials and methods

2.1 Materials

Whey protein concentrate (WPC, 80% protein content) was procured from Kalleh Dairy Company (Iran). Citric acid and calcium chloride were procured from Oxoid (UK).

2.2 Methods

2.2.1 Cross-linking Whey Protein Isolate with Citric Acid.

Whey proteins concentrate (5 g) was dissolved in 200 mL of 0.67 mol/L citric acid solution, and the pH was set to 6.8. To enhance cross-linking, the mixture was incubated in a water bath at 50°C for 4 hours while being stirred continuously. After the reaction, the solution was freeze-dried [11].

2.2.2 Whey Proteins Gel Production

Whey proteins concentrate (native and citric acid-modified) was dissolved (7%) in distilled water and the solutions were kept for at least two hours at 4°C while being stirred. The pH was adjusted to 6.6 and $CaCl_2$ was added at concentrations of 20, 30, 40, 50, 60, 70, 80, and 90 mM. The solutions were heated in a water bath at 90 °C for 30 minutes and at the end of heating time the solution was kept at 5°C for 24 hours[5].

2.2.2.1 Viscosity

The viscosity values of the gel samples were determined at 10°C after one day of refrigerated storage using a

In recent years, researchers have worked on chemically altering whey proteins to improve their gelation properties and broaden their applications. Citric acid is one of the most promising agents in this field, as it is a natural, non-toxic, and inexpensive cross-linking agent. The cross-linking mechanism involves the reactivity of carboxyl groups in citric acid with free amino groups in the protein, which results in the production of new amide bonds that enhance the protein structure [6].

Previous research has shown that adding citric acid to whey proteins improves their functional properties, such as increased surface hydrophobicity and disulfide bond formation, which improves gelation capacity and water holding capacity [7][8]. However, the implications of this change on calcium-induced gelation behavior have not been adequately investigated.

Calcium ion concentration is an important aspect in the gelation process because it influences the properties of the produced gel, such as hardness, viscosity and water holding capacity [9]. Calcium ions interact with negatively charged areas of the protein surface, generating ionic bridges that connect protein molecules and form a cohesive gel network [10].

This study aims to prepare gels from native and citric acid-modified whey proteins using different concentrations of calcium chloride and compare the properties of the formed gels to

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Brookfield DVII+ Viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass.), following the procedure described by [12].

2.2.2.2 Water Holding Capacity:

The water holding capacity of the whey gel samples was measured using the method given by [13].

2.2.2.3 Spontaneous Whey Separation

To estimate spontaneous whey separation, a cup of whey protein gel was placed at a 45° angle for two hours at 5° C. The separated whey was then removed from the surface using a syringe, and the cup was reweighed. The technique was carried out in 10 seconds to minimize excessive syneresis, as described by [14].

2.2.2.4 Texture Determination:

The whey protein gel sample's hardness, springiness, and cohesiveness were assessed using a Texture Analyzer (Model CT3-4500, Brookfield Engineering Lab, USA), as reported by [15]. The following operating parameters were used: a TA16 synthetic plastic cylinder was introduced into the whey protein gel sample (volume: 100 ml) to a depth of 20 mm, with a trigger load of 5 g and a test speed of 1 mm/s, at room temperature.

2.2.3 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences between means at a confidence level of $p < 0.05$. All statistical analyses were conducted using SPSS software ([2018], IBM Corp., USA).

3 RESULTS AND DISCUSSION

3.1.1 Spontaneous Whey separation

Figure (1) showed the effect of different concentrations of calcium chloride on spontaneous whey separation of whey protein gels prepared from native and CA modified whey proteins. It is important to observe that the two samples behaved in diametrically opposed ways . At low calcium concentrations (20-30 mM) the amount of whey separated from gel prepared from native whey proteins was the highest , and this value decreased to 1.2 % at 40 mM calcium. This is explained by the fact that the negative charges on the protein surface are neutralized by positive calcium ions, which reduces intermolecular repulsion and permits molecules to assemble into a more cohesive network [16] Additionally, by creating bridges between protein chains, these ions may improve the cohesiveness of the network and its ability to retain water [17],[18]. This outcome is in good agreement with existing fact about salts' capacity to enhance the rheological characteristics of protein gels [19].

The outcomes for the citric acid-modified protein, on the other hand, were very different, with the amount of whey released was small at low calcium concentration. The structure

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lessen some protein gels' capacity to bind water. In summary, the way whey protein reacts to calcium underwent a qualitative change as a result of the citric acid treatment.

Statistical analysis showed a significant difference ($P<0.05$) in whey separation between native and CA‑modified whey protein gels at 20-30 mM calcium , and their behavior changed in opposite ways as calcium concentration increased.The citric acid treatment created pre‑existing crosslinks within the protein, which helped trap water better and reduced whey release at low calcium levels.

of the protein seems to have been profoundly changed by the chemical treatment with citric acid. By interacting with amino groups, the acid, a tricarboxylic molecule, creates new additional ligands and massive protein polymers, serving as a bridge between protein molecules [7],[8]

As a result, the addition of calcium may result in more protein aggregation that effectively retain water rather than the creation of a regular network [20][21] .

This is in line with the findings of Mohammadian [22], who suggested that pre-adjacent crosslinking could

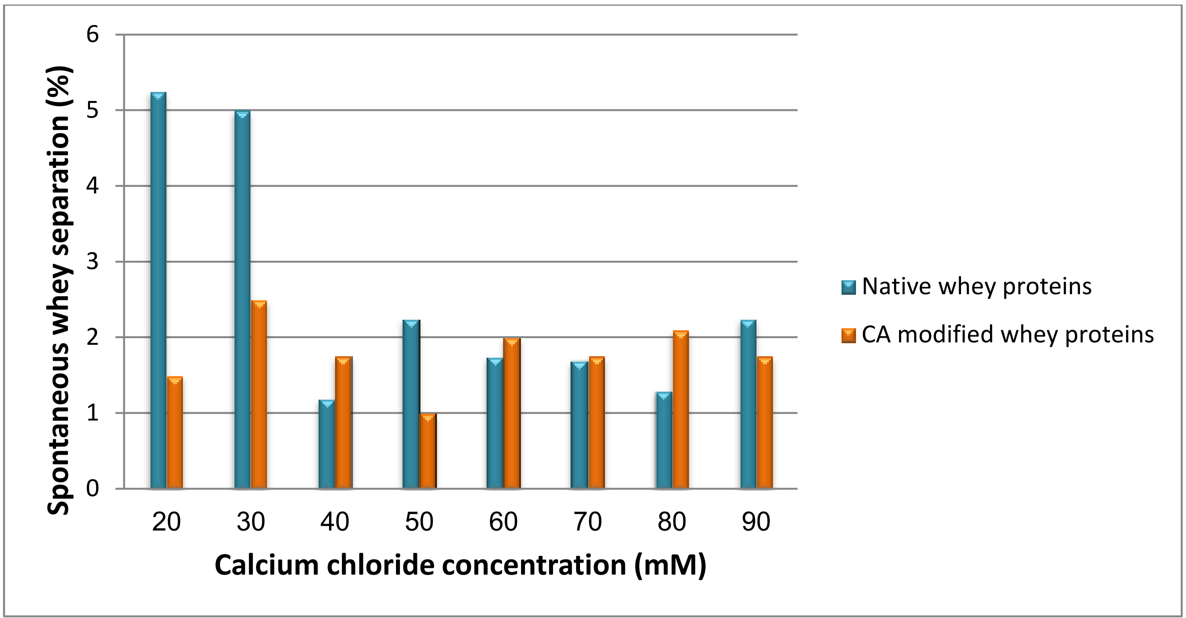

Figure (1):Effect of different concentrations of calcium chloride on spontaneous whey separation of gel prepared native whey proteins and CA modified whey proteins .

3.1.2 Water Holding Capacity

Water holding capacity was determent in both gels prepared from native and CA modified whey proteins using calcium chloride. WHC values in natural whey protein gel vary from 34.0% to 42.0%, gradually decreasing to at least 34.0% at 50 mM and then

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the difference between the two samples (36.0% for the native sample and 59.0% for the modified sample at 40 mM). This could lead to industrial applications that require more stable gel structures [20][22].

A significant difference ($P<0.05$) was found between the two types of gels at all calcium concentrations in this study. The modified protein had much higher WHC (up to 59.0%) compared to the native one (36.0%) at 40 mM calcium .The Cross-linking with citric acid made the gel network tighter and more cohesive, so it could hold more water inside its structure slightly increasing at higher concentrations (figure 2).

On the other hand, CA modified whey protein shows noticeably higher WHC values, ranging from 35.0% to 59.0%, with a rapid increasing trend that peaks at 40 mM at 59.0%. This notable improvement reflects the effect of cross-linking with citric acid in increasing the cohesion of the gel network and its water-holding capacity [7],[8].

The chemical modification greatly increases the protein's WHC in the presence of calcium, as evidenced by

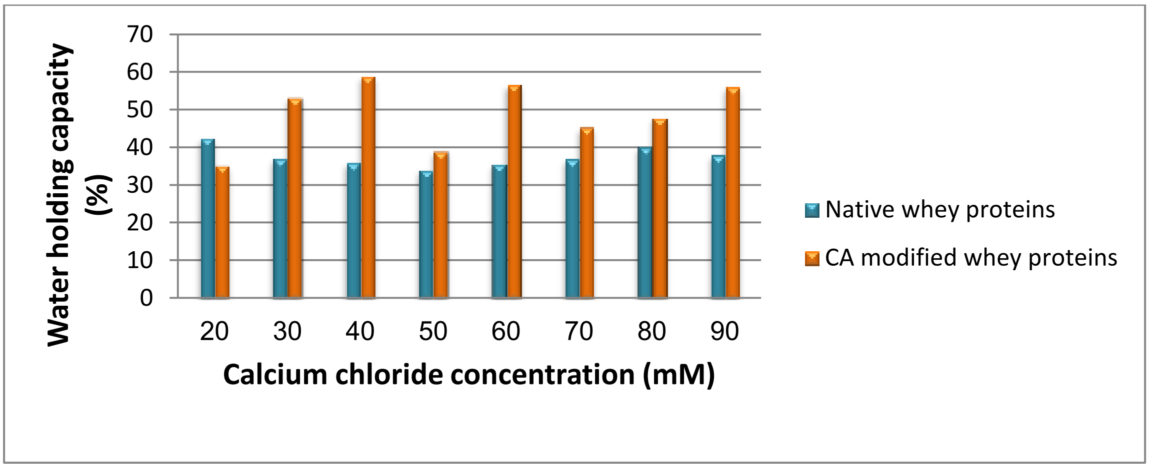

Figure (2): Effect of different concentrations of calcium chloride on water holding capacity of gel prepared native whey proteins and CA modified whey proteins .

3.1.3 Viscosity of protein gel

Changes in viscosity are directly related to changes in the size of the particles.

The viscosity of native whey proteins gels at all calcium concentrations was higher in comparison with CA modified whey proteins gels(figure

In gels, viscosity is a key indicate of particle size. In general, smaller particles make the gel thicker, while larger particles make it thinner. This happens because smaller particles have more surface area and a higher number concentration, which makes the friction between them stronger.

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There was a significant difference ($P<0.05$) in viscosity in all calcium concentration in this study. Native whey protein gels were consistently more viscous than the modified ones across all calcium concentrations. Citric acid modification produced larger protein polymers that had less internal friction, which reduced the gel's resistance to flow.

3).This result can be explained by the fact that citric acid cross-linking results in bigger, more linked protein polymers, which in turn naturally raise flow resistance and internal friction [7],[8].

When comparing the two samples, we find that the modified protein clearly has an advantage in creating a very viscous gel network at the ideal concentrations (particularly 40–60 mM).

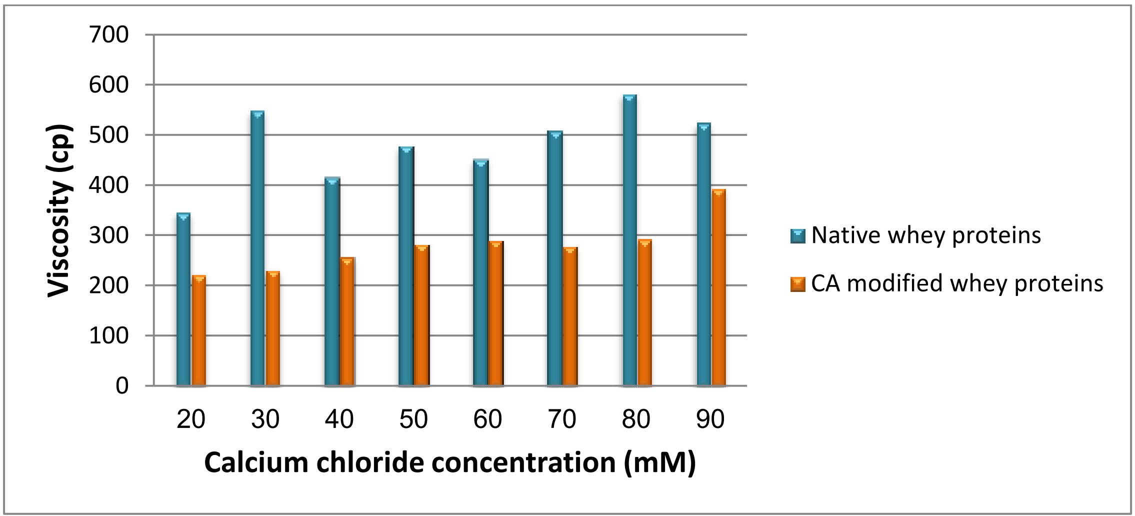

Figure (3): Effect of different concentrations of calcium chloride on viscosity of gel prepared native whey proteins and CA modified whey proteins .

3.1.4 Hardness of protein gel

interconnection density and enhancing mechanical characteristics [17].

[19] proved that adding salts at the optimum amounts improves intermolecular interactions and results in a stronger, more robust network. However, whey protein treated with citric acid behaves differently. Hardness of CA modified whey

Natural whey proteins gel hardness gradually increases with increasing calcium concentration up to 50 mM (figure 4). This is due to the well-known role of calcium ions in strengthening the gel network by neutralizing negative charges and establishing ionic bridges between protein chains, thereby increasing

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Furthermore, pre- crosslinking might make the network more brittle and vulnerable to high salt concentrations, reducing its flexibility and adaptability [21].

This is consistent with [22] discovery that crosslinking can increase the mechanical characteristics of protein gels up to an ideal point before having the opposite impact due to ionic saturation or the production of irregular aggregates.

A significant difference ($P<0.05$) was observed at concentration 20 and 50 mM of calcium. At low levels (≤50 mM), native gels were harder; at high levels (≥60 mM), the modified gels became harder.Calcium ions strengthen the native network at low concentrations, while in modified proteins, pre- crosslinking creates a structure that becomes firmer only at higher calcium levels.

proteins gel at calcium concentration 50 mM and less was lower than the hardness of native whey proteins gels , while at higher calcium concentration (60 mM and higher), the opposite was noticed .

begins at a greater level than typical, then climbs suddenly to a very high peak at 50 mM, before rapidly decreasing as concentration increases[23] found that chemical modification causes structural modifications that improve reactivity with calcium ions at optimum concentrations.

The abrupt decrease in hardness of native whey proteins gel beyond 50 mM can result from the fact that extremely high calcium concentrations can have the reverse effect, resulting in excessive ionic strength and the development of random, irregular protein aggregates that degrade the gel's continuous structure [24].

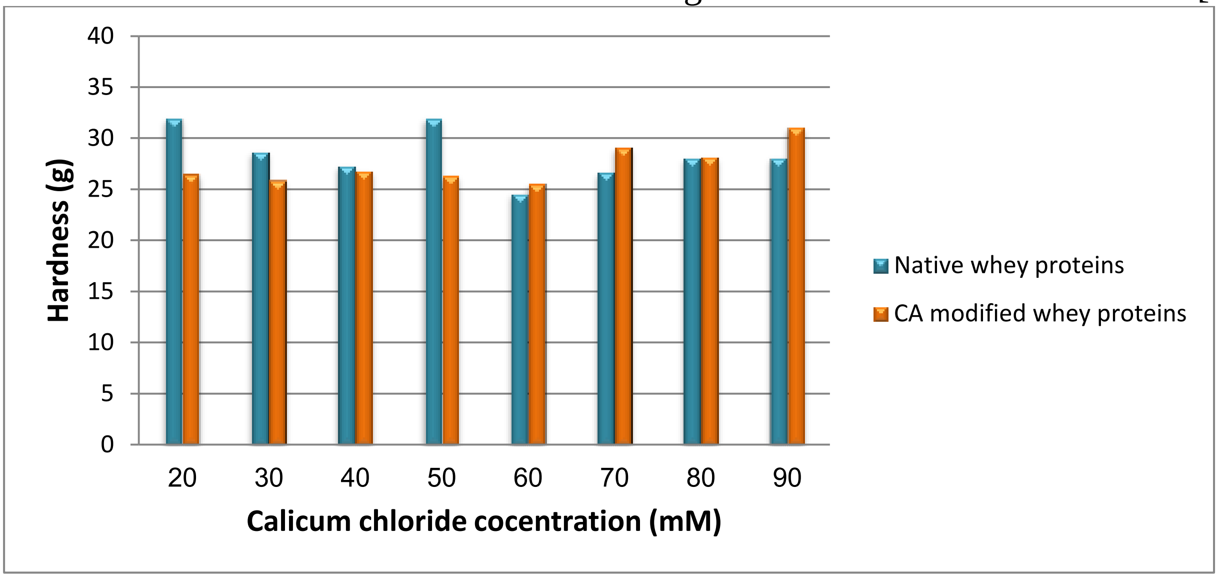

Figure (4):Effect of different concentrations of calcium chloride on hardness of gel prepared native whey proteins and CA modified whey proteins .

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3.1.5 The cohesiveness of protein gel

Cohesiveness of gel determines its internal strength and capacity to maintain integrity, resisting breakage and breakdown of structure under stress. Figure (5) showed that Cohesiveness values in CA modified whey proteins gels were higher in all calcium concentration in comparison with native whey proteins gels.

This rise is due to the fact that cross-linking with citric acid forms more linked and cohesive protein polymers, which naturally increases the strength of internal bonds [7].[8],[23] found that chemical modification has been shown to promote surface affinity and intermolecular contacts, which has a favorable influence on cohesion. Furthermore, too strong surface affinity might cause micro-phase dissociation zones inside the network, disturbing internal connections [24] .The statistical analysis revealed a significant difference ($P<0.05$), with modified protein gels showing higher cohesiveness at every calcium concentration tested.The extra covalent bonds introduced by citric acid treatment increased the internal bond strength and made the gel network more unified.

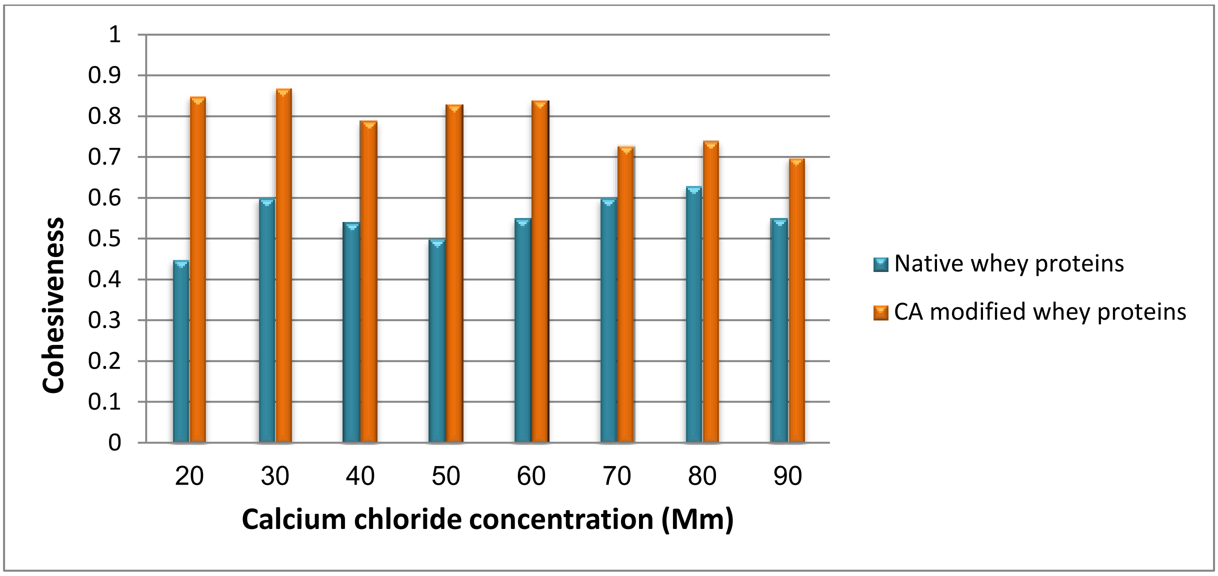

Figure (5): Effect of different concentrations of calcium chloride on cohesiveness of gel prepared native whey proteins and CA modified whey proteins .

3.1.6 Springiness of protein gel

Springiness is a texture property that shows how well gels can return to their original shape and height after being compressed. From figure (6) we noticed that at all calcium concentration, springiness of CA modified whey proteins gel was higher than springiness of native whey proteins gels.

In native whey proteins gel ,calcium ions have an important part in the

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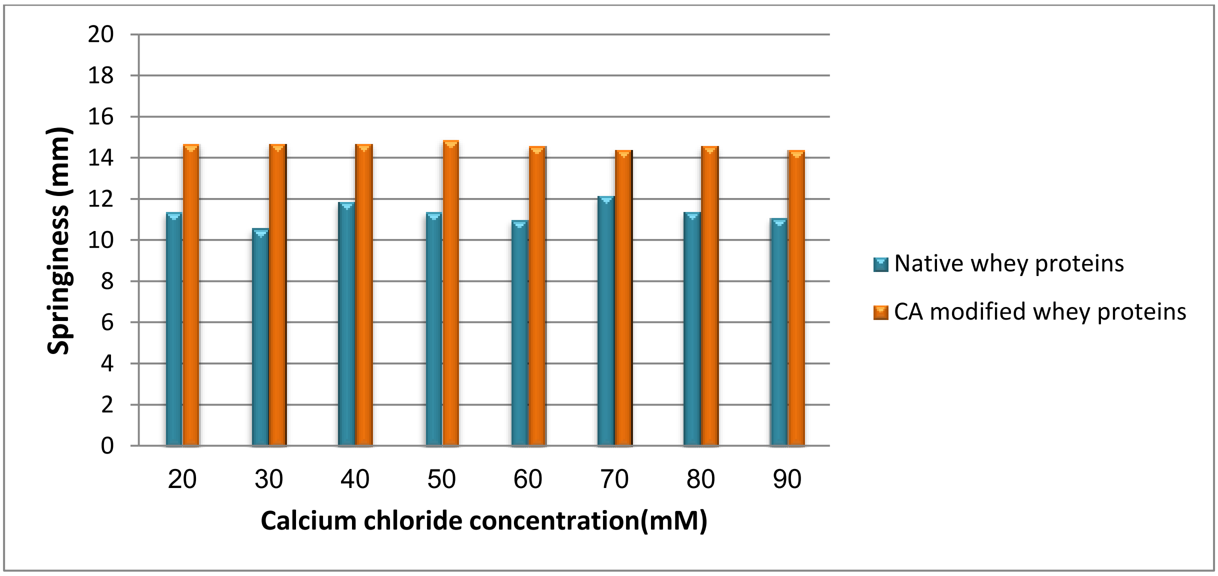
more ordered structure and natural springiness even before calcium is added, due to the new covalent bonds that form a flexible framework [7],[8] . A significant difference ($P<0.05$) was found between springiness values of native and CA modified whey protein gels at all calcium concentrations. Modified whey protein gels were more springy than native ones across all calcium concentrations. Pre‑ crosslinking with citric acid gave the protein a more ordered and naturally elastic structure, even before calcium was added.

formation of a more organized and elastic gel network, which accounts for the continual improvement. Ca^{+2} ions neutralize negative charges and build ionic bridges between protein chains, resulting in a balanced structure that can withstand stress and return to its original condition [17]

However, whey protein treated with citric acid had higher springiness values . The large initial increase in springiness is explained by the fact that crosslinking with citric acid results in protein polymers with a



Figure(6): Effect of different concentrations of calcium chloride on springiness of gel prepared native whey proteins and CA modified whey proteins .

Conclusion

water-holding capacity .These enhancements are explained by the efficient cross-linking of protein molecules with citric acid, which encourages the creation of a more cohesive and stable three-dimensional gel network. The results demonstrate the potential of citric acid as a natural,

This work effectively showed that the rheological properties of whey protein gels generated by calcium ions are much improved by citric acid modification. When compared to native whey protein, the modified whey protein demonstrated better cohesion, hardness, viscosity, and

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safe, and efficient cross-linking agent for enhancing whey protein gelation behavior.

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