

**RIPENING ACCELERATION AND QUALITY IMPROVEMENT OF GOUDA  
 CHEESE WITH ADDING MILK SOMATIC CELLS  
 BY**

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**ABSTRACT**

*The* effect of adding proteolytic enzymes associated with somatic cells (SC) from healthy cows milk on Gouda cheese ripening and quality was studied. Gouda cheese treatments were made from normal mixed milk (cows and buffaloes, 1:1) to which SC was added at levels of  $\sim 2, 4$  and  $6 \times 10^5$  cells  $\text{ml}^{-1}$  (T2, T3 and T4, respectively), before pasteurization, compared with control without SC addition (T1). Coagulation time, yield, protein & fat recovery and chemical composition analysis of Gouda cheese treatments were slightly affected by addition of SC. A highly significant ( $p < 0.01$ ) increase were observed for proteolysis and lipolysis during ripening by adding SC as indicated by ripening indices (SN, SN/TN, soluble tyrosine & tryptophane and TVFA). Firmness values indicated that all cheese treatments with SC showed more desirable texture along ripening ( $p < 0.05$ ). Also, SDS-polyacrylamide gel electrophoretic patterns and sensory quality confirmed that proteolytic enzymes of SC were contributed directly to proteolysis of protein in cheese treatments (T4 followed by T3), which ripened within 60 days and enhanced the cheese quality.

**INTRODUCTION**

Gouda cheese is a semi-hard cheese with few eye-holes which should contain not less than 48% fat content in the dry matter and not more than 43% moisture content (Codex, 1966), it has been widely spread in many countries (Scott, 1998). Recently, this type of cheese has been increased in Egyptian local market. Nowadays, cheese making involves high capital investment, the running costs and interest charges involved in cheese storage represent a significant proportion of the total costs. The economic advantage of rapid development of more intense cheese flavour in shorter periods would be substantial. Maturation of cheese is a complex process involving the breakdown of protein and to a lesser extent of milk fat. Furthermore, impending legislation may impose mandatory pasteurization in some countries or heat treatments of milk to incorporate the whey proteins into cheese, with the consequence of slowing down the process of maturation. Thus, there is a need to identify an accelerated ripening process capable of producing a cheese with

characteristics equivalent to standard matured cheese (Trepanier *et al.*, 1992). Development of the flavours characteristics can occur partially, by bacteria and enzymes through different metabolic pathways, as well as, changes in the texture and body of the cheese matrix (Kamaly *et al.*, 1989). Adding proteolytic enzymes to cheese is an effective way to accelerate cheese ripening.

Normally, milk contains different amounts of SC, but in case of bacterial infection or other inflammation processes of mammary tissues (*i.e.* mastitis, stage of lactation, season, milk yield, and number of lactations), the number of SC in milk dramatically increases (Albenzio *et al.*, 2002 and Jaeggi *et al.*, 2003). This increase in SC count results from transfer of white blood cells from the blood into the milk (Pirisi *et al.*, 2000). Bastian *et al.* (1991) suggested that the flow of enzymes from blood into milk increases in early lactation.

The SC contain a wide range of hydrolytic enzymes that includes both acids (cathepsins D and B) and neutral (elastase and cathepsin G) proteases (Azzara and Dimick, 1985). The acid proteinase, cathepsin, is probably the lysosomal proteinase in milk (Hurley, 2000). SC populations in milk consist of polymorphonuclear leukocytes, macrophages and lymphocytes (Burvenich *et al.* 1995). Plasmin (alkaline milk proteinase and heat stable protease) plays the main role among the proteolytic enzymes in milk. Plasmin is normally found in milk and is found in larger quantities in high-SC count milk. On the other hand, the increased SC count is correlated with increased amounts of lipase (lipoprotein lipase) in milk, (Barbano *et al.* 2006).

Pasteurization reduce but did not eliminate the enzymatic activity associated with the SC (Marino *et al.*, 2005).

Munro, *et al.*, (1984) and Barbano *et al.* (1991) demonstrated that cheeses made from mastitic milk, generally having poor quality when mature than those made from normal milk, apparently due to an alteration in milk protein composition and mineral balance.

Therefore, the objectives of this study were to examine the proteolytic activity of SC from healthy cow's milk, determine the effect of SC added to normal raw mixed milk on ripening acceleration and quality of Gouda cheese.

## MATERIALS AND METHODS

### 1. Manufacture of Gouda cheese:

Gouda cheese was made according to the method of Scott (1998), using standardized fresh mixed cow's and buffalo's milk (1:1) 3.5 % fat; using commercial of mesophilic aromatic starter culture, type LD. Multiple mixed strain culture containing *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *diacetylactis*, (Chr. Hansen's Lab., Denmark). The starter was added according to manufacturer instructions. Resultant cheese treatments were then kept for ripening (at 10-12°C and 85-95% relative humidity) for 3 months and analyzed when fresh and after 1, 2 and 3 months of ripening period.

[Mixed milk composition was 87.44 % moisture, 3.5 % fat, 3.2 % protein, 0.16 % NaCl, 0.14 % Titratable acidity (TA) and 6.42 pH value].

### 2. Collection of somatic cells:

SC were collected by centrifugation (1000 x g 20 min/4°C, using Harrier 18/80 refrigerated, UK) of healthy cows milk. The SC were collected at the beginning of milking process (obtained from the herd of the Fac. of Agric. and Fac. of Veterinary medicine at Moshtohor, Benha Univ.), and the resultant pellets (*i.e.*, SC) were resuspended in buffere phosphate saline (BPS) at pH 6.8, as described

by Verdi and Barbano (1991). The count of SC in stock, after dilution in BPS, was determined using a Fossomatic 5000 equipment (Type 713, Operators Manual, Food First in Food analysis, 69 Stangerupgade, DK 3400 Hillered) and was found to be  $\sim 6 \times 10^7$  cells  $\text{ml}^{-1}$ . The stock of SC was kept freezed at -5°C until using, in order to disrupt cells and release intracellular enzymes.

### 3. Experimental design:

Four treatments (20 kg each) were performed by using normal raw mixed milk (cow's and buffalo's milk, 1:1) obtained from the herds of Faculty of Agriculture, Moshtohor, Benha University. The stock of SC was added to three batches of milk at levels equivalent to  $\sim 2, 4$  and  $6 \times 10^5$  cell  $\text{ml}^{-1}$  (T2, T3 and T4, respectively). The control cheese was made without adding SC (T1). Equivalent amount of BPS buffer (with SC) was added in all treatments except in control the BPS buffer was added without SC. Milk in all treatments was heat treated (at  $\sim 72^\circ\text{C}$  for 15-20 sec.) before cheese making. The experimental replicated on three occasions and the analysis were duplicated.

### 4. Cheese yield:

The yield of cheese is a mathematical expression for the quantity of cheese obtained from a given quantity of milk as the formula given by Fox *et al.* (2000).

**5. Recovery of the cheese milk constituents:**

The distribution of cheese milk constituents into cheese were calculated according to the method applied by Rao and Renner (1988).

**6. Chemical analysis:**

The moisture and salt contents of cheese were determined according to British Standards Institution (BSI, 1989). TA, fat and soluble nitrogen (SN) content of milk and cheese were determined by the method described by Ling (1963). Total nitrogen (TN) was determined by the method of International Dairy Federation (IDF, 1991). Soluble tyrosine & Tryptophan contents (s-tyrosine & s-tryptophane) were determined by the method of Vakaleris and Price (1959). The total volatile fatty acids (TVFA) of cheese were determined by the distillation method described by Kosikowski (1978), values were expressed as ml (0.1N) NaOH/100g cheese.

The pH values of the milk and cheese treatments were measured using a glass electrode digital pH meter, type (Orion Research model SA720) USA.

**7. Electrophoretic patterns of Gouda cheese treatments:**

SDS-polyacrylamide gel electrophoresis was performed using the system of

Laemmli (1970) to identify their protein profiles. Standard protein marker medium range from 29 to 205 KDa (Fermentas. Com) was used.

**8. Cheese firmness:**

Cheese firmness of cheese was measured using the Penetrometer Model Koehler Instruments Co., (USA) controller as described by Kammerlehner and Kessler (1980), the depth of penetration (0.1 mm = penetrometer unit) was measured after 5 sec at ~25°C (using cone weight 30 g and cone angle 45°. The higher record by the penetrometer reading, the less firmness of cheese.

**9. Sensory evaluation:**

Gouda cheese samples were evaluated organoleptically by 10 of the staff members of Food Science Department, Faculty of Agriculture, Moshtoher, Benha Univ.; Scoring was carried out as recommended by El-Kenawy (1977) which include flavour (50), body & texture (35) and appearance (15) as well as the overall acceptability.

**10. Statistical analysis:**

Statistical analysis was done according to the method described by Clark and Kempson (1997).

## RESULTS AND DISCUSSION

Results presented in Table (1) show the coagulation time, yield, protein & fat recovery and firmness values of Gouda cheese either control (T1) or with adding SC at levels of ~ 2, 4 and 6 x 10<sup>5</sup> cells ml<sup>-1</sup> (T2, T3 and T4, respectively).

These results indicate that, cheese treatments made with SC (T3 and T4) presented significant increase in coagulation time (p<0.05) and insignificant increase in cheese yield (p>0.05) compared with control cheese. The growth of starter culture inhibited by leukocytes of high SC which produce antimicrobial factors (Sordillo and Streicher, 2002) and so delay the fall in pH during manufacture and consequently, the action of

the rennet which led to slight increase in the coagulation time. Mazal *et al.* (2007) observed, during manufacture Prato cheese (similar Dutch Gouda cheese) from high-SC count milk, an increase in pH value of 0.25 units than that from low-SC count milk. This higher pH affected the action of the rennet and significantly increased the coagulation time. Grandisson & Ford (1986) and Mazal *et al.* (2007) detected no effect of the SC level on the yield of Prato cheese manufactured from milk containing SC count from 46 x 10<sup>3</sup> to 2 x 10<sup>6</sup> cells / ml.

Protein and fat recovery of cheese treatments made with SC had highly significant (p<0.01) decrease compared with control cheese (Table1). These results agree with

those of Barbano *et al.* (1991) who reported significant lower protein and fat recovery in cheese made from high-SC milk. High SC count in cheese milk has been associated with reduced protein recovery in cheese (Cooney *et*

*al.*, 2000 and Marino *et al.*, 2005). On contrary, Mazal *et al.* (2007) found that, there was insignificant differences in protein and fat recovery of Prato cheese made from SC milk at levels of  $< 2$  and  $> 6 \times 10^5$  cell  $\text{ml}^{-1}$ .

**Table (1): Effect of adding different levels of SC on coagulation time, yield, potein & fat recovery and cheese firmness of Gouda cheese**

Parameter	Treatments				L.S.D. (5%)
	T1	T2	T3	T4	
Coagulation time (min)	32 <sup>c</sup>	34 <sup>bc</sup>	37 <sup>ab</sup>	39 <sup>a</sup>	3.782
Cheese yield %	13.14	13.25	13.34	13.38	--
Protein recovery %	89.84 <sup>a</sup>	88.75 <sup>ab</sup>	87.67 <sup>b</sup>	85.64 <sup>c</sup>	1.304
Fat recovery %	97.59 <sup>a</sup>	96.52 <sup>b</sup>	95.67 <sup>c</sup>	94.70 <sup>d</sup>	0.6746
<b>Cheese firmness*</b>					
Fresh	38 <sup>E</sup>	40 <sup>D</sup>	43 <sup>B</sup>	45 <sup>A</sup>	
30 days	36 <sup>F</sup>	37.5 <sup>E</sup>	40 <sup>D</sup>	42 <sup>C</sup>	
60 days	34.5 <sup>G</sup>	36 <sup>F</sup>	38 <sup>E</sup>	40 <sup>D</sup>	
90 days	33 <sup>H</sup>	35 <sup>G</sup>	36 <sup>F</sup>	38 <sup>E</sup>	
L.S.D (5%)	0.8486				

Curd firmness\* = as pentameter reading (x 0.1 mm / 5 sec)

The same letters on column for every properties and the column without letters are insignificant.

T1: Control cheese without additives.

T2 : Cheese with  $2 \times 10^5$  cells  $\text{ml}^{-1}$  SC

T3: Cheese with  $4 \times 10^5$  cells  $\text{ml}^{-1}$  SC.

T4 : Cheese with  $6 \times 10^5$  cells  $\text{ml}^{-1}$  SC

The highest firmness value was recorded for control cheese (T1), while the addition of different levels of SC reduced the firmness (T4 followed by T3 then the later was T2) when fresh and during the ripening period. Firmness differences between treatments during ripening period were increased significantly ( $p < 0.05$ ). This may be due to the higher level of proteolysis which attributed to weaken the structure leading to cheese softening through the breakdown of the casein matrix, especially the hydrolysis of  $\alpha_{s1}$ -casein (Marino *et al.*, 2005). As well as, moisture changes in cheese resulting in a firmer texture which led to alteration in the casein matrix (Tunick *et al.*, 1991). However, Grandisson and Ford (1986) demonstrated negative correlation between the SC count and cheese firmness.

#### Chemical composition of Gouda cheese treatments

Composition analysis of Gouda cheese treatments made from milk and milk with adding SC showed that, moisture contents were in the expected range for Gouda

cheese, this parameter was slightly increased by the higher levels of SC addition (Table 2). These results are in agreement with those of Marino *et al.* (2005) who found insignificant differences between the moisture contents of control Cheddar cheese and Cheddar cheese made from milk with adding SC. The moisture content of Gouda cheese treatments decreased during ripening and this may be due to the biochemical changes and lactic acid development, which cause curd contraction and expulsion of aqueous phase of cheese. Also, may be due to the evaporation of some moisture content during ripening period. These results are in agreement with those obtained by Hammad *et al.* (2008).

The fat/DM, salt-in-moisture, and TA content of cheese were slightly affected by the addition of SC, but the differences between treatments were insignificant ( $p > 0.05$ ). These results agree with Marino *et al.* (2005) and Mazal *et al.* (2007). In respect with, the TN content of cheese treatments was reduced by SC addition compared with control cheese. This may be related to the slight increase of

moisture content of cheese made from milk with adding SC. High SC count in milk was associated with reduced protein recovery in cheese (Cooney *et al.*, 2000 and Marino *et al.*, 2005).

increased up to the end of ripening period due to the moisture decrease. These results are in agreement with that obtained by Hammad *et al.* (2008). The gradual increase in the TA of all cheese treatments could be explained by the lactose fermentation by lactic acid bacteria. Similar results were obtained by Rymaszewski and El-Tanboly (1990).

As ripening progressed, the, fat/DM, salt-in-moisture, TA, TN % were gradually

**Table (2): Effect of adding different levels of SC to cheese milk on the chemical composition and pH values of resultant Gouda cheese.**

Parameter	Ripening period (days)	Treatments			
		T1	T2	T3	T4
Moisture %	Fresh	48.17	48.46	49.19	49.69
	30	43.35	43.76	44.54	45.14
	60	40.12	40.66	41.74	42.12
	90	37.62	38.26	39.44	39.82
	L.S.D (5%)	--			
Fat/DM%	Fresh	50.16	49.48	49.38	49.29
	30	50.31	49.79	49.59	49.39
	60	50.60	49.88	49.78	49.59
	90	51.13	50.86	50.66	50.51
	L.S.D (5%)	--			
Salt-in-moisture %	Fresh	3.11	3.3	3.52	3.58
	30	5.91	6.12	6.29	6.42
	60	7.98	8.31	8.51	8.78
	90	9.12	9.44	9.58	9.79
	L.S.D (5%)	--			
TA %	Fresh	1.06	1.04	1.04	1.03
	30	1.70	1.66	1.62	1.58
	60	2.21	2.18	2.15	2.12
	90	2.50	2.48	2.44	2.40
	L.S.D (5%)	--			
pH value	Fresh	5.28 <sup>GH</sup>	5.33 <sup>BCG</sup>	5.37 <sup>CDE</sup>	5.25 <sup>HIJ</sup>
	30	5.09 <sup>M</sup>	5.18 <sup>KL</sup>	5.20 <sup>K</sup>	5.14 <sup>LM</sup>
	60	5.26 <sup>HI</sup>	5.30 <sup>FGH</sup>	5.35 <sup>DEF</sup>	5.22 <sup>JK</sup>
	90	5.40 <sup>BCD</sup>	5.42 <sup>ABC</sup>	5.44 <sup>AB</sup>	5.46 <sup>A</sup>
	L.S.D (5%)	0.05273			
TN%	Fresh	3.43	3.36	3.30	3.25
	30	3.90	3.85	3.78	3.72
	60	4.15	4.09	3.98	3.94
	90	4.35	4.29	4.16	4.11
	L.S.D (5%)	--			

The same letters on column for every properties and the column without letters are insignificant.

T1: Control cheese without additives. T2 : Cheese with  $2 \times 10^5$  cells ml<sup>-1</sup> SC

T3: Cheese with  $4 \times 10^5$  cells ml<sup>-1</sup> SC. T4 : Cheese with  $6 \times 10^5$  cells ml<sup>-1</sup> SC

According to the pH values there were noticeable decreases of all treatments during the first month of ripening, then they gradually increased after the second and third month. The differences in pH values between treatments during ripening period were significant ( $p < 0.05$ ). The gradual increase in the pH which was noticed after the 2<sup>nd</sup> and 3<sup>rd</sup> month, could be attributed to a further breakdown of lactic acid and forming basic compounds as well as basic amino groups through the protein degradation upon advanced ripening. Similar results were reported by Rymaszewski & El-Tanboly (1990) and Hammad *et al.* (2008).

#### Ripening indices of Gouda cheese treatments

During cheese ripening, the major biochemical changes are fermentation of lactose, degradation of proteins, hydrolysis of fat with production of volatile compounds. The activity of proteolytic and lipolytic enzymes in cheese was indicated from the levels of SN, SN/TN, s-tyrosine, s-tryptophane and TVFA content during ripening, respectively. These ripening indices were higher in cheese made from milk with adding SC (T2, T3 and T4) than in control cheese (T1) Table 3. Gouda cheese (T2) had slightly lower ripening indices as compared with treatments T3 and T4, this suggest a relationship between the presence of elevated levels of added SC in milk and the levels of proteolysis in cheese, which may be attributed to the proteolytic activity of SC enzymes. Proteolysis and lipolysis parameters differences between treatments during ripening were highly significant ( $p < 0.01$ ). The results are consistent with those of Cooney *et al.* (2000), who reported a higher level of SN in Swiss-type cheese made from milk with elevated levels of SC. Also, Marino *et al.* (2005) found that, the activity of lysosomal enzymes in Cheddar cheese during ripening was apparent from the high levels of proteolysis in cheese made from milk with adding SC than the control cheese. On the other hand, highest TVFA content was observed for cheese made from milk with the high level of added SC (Table 3), this may attribute to increase of SC level which correlated with lipase (lipoprotein lipase) increase and heat-stable protease

(plasmin) in milk (Barbano *et al.*, 2006). Furthermore, the higher rate of protein degradation which associated with more accumulation of free amino acids may serve as precursors for volatile fatty acids (Nakae and Elloitt, 1965)

A gradual increase in S.N, SN/TN, soluble tyrosine, soluble tryptophane and TVFA content in all cheese treatments was observed over all the ripening period. These could be attributed to the protein degradation; the formation of soluble nitrogenous compounds and partially the decrease of the moisture content.

#### Electrophoretic patterns of Gouda cheese treatments

During cheese ripening, degradation of proteins is the most important phenomenon as they are partially converted from insoluble to soluble forms and broken down to proteoses, peptones, polypeptides and amino acids.

The electrophoretic patterns of Gouda cheese treatments made from control milk and milk with adding SC when fresh and after 60 days of ripening are illustrated in (Fig. 1).

There are no discernible changes in the electrophoretic patterns of cheese protein for all treatments when fresh (lanes 1 to 4). The electrophoretic patterns after 60 days of cheese ripening revealed the degradation of protein was most pronounced in T3 and T4 (lanes 7 and 8) in comparing with T2 and T1 (lanes 6 and 5). The protein degradation can be detected with respect to either numbers or intensity of the different protein bands, also there were numbers of protein bands not observed or present in ripened cheese made with SC (lanes 6, 7 and 8). Marino *et al.*, (2005) whose demonstrated that, during ripening of Cheddar cheese samples made from milk with adding SC, the levels of  $\alpha_{s1}$ - and  $\beta$ - casein were progressively hydrolysed. Degradation of casein by leukocyte proteinases of SC in the order of  $\alpha_{s1} > \beta > K$ - casein (Grieve and Kitchen, 1985). Le Roux *et al.* (2003) found that elevating the SC count can alter the protein fractions distribution. However, plasmin can rapidly cleave both  $\beta$ -casein into  $\gamma$ -casein and smaller polypeptides (Auldust *et al.*, 1996).

Table (3): Effect of adding different levels of SC to cheese milk on the ripening indices of resultant Gouda cheese.

Parameter	Ripening period (days)	Treatments			
		T1	T2	T3	T4
SN%	Fresh	0.267 <sup>HI</sup>	0.274 <sup>HI</sup>	0.274 <sup>HI</sup>	0.277 <sup>HI</sup>
	30	0.422 <sup>G</sup>	0.534 <sup>EF</sup>	0.582 <sup>DE</sup>	0.628 <sup>CD</sup>
	60	0.510 <sup>F</sup>	0.623 <sup>CD</sup>	0.667 <sup>C</sup>	0.758 <sup>B</sup>
	90	0.553 <sup>EF</sup>	0.656 <sup>C</sup>	0.745 <sup>B</sup>	0.849 <sup>A</sup>
L.S.D (5%)		0.05273			
SN/TN %	Fresh	7.78 <sup>L</sup>	8.07 <sup>KL</sup>	8.30 <sup>JK</sup>	8.52 <sup>J</sup>
	30	10.82 <sup>I</sup>	13.92 <sup>F</sup>	15.39 <sup>B</sup>	15.94 <sup>D</sup>
	60	12.29 <sup>H</sup>	15.16 <sup>E</sup>	17.76 <sup>C</sup>	19.24 <sup>B</sup>
	90	12.71 <sup>G</sup>	15.29 <sup>E</sup>	17.91 <sup>C</sup>	20.66 <sup>A</sup>
L.S.D (5%)		0.3208			
S. tyrosine (mg/100g cheese)	Fresh	9.11 <sup>M</sup>	9.40 <sup>M</sup>	9.70 <sup>M</sup>	9.90 <sup>M</sup>
	30	51.26 <sup>L</sup>	64.51 <sup>K</sup>	72.42 <sup>J</sup>	78.50 <sup>I</sup>
	60	88.91 <sup>H</sup>	116.13 <sup>G</sup>	132.58 <sup>F</sup>	155.15 <sup>D</sup>
	90	144.45 <sup>E</sup>	165.89 <sup>C</sup>	186.83 <sup>B</sup>	217.81 <sup>A</sup>
L.S.D (5%)		3.656			
S tryptophan (mg/100g cheese)	Fresh	5.00 <sup>M</sup>	5.27 <sup>M</sup>	5.20 <sup>M</sup>	5.20 <sup>M</sup>
	30	26.84 <sup>L</sup>	34.20 <sup>K</sup>	45.10 <sup>J</sup>	51.40 <sup>I</sup>
	60	48.70 <sup>I</sup>	65.15 <sup>G</sup>	74.10 <sup>F</sup>	85.30 <sup>D</sup>
	90	78.65 <sup>E</sup>	90.85 <sup>C</sup>	100.55 <sup>B</sup>	122.5 <sup>A</sup>
L.S.D (5%)		2.162			
T.V.F.A (0.1 ml N NaOH/100g cheese)	Fresh	6.10 <sup>I</sup>	6.00 <sup>I</sup>	6.20 <sup>I</sup>	6.20 <sup>I</sup>
	30	17.25 <sup>H</sup>	20.25 <sup>G</sup>	24.85 <sup>F</sup>	29.35 <sup>E</sup>
	60	25.50 <sup>F</sup>	29.25 <sup>E</sup>	35.45 <sup>D</sup>	39.50 <sup>C</sup>
	90	29.50 <sup>E</sup>	38.95 <sup>C</sup>	45.65 <sup>B</sup>	51.25 <sup>A</sup>
L.S.D (5%)		2.368			

The same letters on column for every properties and the column without letters are insignificant.

T1: Control cheese without additives.

T2 : Cheese with  $2 \times 10^5$  cells  $\text{ml}^{-1}$  SC

T3: Cheese with  $4 \times 10^5$  cells  $\text{ml}^{-1}$  SC.

T4 : Cheese with  $6 \times 10^5$  cells  $\text{ml}^{-1}$  SC

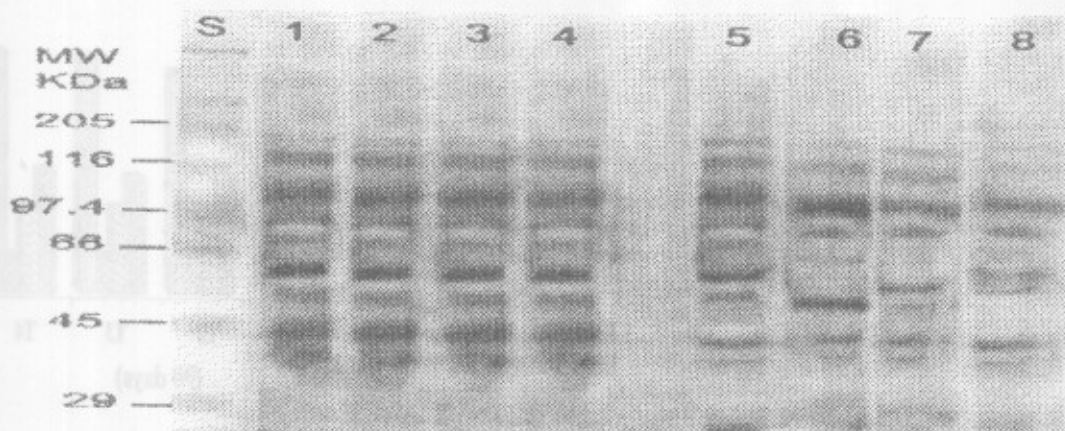
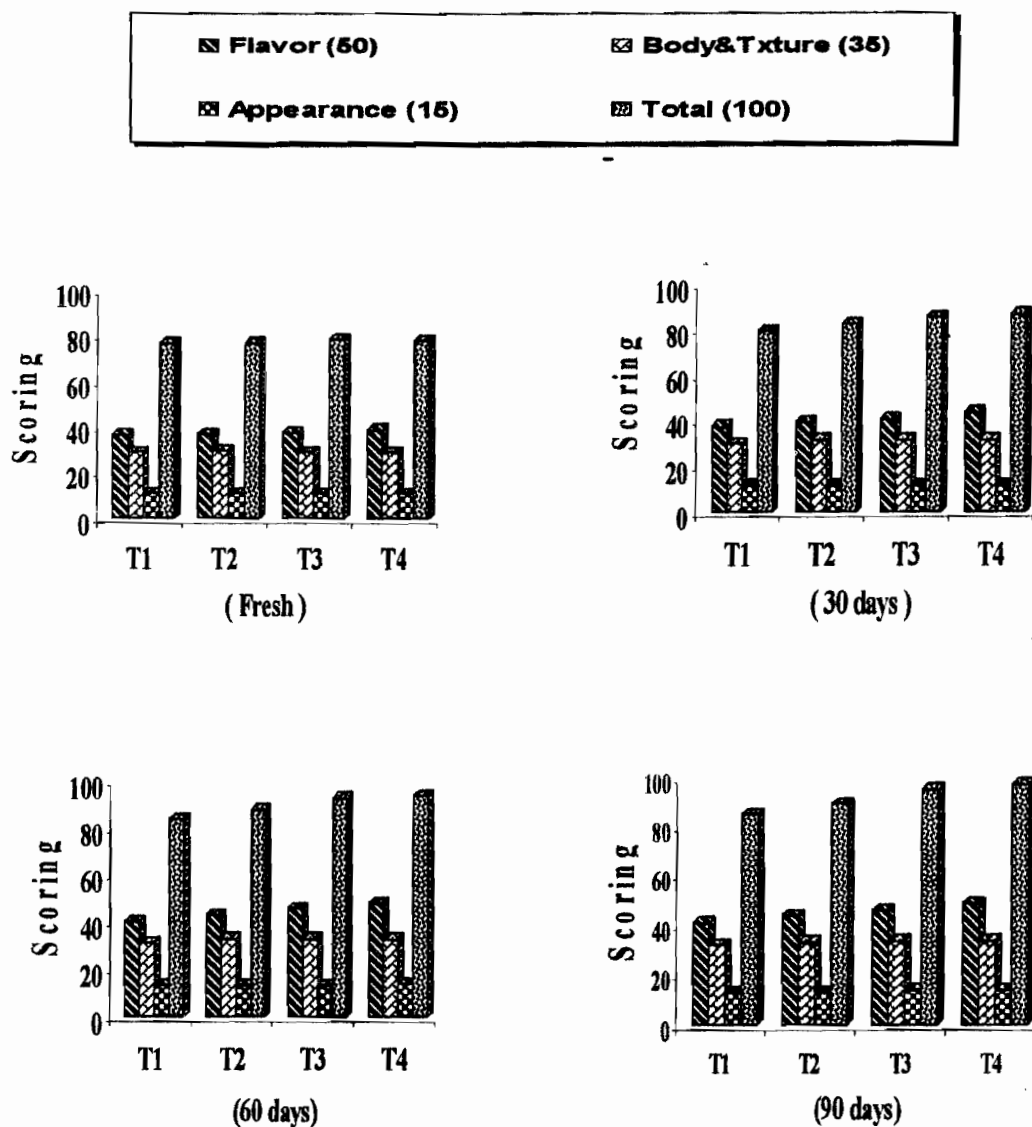


Fig. (1): SDS-PAGE of Gouda cheese made from control milk (lane 1) and made from milk with adding SC at levels 2, 4 and  $6 \times 10^5$  cells  $\text{mL}^{-1}$  (lanes 2,3 and 4, respectively) when fresh. Lanes 5-8 as lanes 1-4 but after 60 days ripening. Lane S (standard marker).

**Organoleptic properties of cheese:**

The organoleptic properties of Gouda cheese made from different treatments and their corresponding scores are presented in Fig. (2). The scoring of cheese was carried out at four different stages fresh, 30, 60 and 90 days. The obtained results reveal that the fresh cheese of all treatments had nearly the same score points for organoleptic characteristics. After 30 days of cheese ripening and during ripening period, the treatments T4 followed by T3 then T2 gained higher score points for organoleptic characteristics than T1. The

statistical analysis of variance showed highly significant differences ( $p < 0.01$ ) between all treatments of Gouda cheese for characteristics. It could be attributed to the higher levels of soluble nitrogenous compounds produced by the enzymes of SC which contributed to improve the cheese structure and also the increase of TVFA as it was confirmed by the chemical analysis. Mazal *et al.* (2007) reported that, the Prato cheese made from high SC count milk had a higher level of proteolysis during ripening, which could compromise the typical sensory quality of the product.



**Fig (2): Effect of adding different levels of SC to cheese milk on the organoleptic characteristics of resultant Gouda cheese.**

T1: Control cheese without additives

T2: Cheese with  $2 \times 10^5$  cells  $\text{ml}^{-1}$  SC

T3: Cheese with  $4 \times 10^5$  cells  $\text{ml}^{-1}$  SC

T4: Cheese with  $2 \times 10^5$  cells  $\text{ml}^{-1}$  SC



In conclusion, it was clear from the ripening indices, electrophoretic patterns and organoleptic characteristics that ripening of Gouda cheese treatments, which made from milk with adding SC (levels  $6 \times 10^5$  cells  $ml^{-1}$

followed by  $4 \times 10^5$  cells  $ml^{-1}$ ) were accelerated and the cheese quality was improved within 60 days in comparing with other treatments.

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### إسراع تسوية وتحسين جودة جبن الجودا بإضافة الخلايا الجسدية المجمعة من اللبن

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درس تأثير إضافة الإنزيمات المحللة للبروتين الموجودة بالخلايا الجسدية المجمعة من لبن أبقار سليمة صحياً على تسوية وجودة جبن الجودا. صنعت جبن الجودا من لبن طبيعي خليط (لبن بقري وجاموسي 1:1) أضيف إليه الخلايا الجسدية بتركيزات 2، 4، 6، 10 × 51 خلية / مل (معاملات 2، 3، 4 و 6 على الترتيب) قبل البسترة، إضافة إلى جبن المقارنة بدون إضافات (معاملة 1). أشارت النتائج إلى حدوث تأثير طفيف في وقت التجبن والتصافي والمحتجز من البروتين والدهن في الجبن (Recovery) في معاملات جبن الجودا المصنع بإضافة الخلايا الجسدية. كما وجد أن التحلل البروتيني والدهني (بقياس مؤشرات التسوية المتمثلة في النتروجين الذائب و النتروجين الذائب / النتروجين الكلي والتيروسين والتربتوفان الذائبين والأحماض الدهنية الكلية الطيارة) ازدادت بإضافة الخلايا الجسدية. كما أظهرت قيم التماسك أن كل معاملات الجبن المصنعة من لبن مضاف إليه الخلايا الجسدية أعطت جبن ذو تركيب جيد خلال مراحل التسوية. وقد أوضح التحليل بجهاز الفصل الكهربائي للبروتين (Electrophoresis) وكذا التقييم الحسي أن لإنزيمات التحلل البروتيني الموجودة بالخلايا الجسدية تأثير مباشر على التحلل البروتيني للجبن ولذلك فإن المعاملة 4 يليها المعاملة 3 حدث بهما إسراع وتمام التسوية بعد 60 يوم من التخزين، كما تحسنت بهما الجودة العامة للجبن الناتج.