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Reducing Goat milk Flavor by Beta – Cyclodextrin (β-CD) and its Effect on Texture and Microstructure in Soft Goat Cheese

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ABSTRACT

Although goat milk and their processing products were recommended for human consumption as a medicinal food, their strong goaty flavor limits their acceptability among the majority of consumers especially in Egypt. In this respect, Beta-Cyclodextrin (β-CD) was reported to be effective in reducing the intensity of this flavor and as a result the commercial value of goat milk products was enhanced. In this research, the effect of reducing goat milk flavor in soft goat cheese by β-CD and their effect on texture and microstructure were investigated. The obtained results showed that the addition of β-CD at different concentrations (0.25g, 0.5 or 1.0% of milk) effectively trapped the short chain fatty acids and made goaty flavor un-sensed. The increase in moisture% and decreased in total solids% as a result of β-CD addition was also noticed. The addition of β-CD (0.25%) to goat milk cheese lead to an increase in firmness, cohesiveness, gumminess and chewiness and a decrease in springiness and resilience when compared to control. Electron micrographs of different goat cheese samples showed obvious microstructural changes in the protein network as affected by β-CD and these changes were Compatible with the changes in moisture and firmness.

Keywords: goat cheese, Beta – Cyclodextrin, goaty flavor, Texture, Microstructure.

INTRODUCTION

Goat milk and its conversion added value to the products made of it. (Yangliar, 2013) FAO 2014 The total population of goats attained more than billion heads in the world, and about 4 millions in Africa. The Number in Egypt about half million. The annual production of goat milk in Egypt is ranges from about 12,000-13,000 tons (Tantawy et al. 2006, Ryffel et al., 2008, Ribeiro and Ribeiro, 2010 and FAO 2014) Large amount of goat milk are usually processed by farmers and locally marketed (Pirisi et al., 2007, Igwegbe et al., 2015). Goat milk and its products are characterized by its medicinal, physiological and functional properties. It is considered as an excellent source of food for both infants and elderly people due to its digestibility and its content of huge numbers of of small fat globules (El-Tantawy et al. 2006, Ribeiro and Ribeiro 2010 Queiroga 2013, Igwegbe et al., 2015). Consuming goat milk could protect people fromcertain diseases (Ribeiro and Ribeiro, 2010).

Making cheese from goat milk is of an added value products ,which are desirable for numerous individuals. And were accepted by several consumers Goat chease is usually characterized with the presence of certain volatile free fatty acids, which add to it strong flavor (El-Tantawy et al. 2006, Ryffel 2008,Sadooghy-Saraby 2011, Yangliar 2013, and Igwegbe et al., 2015) However, Such strong flavor of goat cheese due to the presence of certain branched-chain fatty acids (4-Methyloctanoic and 4- ethyloctanoic acids,which) results in limitation of its consumption among most of the Egyptian individuals Sadooghy-Saraby 2011, Queiroga 2013, Ibrahim and Soryal 2014). Masking of the strong flavor of goat milk could be attained by the selection among the breed, lowering fat in goat milk, or by using different flavoring materials, Sadooghy-Saraby 2011), selective breeding to lower goat milk fat content (Harris and springers 2009), masking goaty flavor by using different flavoring materials (Young et al. 2012, and Hamad and Ismail2012), or by using Cyclodextrins (α-CD, β-CD and κ-CD) which are of nontoxic effect, and approved by FAO and WHO. (Gupta 2004 and Sadooghy-Saraby 2011),

Quality and acceptability of goat cheese greatly influenced by the microstructure of cheese and with its flavor(Burgos *et al.* 2016)

MATERIALS AND METHODS

Fresh goat's milk collected from two different small private farms located in Giza Governorate in Egypt were mixed and a one bulk (6.68 pH , 0.16% TA, 15.38% TS, 84.62% moisture, 4.75% Fat, 3.14% Total protein and 0.87% Ash) was kept under freezing conditions for one day before processing. A Commercially available lyophilized culture (Express 0.2, DVS) was from Chr. Hansen Laboratories, Copenhagen, Denmark. Single strength liquid calf rennet was obtained from Dairy unit, Dairy science Department, Faculty of Agriculture, Cairo University and it was added to the milk at rate of 1.5 mL Kg-1 milk. Beta cyclodextrin was purchased from AraChem, a fine chemicals company, the Netherlands.

24 Kg of goat milk was pasteurized at 72˚C/15 sec. then cooled to 48˚C before dividing to four equal portions. The first portion β-CD addition was considered as control (TCD0). B-CD was added to the other three portions in concentrations of 0.25%, 0.5% and 1.0% (treatments TCD0.25, TCD0.5 and TCD1.0) respectively. Then, the starter culture was added (0.2 gm Kg-1 of milk) at 42˚C and incubated at the same temperature for half an hour before the addition of rennet (1.5 ml Kg-1 milk). After complete coagulation, the resulted curds were poured in plastic frames covered by muslin for whey draining overnight. All fresh cheese samples were then analyzed.

The pH values of milk and yogurt samples were measured using a digital pH meter with a glass electrode (Jenway 3305, England). The titratable acidity (%) was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator. Total solids and Ash of the milk and cheese samples and total protein (%) in milk were determined according to (AOAC, 2000). Fat content (%) was determined by Gerber's method.

Determinations were performed in triplicate. Cheese yield was determined as wt. cheese×100/wt. milk.

Fatty acids in fresh soft goat cheese samples were determined in Food Safety and Quality Control Lab (FSQC), Faculty of Agriculture, Cairo University. The fatty acids were methylated and extracted according to the method described in AOAC, (2012). All solvents used for fatty acid extraction were of analytical grade and obtained from Sigma-Aldrich (Germany). The determination was done on the Agilent 5977E GC/MS. An Agilent DB-WAX 30 m × 250 µm, 0.5 µm column was used (p/n 122-7033). The carrier gas (He) flow rate was 30 ml min-1. During chromatography oven temperature raised from 100 to 230˚C at a rate of 12.5˚C min-1. Each peak was quantified by the retention time of reference standards.

The microstructure of different treatments were examined with a Quanta Scanning Electron Microscope (SEM) model FEG 250 electron probe microanalyzer using a magnification of 2.000X for all samples except for T1.0 which the magnification was 4.000X. Cubes of 1mm were fixed at room temperature in a 2.5% glutaraldehyde solution in phosphate buffer (0.1 M, pH 4.6). The fixed samples were washed with 0.1M phosphate buffer and dehydrated in a graded ethanol series (15, 30, 50, 70, 80, 95 and 100%, 30 min in each). Dried sections were mounted on aluminum SEM stubs with double-sided adhesive tape, and vacuum gold coated using S150A Sputter coater- Edwards-England before screening and imaging.

Texture profile analysis (TPA) was conducted using a universal testing machine (Cometech, B type, Taiwan) provided with software. TPA was performed using a cylinder flat-ended probe (P/2 SL) of 2.5 cm of diameter. Three cylindrical sections (4 cm diameter and 1.5 cm height) from each cheese sample were compressed twice using a crosshead speed of 1mm/sec. The second compression was delayed 5 seconds from the first compression. Cheese samples were allowed to equilibrate at room temperature (25 ºC) prior to testing. From the resulting force–time curve, the values for texture attributes, i.e. firmness (N), gumminess (N), chewiness (N), adhesiveness negative force (N), cohesiveness, springiness and resilience were calculated from the TPA graphic. Both, springiness and resilience, give information about the after stress recovery capacity. But, while the former refer to retarded recovery, the latter concerns with instantaneous recovery (immediately after the first compression, while the probe goes up).

Statistical analysis

The data were analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS (SAS Institute, Inc., Cary, NC). Data were subjected to one-way analysis of variance (ANOVA) with comparison of differences between the means of the treatments at the significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Fresh soft goat cheese samples from control (TCD0) and the three other treatments (TCD0.25, TCD0.5 and TCD1.0) were chemically analyzed. The actual yield was calculated and its values for treatments TCD0, TCD0.25, TCD0.5 and TCD1.0 were 40%, 32%, 33% and 34% respectively. The TA% and pH values were recorded to be about 0.9 and 4.9 respectively with no remarkable different between treatments. Table (1) gives the results for the TS (%), Moisture (%), Fat (%), Ash (%) and Fat/DM for all cheese samples. The control cheese sample (TCD0) had the highest Moisture (%) and the lowest TS (%) among all treatments. The addition of β-CD in TCD0.25, TCD0.5 and TCD1.0 samples led to a significant reduction in moisture (%) and an increase in TS (%) when compared to control. Concerning fat content (%), all processed samples contained almost the same percent and there were no significant differences ($P \leq 0.05$) between the samples. The ash% was significantly the lowest in TCD0 sample while there were no significant differences in ash% among the three other treatments. The fat in dry matter (Fat/DM) was significantly decreased than control in treatment two (TCD0.25) as it is directly related to the change in total solids of the cheese. While, there were no significant differences in Fat/DM between the other three treatments. From these results it is important to highlight that the addition of0.25% of β-CD to milk cheese is enough to affect the TS% and moisture % as well as the Fat/DM in soft goat cheese.

Table 1. Chemical composition of soft fresh goat cheese samples

Parameter	TCD_0	$TCD_{0.25}$	$TCD_{0.5}$	TCD1.0
TS (%)	27.0 ± 0.10^{c}	32.4 ± 0.17^{a}	31.9 ± 0.17^{a}	31.1 ± 0.47^{b}
Moisture (%)	73.0 ± 0.10^{a}	67.6 ± 0.17^{c}	68.1 ± 0.17^{c}	68.9 ± 0.47^{b}
Fat (%)	13.5 ± 1.50^{a}	13.0 ± 1.0^{a}	14.5 ± 0.50^{a}	14.3 ± 1.50^{a}
Ash (%)	0.93 ± 0.12^{b}	1.33 ± 0.06^{a}	1.23 ± 0.06^{a}	1.23 ± 0.06^{a}
Fat/DM	50.00 ± 5.70^{a}	40.10 ± 3.30^{b}	45.50 ± 1.80^{ab}	46.10 ± 5.60^{ab}

Means in the same raw having different small superscript letters are significantly different at $P \leq 0.05$.

Fatty acids profile

It is well known that the straight chain fatty acids (C6 to C10) have a goaty note (Brennaned *et al.* 1989). Data presented in Table (2) shows that the addition of β-CD to goat cheese milk led to a decrease short chain fatty acids in soft goat cheese samples when compared to control without the addition of β-CD prior to cheese processing. Also, the decrease in individual SCFAs or their sum was increased as the amount of β-CD increased. These results could be supported by the finding of Sadooghy-Saraby (2011). Who found that although the addition of lipase to goat milk generates free fatty Acids (FFAs) but the presence of β-CD trapped them and made them none sensed and can't be detected by gas chromatography. Also, Young *et al.* (2012) concluded that β-CD is so useful in minimizing goaty flavor in milk and yogurt.

The changes in the texture profile (TPA)of the control and different other soft goat cheese samples with different amounts of β-CD in terms of Firmness, Cohesiveness, Gumminess, Chewiness, Springiness and Resilience are shown in Table (3). Under current study the addition of β-CD significantly affect all texture profile parameters as compared with control when it

was added in a 0.5% (TCD0.5) to cheese milk which was the highest value among all treatments. This result can be supported by the decrease in moisture% (Table 1) as previously explained by Buriti *et al.* (2005) who explained the importance of moisture in influencing the cheese texture. They reported that the high initial moisture weakens the protein network leads to more soft cheese matrix. The Cohesiveness values ranged from 0.29 to 0.43 showed a slightly significant increase in TCD0.25 but it was decreased significantly by increasing the percent of β-CD addition to be 0.29 for TCD0.5 and 0.31 for TCD1.0 with no significant differences between them. Gumminess and Chewiness values (0.86 and 0.65 respectively) of TCD0.25 were significantly (P≤0.05) increased than control (0.23 and 0.20 respectively) while they decreased again by increasing the percent of β-CD to be 0.53 for Gumminess and 0.15 for Chewiness in TCD1.0. As a result of β-CD addition to cheese milk in treatments TCD0.25, TCD0.5 and TCD1.0 the values of both Springiness and Resilience have the same trends. The highest Springiness and Resilience value (P≤0.05) were recorded in control sample TCD0 to be 0.84 and 0.23 respectively. While there were significant decrease in their values in the other treatments especially for TCD1.0 which represented 0.29 for Springiness and 0.13 for Resilience.

Table 2. Fatty acids relative distribution (%) of soft fresh goat cheese samples

Fatty acid	TCD_0	$TCD_{0.25}$	$TCD_{0.5}$	$TCD_{1.0}$
C4:0	2.164	1.647	1.488	1.560
C6:0	3.832	3.144	3.014	2.950
C8:0	5.258	4.606	4.507	4.290
C10:0	12.848	13.974	11.894	12.800
ΣSCFAs	24.102	23.371	20.309	21.600
C11:0	0.160	-	0.151	0.149
C12:0	6.859	6.469	6.610	6.161
C14:0	13.384	13.18	13.552	12.94
C14:1	0.511	0.497	0.543	0.507
C15:0	1.000	0.953	1.062	0.977
C15:1	0.212	0.208	0.230	0.214
C16:0	29.358	32.433	29.492	31.849
C16:1	1.041	0.941	1.140	1.065
C17:0	1.250	1.082	1.254	1.159
C17:1	0.162	-	0.182	0.168
C18:0	13.283	12.637	15.061	13.734
C18:1	7.661	7.204	8.445	7.791
C18:2	0.281	0.263	0.306	0.283
Non identified fatty acids	0.736	0.762	1.140	1.403

ΣSCFAs: sum of short chain fatty acids (C4-C10)

Table 3. Texture profile analysis (TPA) of soft fresh goat cheese samples

Cheese sample	Firmness	Cohesiveness	Gumminess	Chewiness	Springiness	Resilience
TCD0	0.53^{c} ±0.01	0.43^{b} ±0.01	0.23^{c}±0.004	0.20^{b}±0.004	0.84^{a}±0.02	0.23^{a} ±0.004
$TCD_{0.25}$	1.64^{b} ±0.04	0.52^{a} ±0.03	0.86^{a} ±0.04	0.65^{a} ±0.03	0.72^{b}±0.04	0.18^{b} ±0.01
$TCD_{0.5}$	1.94^{a} ±0.03	0.29^{c} ±0.01	0.55^{b} ±0.01	0.21^{b}±0.004	0.36^{c} 0.01	0.18^{b} ±0.004
$TCD_{1.0}$	1.72^{b} ±0.06	0.31^{c} ±0.01	0.53^{b} ±0.02	0.15^{c} ±0.01	0.29^{d}±0.01	0.13^{c} ±0.01

Means in the same column having different small superscript letters are significantly different at $P \leq 0.05$.

The microstructure of goat cheeses was fixed using Scanning Electron Microscope (SEM) and their micrographs are shown together with the decreasing in moisture and increasing in firmness as a percent from control sample (Figure 1. A and B). For TCD0 sample, its micrograph represents a homogeneous structure, with small protein aggregates linked together (black circle) and small pore sizes in it (white arrow). These pores are for water (or whey) which it is physically entrapped in those pores of the gel network and they are depends on aggregation dynamics (Ericili-Cura, 2012) and the more the large protein network formed during and after gel formation the more the water retention in the gel (Walstra *et al.* 1985). Also, the important of water in the gel structure was previously explained by Hermansson, (2008).. The micrographs of cheese samples with different β-CD amounts (TCD0.25, TCD0.5 and TCD1.0) resulted in a different microstructure compared to control. These changes were clearly related to the decrease in moisture % and increase in firmness % as presented in Figure 1. (A). The protein networks appear as separate large aggregates not in a continuous gel network with a big empty cavities in between these aggregates for treatments TCD0.25, TCD0.5 and TCD1.0. Also, the fat appears in a separate contentious areas not connected with those protein aggregates. These observations may be due to the complex formation between β-CD and milk fat in a form of host-guest complex as discussed by Sadooghy-Saraby (2011). He mentioned that in hydrophobic interaction of Cyclodextrins the most non-polar part of the guest molecule (milk fat in our study) is enclosed in Cyclodextrin cavity and the hydrophilic groups of the complexing agent (Cyclodextrin) interact with water insuring the solubility of the complex.

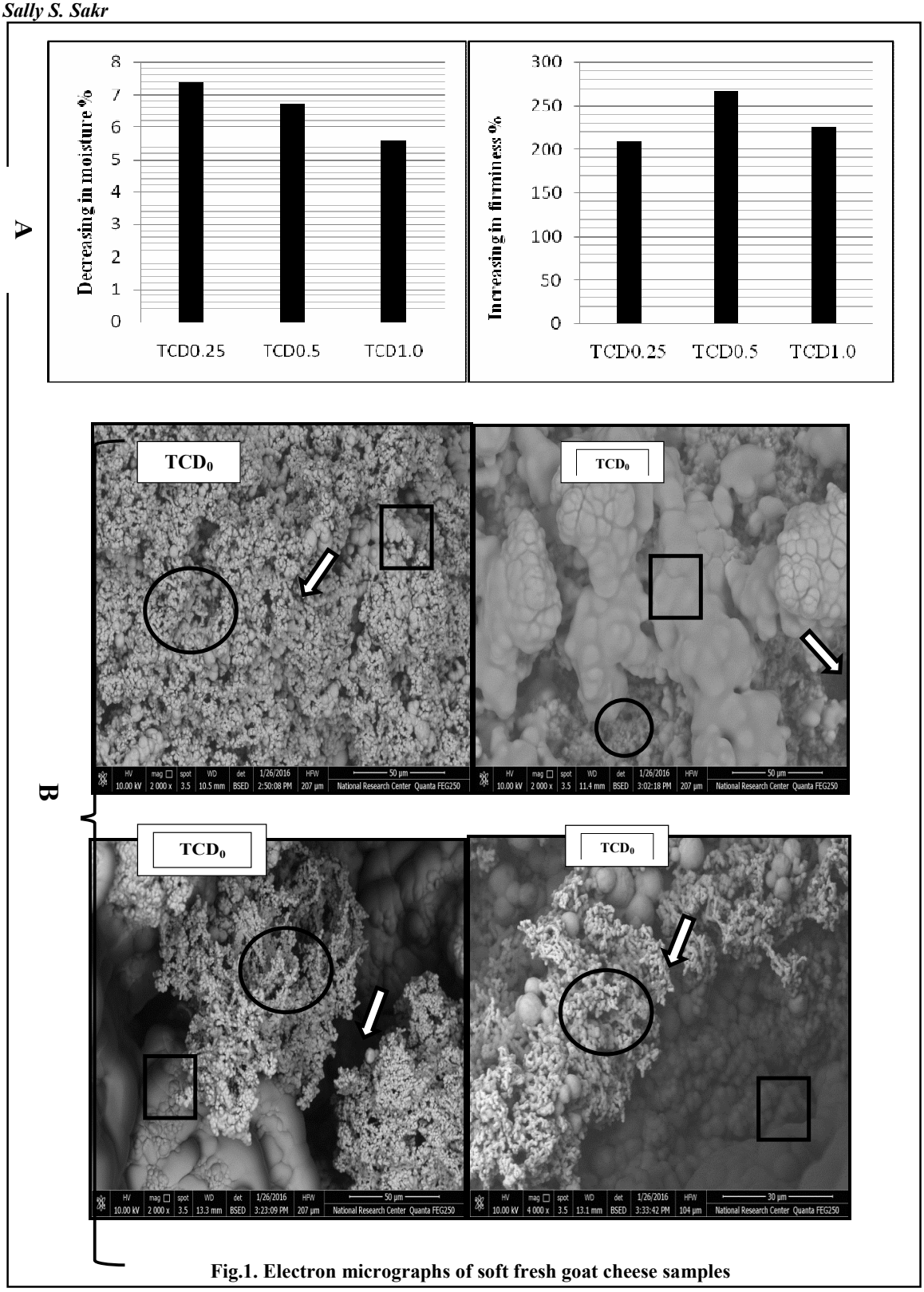

Fig.1. Electron micrographs of soft fresh goat cheese samples

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خفض نكهة لبن الماعز باستخدام البيتا سيكلودكسترين و تأثيرة علي التركيب و البناء الدقيق لجبن الماعز الطري

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على الرغم من التوصيات الخاصة باستهلاك لبن الماعز والمنتجات المصنعة منه كغذاء طبي، إلا أن نكهة لبن الماعز القوية تحد من قبوله للإستهلاك من من قبل غالبية المستهلكين خاصة في مصر، و في هذا الصدد درس الباحثون إستخدام مركب البيتا سيكلودكسترين كمادة فاعلة في إخفاء هذة النكهة والحد من الإحساس بها و ما يترتب عليه من تعزيز القيمة الإستهلاكية و التجارية لمنتجات لبن الماعز، وبناء عليه فقد تم في هذا البحث دراسة تأثير خفض نكهة لبن الماعز في الجبن المصنع منه بواسطة إضافة البيتا سيكلودكسترين وتأثير ذلك على التركيب و البناء الدقيق في الجبن الناتج، وأظهرت النتائج التي تم الحصول عليها إلي أن إضافة البيتا سيكلودكسترين بالتركيزات المختلفة (٠.٢٥ أو ٠.٥ أو ١.٠٪ من اللبن) كان لها دور فعال في خفض نسبة الأحماض الدهنية قصيرة السلسلة مما ترتب عليه خفض الإحساس بالنكهة القوية للبن الماعز، كما لوحظ وجود زيادة في النسبة المئوية للرطوبة مع إنخفاض في النسبه المئوية للمادة الجافة بسبب تلك الإضافه ، و من حيث القوام و التركيب الدقيق فقد تسببت إضافة البيتا سيكلودكسترين (٠.٢٥٪) إلى لبن جبن الماعزفي حدوث تغييرات معنوية في العديد من صفاته التركيبية، كما أظهرت الصور المأخوذة من الميكروسكوب الإلكتروني الماسح أن إضافة البيتا سيكلودكسترين نتج عنها حدوث تغيرات واضحة في شبكة البروتين و كانت هذه التغييرات متوافقة مع التغيرات في نسبة الرطوبة والصلابة في الجبن الطازج و ذلك لكل المعاملات تحت الدراسة عند مقارنتها بالكونترول.