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Monitoring the chemical and textural changes during ripening of Iranian white cheese made with different concentration of glucono delta lactone

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Abstract. The effect of the concentration of glucono delta lactone added to milk on the composition and rheological properties of Iranian white cheese (IWC) was studied during 60 days of ripening in brine. Four treatments of cheese were made using (0, 5, 10, 15 g/7.5 kg milk) concentrations of GDL. As ripening progressed by increasing GDL concentrations moisture content increased whereas their fat, total ash, protein contents decreased. As the concentration of GDL added to milk increased, the value of fracture stress at a given ripening time significantly decreased, leading to a less resistant body against applied stress. The minimum in G' may be consequence of partial loosening of the weak initial gel network due to solubilization of colloidal calcium phosphate or some other physicochemical change in the nature of casein particles.

Key Words: Milk acidification, rheology dynamic, casein, colloidal calcium phosphate, acidifying agent.

Introduction. The initial steps of cheese making involve the coagulation of CN micelles via three possible methods: limited proteolysis (using rennet or other coagulants), acidification (starter culture or addition of acids) and heat (Lucey et al 2003). Acidified milk gels are one of the oldest and the most popular foodstuffs produced throughout the entire world. The popularity of fermented milk products such as fresh acid-coagulated cheese verities is due to various health claims and curative benefits. Irrespective of the commercial importance of these acidified milk products, there is not much information regarding formation, structures and physic-chemical properties of acid-coagulated milk products (Lucey et al 2003). The acidification of milk can be done by using glucono delta lactone (Lucey et al 2003; 1998). The rate of acidification is different between milk acidified with GDL and bacterial cultures; GDL is rapidly hydrolyzed to gluconic acid (especially at high temperatures) whereas after the addition of starter bacteria the pH doesn't usually change very much initially. The final pH that is attained in GDL induced gels is a function of the amount initially added to milk, whereas starter bacteria can continue to produce acid until a very low pH is attained (Lucey et al 1998). As GDL content in cheese increased, storage modulus (G') increased. The objectives of the present paper were to study: 1) the chemical and textural changes that occur in IWC during ripening and 2) investigate the effect of the different concentrations of GDL added to cheese milk on these attributes.

Material and Method

Treatments, cultures, and rennet. Four treatments of cheese were made as follows: cheeses made with 0 g of GDL/7.5kg of milk (C1), cheese made with 5 g of GDL/7.5 kg of milk (C2), cheese made with 10 g of GDL/7.5kg of milk (C3), and cheese made with 15 g of GDL/7.5 kg of milk (C4). Cheese was manufactured in triplicates, each replicate in 1 day using 7.5 kg of milk for each treatment. The GDL (Merck Darmstadt, Germany) which decomposes slowly in aqueous media was used as acidifying agent instead of adding starter bacteria. As coagulant chymosin derived by fermentation of *Aspergillus niger* var. awamori (Standard rennet, Chy-Max, Chr, Hansen Inc., Denmark: 183 International Milk Clotting Units (IMCU)/ml^3) was used at a concentration of 4.5 IMCU/ kg of milk. Rennet was diluted 30-fold with cold water then added to each 7.5 kg batch of milk.

Cheese-making procedure. Fresh raw milk obtained from Animal Husbandry of Urmia University, was batch-pasteurized at 85 ^oC for 30 min (IDF 1997) this time included that required for milk to come up to temperature, normally 2 - 3 min. After heating, milk rapidly cooled to 30 ^oC by immersion in ice water (Lucey et al 2000) then transported carefully to a cheese vat (FT20-MkII cheesse vat, Arm field Ltd., Ringwood, Hampshire, UK). The milk was supplemented with 0.15 g of $CaCl_2$/1 kg of milk and held at 35 ^oC for approximately 45 min after adding of GDL and rennet simultaneously. The curd was cut crossways in cubes of 2 cm^3 when firm (approximately after 45 min). After being cut, the curd was allowed to settle for 3 – 5 min and then gently agitated at a gradually increasing rate for 10 min to avoid fusion of freshly cut curd cubes and facilitate whey expulsion. This was followed by whey draining and pressing the transferred curd into molds (25) initial pressure of 0.3 kPa which gradually increased up to approximately 2.9 kPa at the first hour and held constant to the end of pressing to complete draining. After pressing, the curd was divided into six equal portions which were stored at 23 – 25 ^oC for 19 – 20 h, placed in airtight plastic containers, and covered with 15 % (w/v) brine (brine was beforehand pasteurized at 80 ^oC for 10 min and filtered through a clean cloth after rapid cooling) in a random order. After sealing, the containers were stored first at 23 – 25 ^oC for 24 h and then refrigerated at 5 – 6 ^oC for the ripening period of 8 weeks.

Chemical analysis. Cheese was analyzed for moisture content by vacuum-oven (method number 926.08), and for ash content by dry ash method (AOAC 1997). The fat content of milk and cheese samples was determined by the Gerber method and their total protein contents were determined by measuring total nitrogen using the Kjeldahl method (AOAC 1997) and converting it to protein content by multiplying, by 6.38. All chemical measurements were done in triplicate. Cheese samples were chemically analyzed at the first, forth, eighth week of ripening.

Rheological analysis

Uniaxial compression. The simplest fundamental test, uniaxial compression was performed at the 8th week of ripening using a HTE Universal Testing Machine (S-Series Bench U.T.M. Model H5K-S, Hounsfield Test Equipment Ltd. was followed except that cheese blocks were cut into 24 mm in diameter and UK). The method of Tunick with modifications described by Madadlou et al (2005) was followed except that cheese blocks were cut into 24 mm in diameter and 16 mm in height cylinders and samples were compressed uniaxially with 62.5 % deformation (test end point 10 mm) from the initial height of the sample in one bite. 16 mm in height cylinders and samples were compressed uniaxially with 62.5 % deformation (test end point 10 mm) from the initial height of the sample in one bite.

Dynamic rheological measurements. Small amplitude oscillatory shear measurements were performed with a Universal Dynamic Spectrometer, Paar Physica UDS 200 rheometer (Physica Messtechnik GmbH, D-70567, Stuttgart, Germany). Samples were cut at least 1 cm deep of the cheese blocks at 6 ^oC. These samples were immediately placed in small airtight plastic containers and equilibrated at room temperature (23 ± 1 ^oC)

for at least 4 h. The measuring geometry consisted of 2 parallel plates with a diameter of 25 mm and 1 mm gap size (sample thickness). A small piece of cheese was placed on the lower plate then the upper plate was slowly lowered until the gap size obtained. Excessive cheese was trimmed of carefully with a razor blade and the sample allowed resting for 10 min in the rheometer to allow stresses induced during sample handling to relax. A strain in the linear region (0.1 %) was then selected and a frequency sweep performed as the frequency varying from 0.1 to 10 Hz (Madadlou et al 2005). Mathematical models that describe relationships between structural parameters and deformation outside the linear range have not been well developed. The parameter calculated was: G', the storage modulus which is a measure of elastic nature. Values are the averages of two measurements for three replicates of each cheese. All rheological measurements were performed at the first, fourth, and eighth week of ripening.

Statistical analysis. The experiment was replicated three times in a complete randomized design, which incorporated the three treatments. The analysis of variance (ANOVA) was carried out using the PROC general linear model (GLM) procedure of SAS statistical software package (Version 8.2, SAS Institute, Inc., Cary, NC) to determine the effects of treatment for all variables and find the differences between data means at 5 % significance level.

Results and Discussion

High heat treatment of milk causes the increase of whey protein denaturation level. Increased level of whey protein denaturation results higher moisture of cheese. The product also show poorer curd fusion and lower fracture values during ripening (Singh 1995).

As expected, increasing the concentration of GDL added to milk significantly increased the moisture content. The moisture content of treatments during ripening was increased, that probably due to: 1. proteolysis of cheese (Kaya 2002), 2. amount of chymosin (Prieto et al 2004) and 3. GDL.

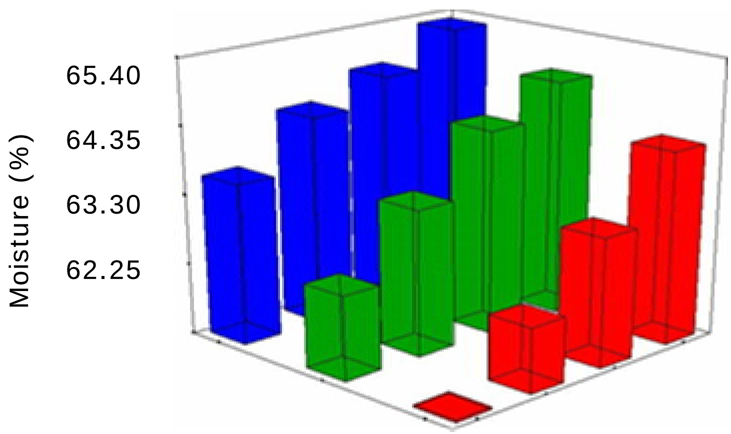

Figure 1. Effect of GDL on the percentage of moisture during the storage of Iranian white cheese.

Heat treatment of milk at a temperature above 70 ºC causes denaturation of whey proteins, some of which associate with casein micelles, involving κ-CN, via hydrophobic interactions and intermolecular disulphide bonds (Singh & Creamer 1992). So the sever heat treatment of milk prevent whey separation as is commonly believed (McKenzie 1971). Mcmahon & Oberg (1999) determined the changes of water during storage and related these changes to cheese microstructure and functionality. The expansion of the protein matrix occurred over the same time span as the decrease in expressible water and indicated that the protein matrix is adsorbing water originally located in fat-serum channels. Also by increasing GDL concentration the moisture content of treatment was increased, this may be due to hydrophilic properties of GDL (Figure 1).

As ripening progressed, protein, fat, total ash contents of the treatments continuously decreased. Madadlou et al (2005) related the decrease in protein content of Turkish white cheese during ripening to diffusion of some proteolysis products from the curd into the brine. Ehsani et al (1999) reported that the amount of total N and NPN in the brine was increased during ripening of IWC. Robinson & Wilbey (1998) reported that about 6 % of the coagulant add to milk is active in the cheese curd and most of them were expelled by whey during manufacture and at cutting stage (Fox 1989) while the percentage of entrapped water increased, so the amount of rennet was increased as result proteolysis by increasing moisture was increased (Figure 2).

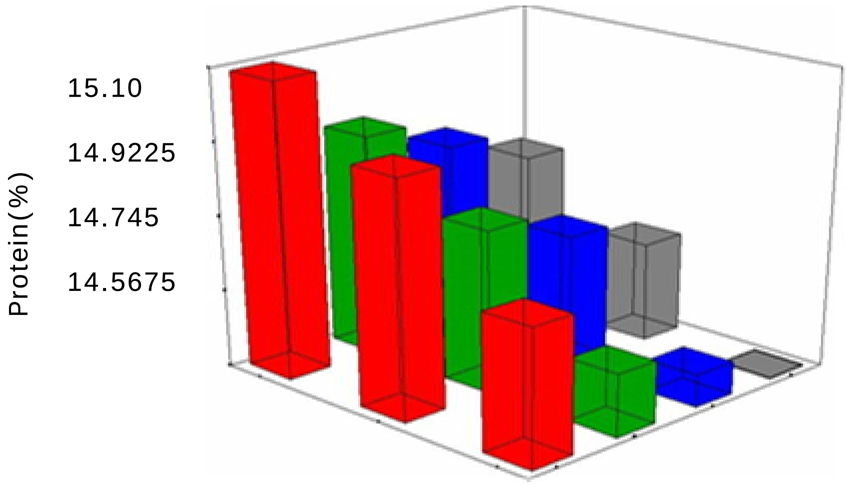

Figure 2. Effect of GDL on the percentage of protein during the storage of Iranian white cheese.

The value of pH at renneting significantly influenced the chemical characteristics of the treatment. As the acidification rate enhanced by increasing the concentration of added GDL to milk, the ash content of the treatments decreased. The decrease in the ash content was due to the solubilization of colloidal calcium phosphate, magnesium and citrate ions (Le Graet & Gaucheron 1999) (Figure 3).

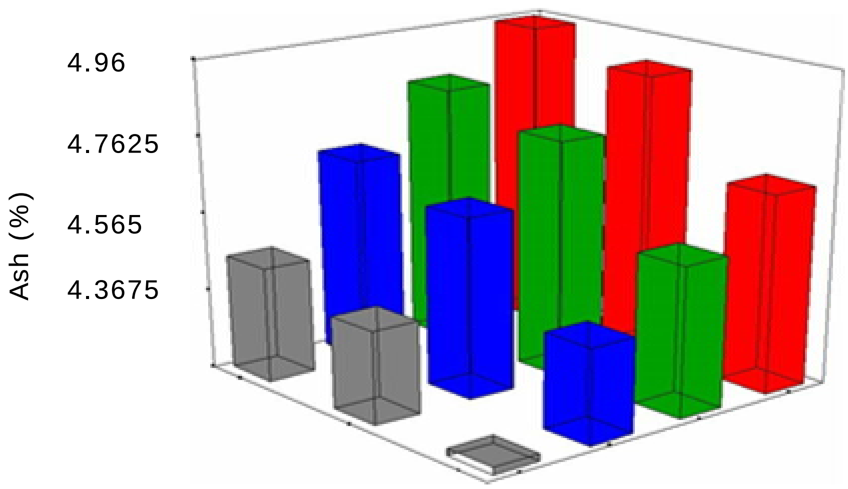

Figure 3. Effect of GDL on the percentage of total ash during the storage of Iranian White cheese.

The decrease in fat content of IWC during ripening to diffusion of some lypolysis products from the curd into the brine and increase in GDL concentrations have no effect on fat content (Figure 4).

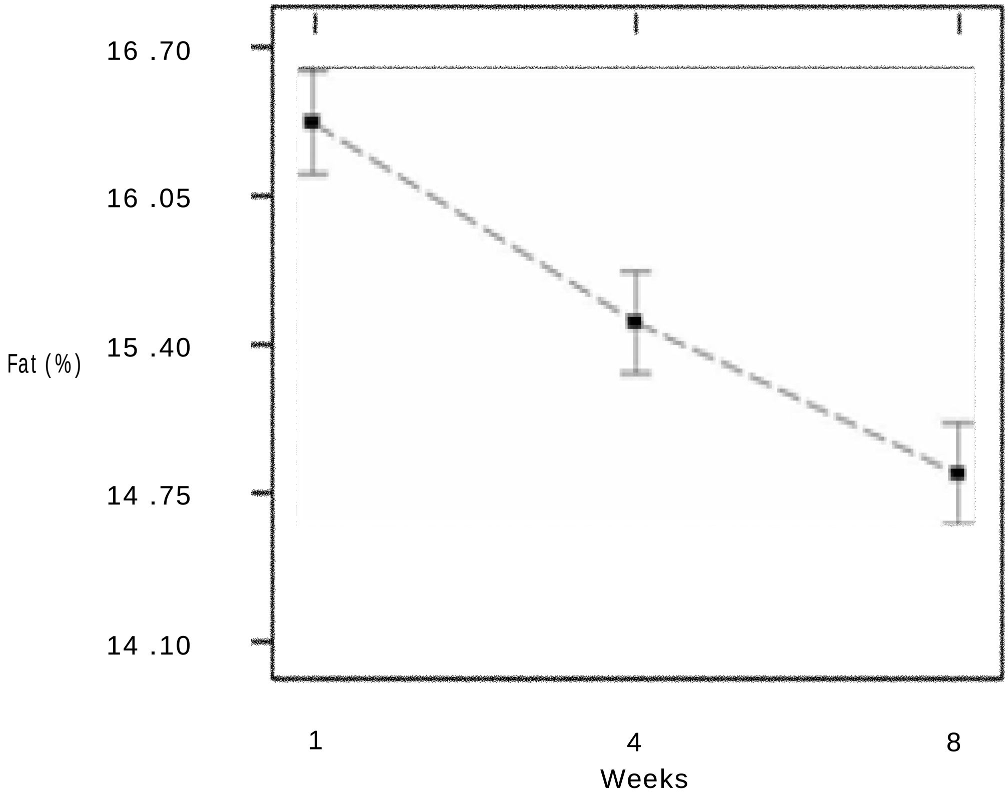

Figure 4. Effect of GDL on the percentage of fat during the storage of Iranian white cheese.

Rheological analysis. The uniaxial compression parameters are shown in figure 5. The longer the ripening period, lower were the values of stress and Hencky strain at fracture for a given treatment. Fat and moisture act as the filler in the casein matrix of cheese texture (Madadlou et al 2005), giving it lubricity and softness. The casein matrix provides the elastic character to cheese texture (Khosrowshahi et al 2006). The decrease in the volume fraction of force-bearing component in cheese microstructure during ripening could account for most of the reduced firmness. It is noteworthy that diffusion of salt into cheese during aging changes the strength of the interactions between protein molecules by screening of charged groups. This reduces electrostatic repulsions between protein molecules but also lessens the number of plus-minus interactions between protein molecules (Lucey et al 2003). The domination of the latter effect likely weakened the structural bonds of the protein matrix of cheese, leading to less resistance against applies stress. According to Kaya (2002), brines with less than 15 % salt could cause weakening of cheese structure. In addition, the slow solubilization of colloidal calcium phosphate undoubtedly had a part in softening the treatments during aging (Lucey et al 2003).

Increasing the concentration of GDL added to milk also decreased cheese fracture stress and made the cheese body weaker. The decrease in fracture stress clearly coincided with the decrease in surface area occupied by the protein fraction in cheese microstructure.

The reduction in the amount of calcium associated with casein molecules would, however, increase electrostatic repulsion between caseins (Lucey et al 2003) and cause a weakening of the structural bonds. This probably took a part in the decrease in value of stress at fracture as the pH at renneting decreased (Figure 5).

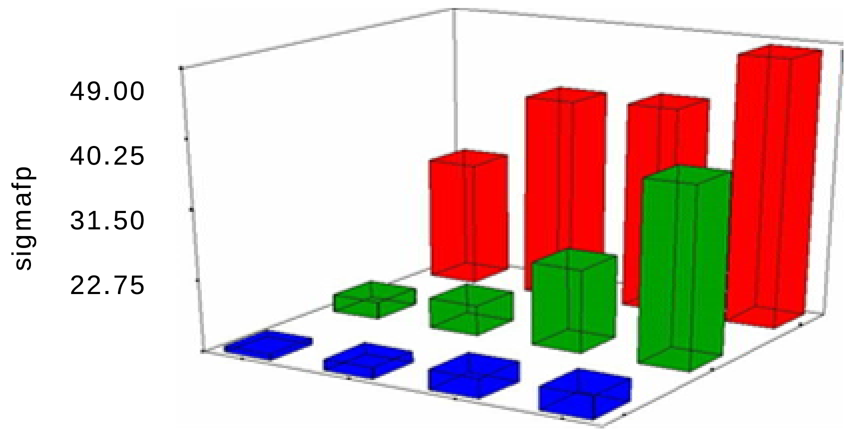

Figure 5. Effect of GDL on the fracture stress during the storage of Iranian white cheese.

Dynamic rheological measurements. Frequency sweep tests were used to determine whether ripening time and increasing the GDL concentration influences the textural characteristics of the cheese. The comparison of G' of treatments is seen in figure 6.

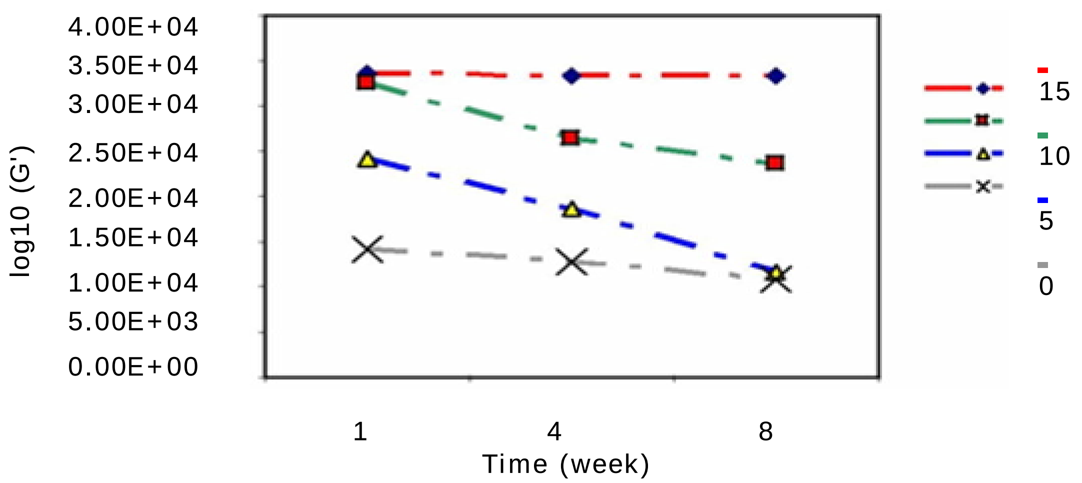

Figure 6. Effect of GDL on the storage modulus (G') during the storage of Iranian White cheese. Grey, blue, green and red marks represent Iranian white cheese made with 0, 5, 10, 15 concentrations of GDL.

Both storage and loss moduli of all treatments were dependent of frequency and demonstrated similar shapes and trends. In acid milk gels the value of G' is always substantially higher than that of G'' for all frequencies (Lucey et al 1998), which indicates a dominant contribution of the elastic component to the viscoelasticity (Lucey & Singh 1997), reflecting the typical behavior for a solid viscoelastic material (Mohsenin 1986). Both storage and loss moduli of 4 cheese were dependent on frequency, reflecting relaxation of more bonds when the timescale of applied stress is longer (Mohsenin 1986). During the acidification of milk many of the physicochemical properties of casein micelles undergo considerable change, especially in the pH range 5.5 - 5.0, including a voluminosity maximum, dissociation of the caseins, solubilization of colloidal calcium phosphate (Lucey et al 2001). An increase in the GDL concentration resulted in a increase in G' because on acidification, casein particles aggregate as a result of charge neutralization, leading to the formation of chains and clusters that are linked together to form a three-dimensional network. Considerable changes occur in casein micelles during acidification such that the casein particles that form a gel at pH ~4.8 do not contain any ccp and have a different character to native casein micelles, as discussed earlier (Lucey et al 1998) (results not shown). The G' value decreased by ripening time may be a consequence of partial loosening of the weak initial gel network due to solubilization of colloidal calcium phosphate or some other physicochemical change in the nature of the casein particles (Lucey et al 1998). Breakdown products of casein are water-soluble and cannot contribute to the framework provided by the protein matrix; therefore, the cheese becomes softer. When protein matrix is breakdown the texture of cheese become softer during ripening (Figure 6).

Conclusions. The concentration of GDL added to milk caused variation in the value of pH at renneting, which in turn affected most of the chemical characteristics of IWC. The study indicated that texture (rheology) and chemical and biochemical composition of IWC changed markedly during 60 days of aging in brine at 5 ºC. The decrease in cheese firmness due to ripening progress and increased concentration of GDL added to milk.
Practical application. In this research a high temperature has been applied which causes an increase in the output efficiency and in the weight of the cheese because of having a higher amount of water in the curd and the addition of w.p. and casein into it which in turn, enhances the nutritional value of the cheese. Also, glucono delta lactone is used as the acidic agent which reduces the time of production and makes a product with a fixed quality. It also increases the efficiency and improves the parameters of the process. It also decreases the synersis and increases the shelf time through preventing the growth of M.O. because of reducing the pH. Because wetness is high in this cheese, it is suitable for hard kinds of cheese like mozzarella and pizza cheese since it enjoys a proper flexibility.

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