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EFFECT OF TRANSGLUTAMINASE ON SOME CHEMICAL, RHEOLOGICAL AND SENSORIAL CHARACTERISTICS OF UF-RAS CHEESE DURING RIPENING

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ABSTRACT

The objective of this study was to investigate the effect of addition transglutaminase TG: (0, 20, 40 and 60U/kg retentate) on some chemical, rheological and sensorial characteristics of UF-Ras cheese over ripening period (90 days). Results demonstrated that the moisture and protein contents were significantly higher in TG cheeses than in the control cheese. The rate of proteolysis (WSN/TN, NPN/TN%) in UF-Ras cheeses treated by TG was slightly higher than in the control cheese. Concerning rheological properties, results showed significantly higher values of hardness, gumminess and chewiness than in the control cheese. TG addition had no significant effect on springiness, cohesiveness and adhesiveness values. Textural characteristics of TG cheeses were at the same level as in control cheeses at 60 days of ripening and no significant effect of TG amount was observed. Sensory analysis revealed that no effect of TG on the flavour and appearance scores. The total scores of TG and control cheese did not differ significantly at 90 days of ripening. Overall, the main effect of TG was to modify the rheological parameters and to increase the protein content of the experimental UF-Ras cheese.

Key words: Transglutaminase, cross-linkage, UF, Ras cheese, ripening period, texture.

INTRODUCTION

Ras cheese is a local hard cheese variety manufactured in Egypt. It being exported to different countries in Middle Eastern. It has a cylinder-shape (round wheels) with a dry crust; its weight is about 10-12 kg, 20 cm height, 20-30 cm diameter, low moisture content (<40 g/100 g cheese), and a minimum fat-in-dry matter (45 g/100 g dry matter). After 3–6 months the cheese will develop an open texture and a sharp, pungent flavour (Hofi *et al.*, 1970).

Application of ultrafiltration (UF) process in cheese-making has many potential benefits as a tool for separation and concentration (Lawrence, 1989). It is used extensively in soft chees manufacture but it is more difficult to produce hard cheeses (Zeman and Zydney, 1996). Considerable work has been done to develop this process for prodcution of hard cheeses, but this process has found only limited applications due to the difficulties in acheiving the desired consistency, texture and taste (Bech, 1993).

Novel applications based on enzyme-modification of milk protein matrix system tended to be promising for improving cheese textural and sensorial characteristics (Di Pierro *et al.*, 2010). The transglutaminase enzyme (TG; protein-glutamine γ-lutamyltransferase, EC 2.3.2.13) has been widely used in the dairy industry to catalyse the formation of new covalent crosslinking bonds between and within casein micelles resulting in form gel with modified rheological characteristics. The mechanism of protein cross-linking induced by TG and the effect of TG on various food proteins have been well-documented (Kuraishi *et al.*, 2001; Di Pierro *et al.*, 2010; Kieliszek and Misiewicz, 2014). Major effects of transglutaminase when used in food processing

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are an increase in firmness, viscosity and imporve water-holding capacity (Kuraishi *et al.*, 2001 ; Mahmood and Sebo, 2009 ; Chen and Rosenthal, 2015).

The objective of this work was to investigate the effect of enzymatic cross-linking induced by transglutaminase (TG) on some chemical, rheological and sensorial characteristics of UF-Ras cheese during ripening period for 90 days.

MATERIALS AND METHODS

Materials

Fresh cow´s milk was supplied by a private farm in Fayoum Governorate, Egypt. Rennet powder (CHY-MAX, 2280 IMCU/ml, Ch. Hansen Lab., Denmark), calcium chloride (food quality grade), table salt (sodium chloride), and yoghurt starter (DVS YC-X11, Ch. Hansen Lab., Denmark) were obtained from the local market. Plant Transgultaminase enzyme (EC 2.3.2.13, enzymatic activity of 80U/g) powder was imported from Poland. Experimental UF-Ras cheeses were produced in the pilot dairy plant of Fac. Agric., Fayoum Univ., Egypt.

Methods of Analyses

UF-Ras cheese manufacturing and cheese sampling

Cow´s milk was pasteurized (72ºC/15 s) and ultrafiltered at 50ºC to concentration factor 2 :1 (El-Shibiny *et al.*, 1991) using UF unit Carbo-sep (Moduel tubular), then the retentate was cooled to 37ºC and the freeze-dried starter cultures (1%) were added directly to retenate. The inoculated retentate (60 kg) was divided into 4 equal portions. One portion (15 kg) was used to produce UF-Ras cheese without TG addition (control), while the other three portions were separately mixed with TG prior coagulation (5min) at a ratio 20, 40, 60U/15 kg retentate, respectively. Coagulation was carried out by rennet addition (2 g/100 kg retentate) and the procedure of cheese making was followed as described by El-Shibiny *et al.* (1991). Cheese ripening was took place at 12±2ºC and a relative humidity of 80-90% for 90 days.

From each batch, cheese samples were taken in triplicate after salting and on the days 15, 30, 60 and 90 of ripening period for chemical, sensorial analyses and on 30, 60, 90 days for rheological determinations.

Chemical analyses

Grated cheese samples were analysed for acidity, moisture, protein, fat, WSN/TN, and NPN/TN contents according to AOAC (1990). Moisture in nonfat substance (MNFS %) was calculated as (% moisture/100-%fat) × 100. All analyses were performed in triplicate and results reported as mean ± standard deviation.

Texture profile analysis (TPA)

Cylindrical cheese samples were compressed to 20% of the original height in two bites on an universal Testing machine (TL-Pro, Food Technology Corporation, Sterling, Verginia, USA) provided with software at a rate of 60 mm/sec., force 1 N at room temperature. The following parameters were evaluated according to the definitions given by the international Dairy Federation, IDF (1991): Hardness (N), Cohesiveness (˷), Springiness (mm), Adhesiveness (N.s), Gumminess (N), Chewiness (N.mm).

Sensory analysis

Ten panelists from staff members of Dairy Department, Faculty of Agriculture, Fayoum University, organoleptically judged the experimental cheeses at 15, 30, 60 and 90 days of ripening period using a score card for evaluation of flavour (60 points), body and texture (30 points), appearance (10 points). Coded samples of approximately 100 g of cheese were partitioned and presented to the panelists at room temperature. Results were expressed as a mean score with standard deviation obtained from the 10 panelists.

Statistical analysis

All data were expressed as mean values ± standard deviation and analyzed using on way analysis of variance (ANOVA) followed by the least significant differences (LSD) test at significant ($P < 0.05$) using XLSTAT statistical software version (2009).

RESULTS AND DISCUSSION

Chemical Characteristics of UF-Ras Cheeses Treated by TG

The chemical characterizatics of UF-Ras cheese samples are presented in Table 1. It is markedly that the acidity values of the experimental UF-Ras cheeses were affected by TG addition, since the differences between samples were statistically significant. This could be attributed to that growth of bacteria which slowed down because the availability of milk proteins to the bacteria was decreased due to the enzymatic cross-linking induced by TG, since the liberation of amino acids into the milk is necessary to the growth of lactic acid bacteria and to the production of lactic acid (Tamine and Robinson, 1999). These findings are in agreement with Ozer *et al.* (2007) who reported that TG enzyme led to an imbalance of the protosymbiosis growth of the yoghurt culture.

It was also observed that the addition of TG led to a significant increase ($P < 0.05$) in the protein and moisture contents in the TG treated UF-Ras cheese than that in untreated cheese (control). Since, the role of TG in the system is the formation of bonds between lysine and glutamine, creating a more complex matrix where additional water molecules can be held (Kieliszek and Misiewicz, 2014). These observations are in agreement with the results obtained by De Sá and Bordignon-Luiz (2010) on the manufacture of milk gels and processed cheese. They found that a marked increase in cheese protein and total solid contents in cheese treated by TG. The increased protein can be explained by the enhancement in serum binding to the gel network reinforced by additional covalent bonds and incorporation of casein fines into the gel network (Hu *et al.*, 2013). Similar results were also observed by other authors (Cozzolino *et al.*, 2003; Bönisch *et al.*, 2008 ; Di Pierro *et al.*, 2010 ; Sayadi *et al.*, 2013).

With regard to the proteolysis, it is obvious that the WSN/TN and NPN/TN ratios of all cheeses gradually increased during the ripening period as a result of protein degradation (Table 1). Moreover, the degree of proteolysis of the crosslinked UF-Ras cheese treated by TG was significantly higher than in untreated UF-Ras cheese in the early stage of ripening (30 and 60 days). This may be attributed to the crosslinked UF-Ras cheese by TG showed higher MNSF% than in untreated cheese, which means an increased in the freedom of moisture, increased the activity of enzymes or microorganisms and thereafter the degree of proteolysis (Hu *et al.*, 2013). It is well known that, the highest MNSF (%), the highest the rate of breakdown (Fox, 1999). In general, increasing amount of enzyme addition into retentate led to the considerable rise in the chemical characteristics of the experimental UF-Ras cheese.

Rheological Characteristics of UF-Ras Cheeses Treated by TG

The Texture Profile Analysis (TPA) parameters of the experimental UF-Ras cheese are given in Table 2. It was observed that UF-Ras cheeses treated by TG were characterized by higher values of hardness, gumminess (hardness x cohesiveness) and chewiness than in untreated cheese samples and further increase in TG amount had adverse effect on these textural parameters. These higher values may be due to inter- and intra-molecular crosslinking of protein created by TG enzyme resulted in more compact structure than in the control one. The interconnection amongst protein strands and particles in cheese texture increases with increasing protein content, strengthening the elastic nature of samples (Sayadi *et al.*, 2013). Also, it can be seen that the crosslinked UF-Ras cheeses obtained by TG did not exhibit any significant changes in the springiness, cohesiveness and adhesiveness attributes of UF-Ras cheese. Prakasan *et al.* (2015) reported that there were no significant effects on these properties of Paneer cheese treated by TG. Similar results have been reported by (Lauber *et al.*, 2000 ; Lorenzen *et al.*, 2002 ; Sayadi *et al.*, 2013). No significant differences were observed in the rheological characteristics of TG cheeses at 60 days of ripening probably because of the proteolysis of TG-catalysed gel network.

Table 1. Chemical characteristics of UF-Ras cheese treated by TG during ripening period

Parameter	Ripening period (day)	TG concentration (U/15 kg retentate)			
		Control	20	40	60
Acidity (%)	**15**	2.02±0.08^{a}	1.95±0.05^{ab}	1.83 ±0.06^{bc}	1.72±0.08^{c}
	30	2.10±0.10^{a}	2.03 ±0.06^{ab}	1.95±0.05^{b}	1.92±0.03^{b}
	60	2.22 ±0.03^{a}	2.12 ±0.03^{b}	2.03±0.03^{c}	1.98±0.03^{c}
	90	2.82±0.03^{a}	2.77±0.03^{ab}	2.75±0.00^{bc}	2.70 ±0.05^{c}
Moisture (%)	**15**	35.21 ±0.18^{b}	35.95±0.80^{ab}	36.53 ±0.55^{a}	36.60±0.38^{a}
	30	32.25±0.23^{c}	32.87±0.29^{bc}	33.24 ±0.59^{ab}	33.55±0.15^{a}
	60	30.72±0.26^{a}	30.80±0.08^{a}	30.84 ±0.20^{a}	31.03±0.25^{a}
	90	29.81±0.15^{b}	29.97 ±0.45^{b}	30.25±0.45^{ab}	30.80 ±0.22^{a}
Protein (%)	**15**	25.46 ±0.06^{d}	26.35±0.06^{c}	27.71 ±0.35^{b}	29.45±0.61^{a}
	30	26.35 ±0.45^{d}	27.29±0.13^{c}	29.03±0.32^{b}	30.28±0.29^{a}
	60	27.65 ±0.26^{b}	.28.54 ±0.19^{b}	29.92±0.06^{a}	31.03 ±1.29^{a}
	90	29.03±0.32^{c}	29.26 ±0.19^{c}	29.96±0.26^{b}	31.69±0.18^{a}
MNFS (%)	**15**	52.49±0.43^{ab}	53.30±0.98^{a}	53.59±0.45^{a}	51.62±0.67^{b}
	30	49.01±0.39^{ab}	49.68±0.75^{a}	49.37±0.81^{a}	47.93±0.41^{b}
	60	47.50±0.59^{a}	47.32±0.04^{a}	47.03±0.39^{a}	45.93±1.39^{a}
	90	46.46±0.30^{a}	46.37±0.86^{a}	46.44±0.58^{a}	45.61±0.32^{a}
WSN/TN (%)	**15**	6.01±0.49^{c}	8.48±0.87^{b}	9.40±0.37^{b}	10.61±0.54^{a}
	30	7.26±0.12^{c}	9.55±0.52^{b}	12.31±0.90^{a}	13.03±0.53^{a}
	60	12.66±0.56^{c}	13.82±0.53^{c}	16.42±1.56^{b}	18.69±0.73^{a}
	90	14.29±1.26^{c}	18.32±1.58^{b}	20.37±1.03^{ab}	21.60±2.03^{a}
NPN/TN (%)	**15**	3.51±0.45^{c}	4.10±0.25^{c}	4.83±0.34^{b}	5.56±0.25^{a}
	30	4.38±0.31^{b}	5.59±0.23^{ab}	5.38±0.71^{a}	5.90±0.70^{a}
	60	5.65±0.76^{b}	6.39±0.27^{b}	6.72±0.76^{ab}	7.94±0.96^{a}
	90	7.95±0.37^{c}	8.39±0.75^{bc}	9.69±0.83^{ab}	10.57±0.76^{a}

Mean ± SD with different letters in the same row represent statistically significant difference ($P < 0.05$) between values

MNFS: Moisture in nonfat substance

Table 2. Rheological parameters of UF-Ras cheese treated by TG during ripening period

Parameter	Ripening period (day)	TG concentration (U/15 kg retentate)			
		Control	20	40	60
Hardness (N)	**30**	41.50±11.90^{b}	79.20±24.90^{a}	67.20±39.00^{a}	84.40±17.40^{a}
	60	23.40±4.58^{c}	44.40±13.40^{b}	39.20±13.00^{b}	52.10±17.10^{a}
	90	13.75±0.07^{a}	27.25±6.58^{a}	16.55±8.27^{a}	20.50±8.20^{a}
Adhesiveness (N.s)	**30**	0.35±0.08^{b}	0.20±0.07^{b}	0.13±0.06^{b}	0.67±0.12^{a}
	60	0.09±0.02^{a}	0.07±0.01^{a}	0.07±0.03^{a}	0.08±0.01^{a}
	90	0.02±0.01^{a}	0.02±0.01^{a}	0.03±0.01^{a}	0.02±0.01^{a}
Cohesiveness (-)	**30**	0.69±0.01^{a}	0.59±0.05^{a}	0.52±0.08^{a}	0.65±0.09^{a}
	60	0.70±0.02^{a}	0.67±0.02^{a}	0.72±0.02^{a}	0.70±0.03^{a}
	90	0.71±0.05^{a}	0.70±0.00^{a}	0.70±0.04^{a}	0.72±0.04^{a}
Springiness (mm)	**30**	3.43±0.32^{a}	2.96±0.12^{a}	3.67±0.59^{a}	3.24±0.38^{a}
	60	1.66±0.15^{a}	1.62±0.04^{a}	1.88±0.12^{a}	1.63±0.15^{a}
	90	0.75±0.08^{b}	0.89±0.03^{a}	0.92±0.00^{a}	0.93±0.01^{a}
Gumminess (N)	**30**	28.6±8.54^{b}	46.20±12.80^{a}	36.30±25.50^{a}	54.30±4.19^{a}
	60	16.40±3.38^{c}	29.70±9.31^{b}	28.40±9.52^{b}	36.50±11.80^{a}
	90	9.65±0.78^{a}	19.15±4.60^{a}	11.70±6.36^{a}	14.95±6.72^{a}
Chewiness (N.mm)	**30**	99.52±38.60^{b}	137.87±42.50^{a}	140.95±115.00^{a}	175.20±6.90^{a}
	60	27.67±7.53^{c}	48.01±14.70^{bc}	54.09±20.30^{ab}	60.77±23.90^{a}
	90	7.27±1.38^{a}	17.16±4.60^{a}	10.74±5.80^{a}	13.85±6.34^{a}

Mean±SD with different letters in the same row represent statistically significant difference ($P<0.05$) between values

Sensory Characteristics of UF-Ras Cheeses Treated by TG

The sensory evaluation of UF-Ras cheese samples are presented in Table 3. It can be seen that appearance and flavour scores of the experimental UF-Ras cheese were not affected by TG addition. This indicate that TG addition did not affect on the acceptability of product by consumer. TG cheese had lower texture scores resulting in rigid of cheese. These results are in agreement with Prakasan *et al.* (2015) who studied the effect of microbial TG treatment on Paneer cheese and observed that the addition of microbial TG resulted in reduction of texture score and overall score due to formation of rigid gel.

Conclusion

Enzymatic crosslinking of milk protein by TG increased the moisture-to-protein ratio of the experimental UF-Ras cheese leading to increased textural hardness, gumminess and chewiness values. UF-Ras cheese treated by TG had a lower total scores than in the control, but still satisfactory level of sensory characteristics with a certain extent of hard texture.

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Table 3. Sensory attributes of UF-Ras cheeses treated by TG during the ripening period

Parameter	Ripening period (day)	TG concentration (U/15 kg retentate)			
		Control	20	40	60
Flavour (60 points)	15	54.30±3.92^{a}	52.80±5.09^{a}	50.70±5.23^{a}	50.80±6.18^{a}
	30	52.60±3.50^{a}	53.00±4.57^{a}	52.20±3.49^{a}	51.90±4.72^{a}
	60	56.10±2.77^{a}	57.00±2.71^{a}	54.40±3.10^{ab}	53.00±3.97^{b}
	90	53.90±6.94^{a}	54.30±5.14^{a}	54.10±4.41^{a}	50.60±5.68^{a}
Body& Texture (30)	15	28.80±0.92^{a}	27.40±2.84^{ab}	25.30±2.50^{b}	25.20±3.43^{b}
	30	28.40±1.17^{a}	26.80±2.86^{ab}	24.60±2.27^{c}	24.70±2.95b^{c}
	60	28.30±1.34^{ab}	28.40±1.51^{a}	26.80±1.75^{b}	23.70±2.00^{c}
	90	26.50±3.69^{a}	25.00±4.76^{ab}	25.60±3.50^{a}	21.40±5.72^{b}
Appearance (10)	15	9.30±0.82^{a}	9.20±0.92^{ab}	8.00±1.63^{bc}	7.70±1.77^{c}
	30	9.10±0.74^{a}	8.80±0.92^{a}	8.50±0.85^{a}	8.60±1.23^{a}
	60	9.20±0.63^{a}	9.10±0.74^{a}	8.80±0.92^{a}	8.90±0.97^{a}
	90	9.30±0.67^{a}	8.70±0.95^{a}	8.60±0.84^{a}	8.70±1.16^{a}
Total score (100)	15	92.40±4.03^{a}	89.40±7.07^{ab}	84.00±8.07^{b}	83.70±9.98^{b}
	30	90.10±4.43^{a}	88.60±7.32^{a}	85.30±5.33^{a}	84.80±7.25^{a}
	60	93.60±4.22^{ab}	94.50±4.67^{a}	90.00±4.52^{b}	84.30±5.98^{c}
	90	89.70±7.86^{a}	88.00±9.25^{a}	88.30±5.93^{a}	86.70±10.19^{a}

Mean ± SD with different letters in the same row represent statistically significant difference ($P < 0.05$) between values

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تأثير إنزيم الترانس جلوتاميناز علي بعض الخصائص الكيميائية والريولوجية والحسية للجبن الراس الناتج بالترشيح الفائق خلال التسوية

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أجريت هذه الدراسة بهدف تقييم تأثير إضافة إنزيم الترانس جلوتاميناز (صفر، ٢٠ ، ٤٠ ، ٦٠ وحدة إنزيم) على بعض الخصائص الكيميائية والريولوجية والحسية للجبن الراس الناتج بالترشيح الفائق خلال التسوية لمدة ٩٠ يوما، وقد أظهرت نتائج هذه الدراسة ارتفاع ملحوظ خلال فترات التسوية للجبن المعاملة بالإنزيم في نسب كلا من الرطوبة، البروتين، وكذلك قيم معدلات التسوية متمثلة في النسبة المئوية للنتيروجين الذائب في الماء والنسبة المئوية النتروجين غير البروتيني وانخفاض معدل الحموضة في الجبن المعاملة بالإنزيم عن الجبن الكنترول، كما تبين من المعاملة بالإنزيم حدوث ارتفاع ملحوظ في قيم الريولوجي خلال فترات التسوية عند ٣٠ ، ٦٠ يوم ثم تبين بعد ذلك عدم وجود أي فروق معنوية بين المعاملات عند تقدم التسوية حتى نهاية ٩٠ يوم، وحصلت الجبن المعاملة بالإنزيم على درجات تحكيم أقل من جبن الكنترول وقد لوحظ أثناء التحكيم إلى أن الجبن له قوام وتركيب صلب نسبيا مقارنة بجبن الكنترول، ولا يوجد فروق معنوية بين المعاملات المختلفة من حيث اللون والنكهة.

المحكمون :

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