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Effect of milk proteins aggregation using Transglutaminase and Maillard reaction on Ca^{2+} milk gel

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Abstract - ***Effect of transglutaminase, Maillard reaction induced crosslinking and combination of transglutaminase and Maillard reaction induced crosslinking between whey proteins and caseins in milk on calcium milk gel properties were investigated. Treatment of milk with transglutaminase, Maillard reaction and transglutaminase + Maillard reaction cause to the appearance of new high MW protein bands. Water holding capacity , gel strength and sensory scores of gel samples increased and spontaneous whey separation decreased in calcium-induced milk gel made from transglutaminase and combination of transglutaminase and Maillard reaction treated milk compare with calcium-induced milk gel made from untreated milk alone.***

Keywords: calcium milk gel, Maillard reaction, transglutaminase, crosslinking

1. INTRODUCTION

Generally there are two major methods for milk coagulation, by adding rennet or using acids [1], Siamand, *et al.* were able of producing milk gel by adding calcium chloride and elevating milk temperature to 85 °C. The best texture and sensory properties was noticed for gels made with the addition of 13.5 mM of calcium chloride [2].
Development of calcium milk gel processing will provide milk industry with another useful product, with high nutritional value and high acceptability which can help to increase dairy product consumption.
Reaction between proteins through formation of covalent bonds leading to the formation high molecular weight aggregations is called crosslinking [3]. Under conditions such as treatments at high temperature, alkaline pH, and enzyme activity, cross-linking can occur. During milk processing, many types of crosslinking can develop in proteins .The first type of cross-linking in milk proteins is interactions via formation of disulfide bonds [4]. Besides, dehydroalanine formation may cause protein association by means of creation of lysinealanine and histidinealanine [5].
Besides that, Maillard reaction has the ability to induce protein aggregation through its advanced products [6].
Many factors can effect Maillard reaction ratio in foods such as temperature, humidity, pH, and nature of the reactants. Temperature and humidity are the most important factors affecting MR occurrence during food processing and storage [7, 8].
Role of induced Maillard reactions in milk on yoghurt was studied and found that yoghurt made from milk with induced Maillard product had better sensory properties and texture in comparison with control yoghurt [9].
Transglutaminase has recently been applied in the food industry. The main application of this enzyme is to improve the functional properties of protein [10]. Transglutaminase induce the reaction between the amino acid glutamine in proteins and various primary amines such as that of lysine.
Many researchers confirmed that Transglutaminase is able to induce crosslinking in food proteins leading to change the known properties of foods like their nutritional, textural and sensory properties [11 12 13].
The purpose of this research aimed to examine the role of Maillard reaction crosslinking and Transglutaminase treatment on physiochemical and sensory properties of calcium–milk gel.

2. MATERIALS AND METHODS

2.1. Materials

Dried milk (Al Mudhish , 26% protein , 28% fat and 27% lactose) produced by Oman Food stuff Factory was purchased from local market . Transglutaminase (microbial) was obtained from Ajinomoto (Japan).

2.2. Modification of milk proteins by Maillard reaction

Milk powder (26% protein, 28% fat and 27% lactose) was subjected to Maillard reaction at 70 °C and 65% relative humidity for 4 hours [14].

2.3. Enzymatic modification of milk

Milk powder and Maillard modified milk powder was dissolved in water (13%) and Transglutaminase was added (50 unit enzyme/g protein) .The solution was hold at 40±1 ^{o} C for 4 hours.

2.4. Milk gels production

Reconstituted milk temperature was elevated to 85 °C and kept at this degree for 20 min, after that milk was cooled to 20 ±2 °C and 13.5mM calcium chloride was added , after that the temperature was raised to 85±1 °C and kept at this degree for 20 min for gel formation . Gel samples were kept at 5-7 °C for 28 days.

2.5. Electrophoresis

Electrophoresis in reducing conditions for samples were done following Laemmli method [15].

2.6. Gel strength

Texture Analyzer (CT3 (4500), Brookfield engineering lab) at 20 ± 2 °C was used for the determination of the strength of milk gels. The instrument parameters were: a cylinder (radius = 1 cm) was press into gel to a depth of 2 cm with 5.0g trigger and speed of 0.1 cm/sec.

2.7. Water holding capacity

Milk gel ability to hold water holding was recorded according to Doleyres*, et al.* [16].

2.8. Spontaneous whey separation

Whey separated internally from gels was collected and measured [17].

2.9. Sensory evaluation

Gels were tested by 8 panelists from technical college of applied sciences. Sensory evaluation degree was divided for the properties: flavor, body, texture, bitterness and color.

2.10. Statistical analysis

The results of all experiments were the mean of 3 replications. Variations among treatments were tested using LSD [18].The level of significant was $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of Transglutaminase and Maillard reaction on milk proteins

Cross-linking in milk proteins can change the characteristics of proteins. Protein polymerisation can improve the texture of yoghurt [19], cheeses and milk proteins films [10, 20].

Electrophoresis for proteins in milk treated with transglutaminase, Maillard reaction and transglutaminase + Maillard reaction are summarized in Fig. 1. SDS-PAGE bring to light that treatment of milk with transglutaminase ,Maillard reaction and transglutaminase + Maillard reaction cause the synthesis of aggregated protein bands. Formation of these aggregates was a result of proteins crosslinking and these bands had higher molecular weights in comparison with native milk proteins. Proteins polymerization bands were clear in milk samples treated with transglutaminase, Maillard reaction and transglutaminase + Maillard reaction, however the density of bands shows that the concentration of crosslinked proteins was lower in Maillard reaction treated milk in comparison with other samples, probably due to the high glutamyl residues in milk proteins and the high content of lysine promote protein polymerization stimulated by TG [21]. In addition, in Maillard reaction treated milk, the formation of advanced MR outcomes may cause synthesis of aggregated protein mixtures [22].

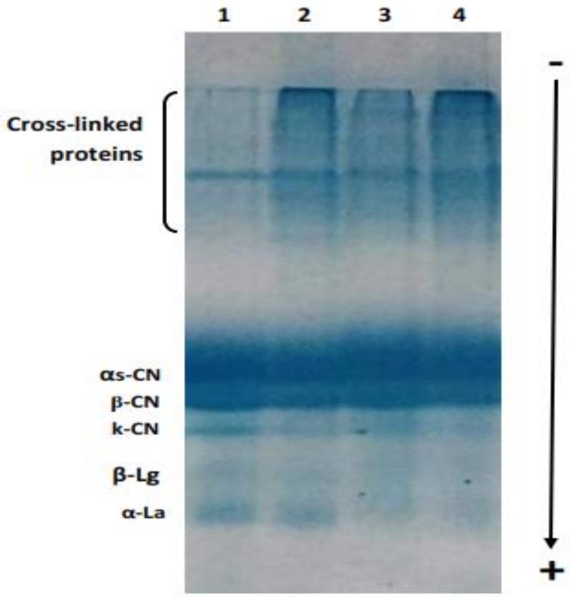

Figure 1 Reducing electrophoresis of milk after treatment with transglutaminase and Maillard reaction, (1) control (2) transglutaminase treated milk (3) Maillard modified milk (4) Maillard and transglutaminase treated milk

The high glutamyl residues in milk proteins and the high content of lysine promote protein polymerization stimulated by TG [21]. In addition, in Maillard reaction treated milk, the formation of advanced MR outcomes may cause synthesis of aggregated protein mixtures [22]. Electrophoresis results showed that crosslinking increased in milk samples treated with transglutaminase, Maillard reaction and transglutaminase + Maillard reaction and this crosslinking reduce the pore size of the protein network, which affect the textural properties of resulting gels [6].

3.2. Effect of Transglutaminase and Maillard reaction on pH of calcium milk gels

Changes in pH values of calcium milk gels during storage are given in Figure (2). pH values of gel samples made from controlled milk , Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk were 6.21 , 5.61 , 6.13 and 6.09 respectively after 1 day of storage at 7 ^{o}C , and during storage for 28 d, the pH of the gels prepared decreased to 5.87 , 5.05 ,5.82 and 5.72 respectively .

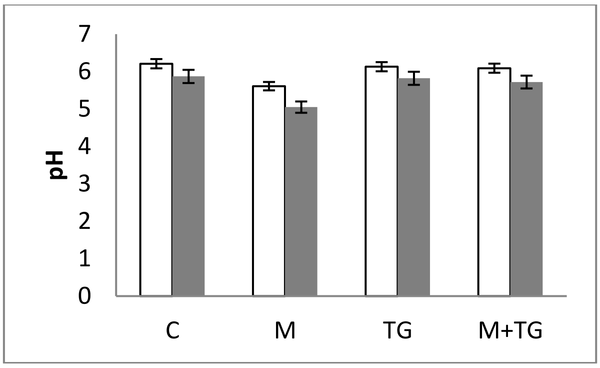

Figure 2: pH of gel samples during storage 1 day ( ) and 28 day ( ). (C) Control, (M) Maillard modified milk, (TG) transglutaminase treated milk and (M+TG) Maillard and transglutaminase treated milk.

The low pH values of gel samples after 1 d of storage is caused by the effect of high temperature .On-Nom, *et al.* [23] found that milk pH decreased from 6.67 to 6.08 after heat treatment at 80°C for 20 minute while Chandrapala, *et al.* [24] observed a drop in milk pH from 6.55 to 6.09 after milk heating at 90°C for 10 minute. Beside that the higher decrease in pH of Maillard reactions gel samples is caused by the activity of Maillard reactions in formation acids like formic acid (6). The decreases in pH of calcium milk gels during cold storage may result from Ca^{+2} role in reducing negative charges of caseins [25].

3.3. Spontaneous whey separation of calcium milk gels

Spontaneous whey separation was determined by measuring the volume of whey separation on the top of the samples. Spontaneous whey separation (%) of milk gel samples made from control, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk were 1, 12, 0.5 and 0.4 ml respectively after 1 day of storage at 7 °C .These values changed during storage at 7 °C until they reach to 0.5, 10, 0.5 and 0.5ml respectively, after 28 day of storage (Figure 3).

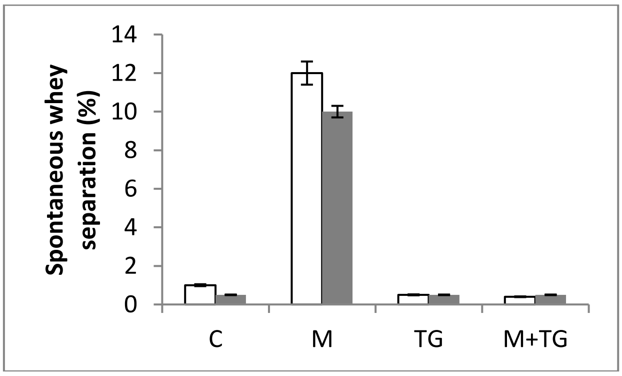

Figure 3 Spontaneous whey separation (%) of gel samples, 1 day ( ) and 28 day ( ). (C) Control, (M) Maillard modified milk, (TG) transglutaminase treated milk and (M+TG) Maillard and transglutaminase treated milk

The high spontaneous whey separation ($P < 0.05$) of calcium-induced milk gel sample made from Maillard modified milk is due to the increase in its acidity which cause to form an open texture gel, leading to increase spontaneous whey separation and decrease WHC [26].

3.4. Water holding capacity of calcium milk gels

WHC has been defined as the amount (g) of water held per 100 g of protein. The quantity of moisture kept by a protein depends upon its primary structure (especially the number of hydrophilic amino acids), conformation of proteins, surface properties and processing history [27].

The WHC of the calcium–milk gels made with control, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk were 21, 12, 38.5and 38%, respectively in the first day of storage (Figure. 4).

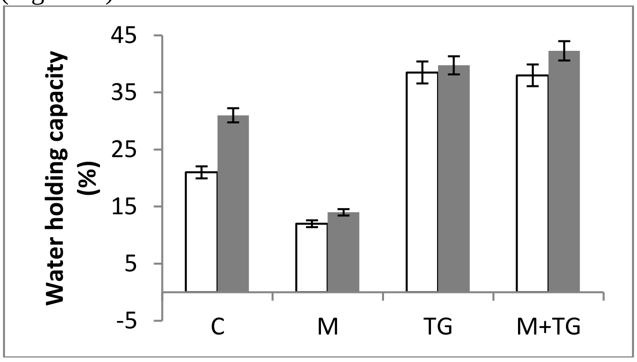

Figure 4: Water holding capacity (%) of gel samples.1 day ( ) and 28 day ( ). (C) Control, (M) Maillard modified milk, (TG) transglutaminase treated milk and (M+TG) Maillard and transglutaminase treated milk.

These results confirm the capability of calcium–milk gels to hold liquids and this binding increased with using transglutaminase in preparation.

The WHC of the gels increased with storage time for all treatments used. After 28 days at 7°C, the WHC of calcium–milk gels made with control , Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk were 31, 14, 39.7and 42.2%, respectively. This enhancement in WHC during storage is related to the effect of calcium in increasing the quantity and intensity of bonds between milk proteins [28].Besides that, the higher WHC in calcium–milk gels made with Maillard and transglutaminase treated milk is related to the function of MR in induce interactions between milk proteins which make calcium–milk gel proteins network stronger and have more ability to keep water [9].

3.5. Gel strength of calcium milk gels

Determination of gel strength is an indicator to the intensity of gel structure [29].

Gel strength (Pa), is shown in Figure 5. The gel strength of calcium–milk gels made with control, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk were 733, 1110, 1125 and 1090 Pa respectively after 1 day of storage at 7°C. These results indicate that gel strength increased with using Maillard reaction and transglutaminase for preparation of the gels; this increase may be related to the effect of these treatments in increasing crosslinking between milk proteins [10 30].

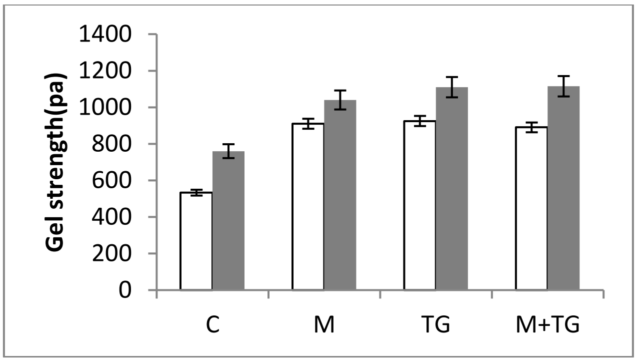

Figure 5: Gel strength (pa) of gel samples 1 day ( ) and 28 day (.) (C) Control, (M) Maillard modified milk, (TG) transglutaminase treated milk and (M+TG) Maillard and transglutaminase treated milk.

Gel strength increased gradually during storage at 7°C for all treatments used in this study. After 28 days of storage, gel strength of the gels manufactured with control, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk reached 960, 1240, 1310 and 1315 Pa, respectively. These results indicate that Ca^{+2} role on formation of cross-linked milk proteins continues during storage ,and this result from the fact that the positive charges of Ca^{+2} can neutralize casein negative charges and form bridges between casein micelles[25].

Sensory properties of calcium–milk gels

The sensory results of gel samples prepared from control, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk during storage at 7 °C for 28 days is summarized in Figure 6.

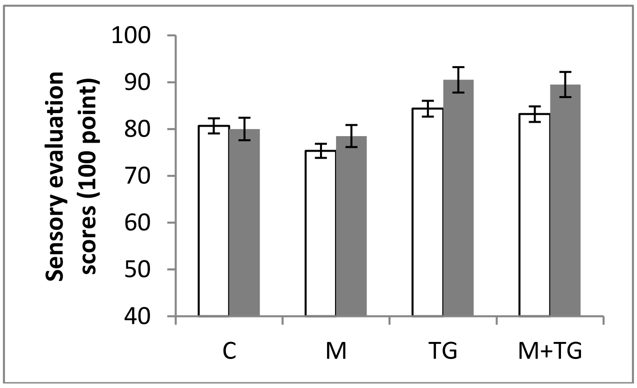

Figure 6: Sensory evaluation scores (100) of gel samples. 1 day ( ) and 28 day ( ). (C) Control, (M) Maillard modified milk, (TG) transglutaminase treated milk and (M+TG) Maillard and transglutaminase treated milk.

Sensory studies showed that the total scores after 1 day of storage at 7 °C were 80.6 ,75.3 ,84.3 and 83.1 for gels control , Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk respectively and these values changed to 80 , 78.5 ,90.5 and 89.5 at the end of storage period at 7 °C respectively .

Among all calcium–milk gels prepared in this study, the gel prepared from Maillard modified milk got the lowest sensory scores by the panelists and this is related to separation of whey on the upper layer of gel.

The increment in sensory evaluation score during storage is due to the effect of added calcium and Millard reaction in induce cross linking of milk proteins which increase the bonding between milk proteins and make milk gel proteins network stronger [28 30].A similar result was obtained by [2] with calcium–milk gel prepared using 13.5 mM calcium chloride .

4. Conclusions

Gels were made from control milk, Maillard modified milk, transglutaminase treated milk and Maillard and transglutaminase treated milk using high temperature treatment and Ca^{+2} .

Treatment of milk with transglutaminase and Maillard + transglutaminase increase the cross-linking between milk proteins and this improve gel strength, WHC and sensory evaluation score of the gels.

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