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Evaluating Milk Coagulants from Seeds of *Solanum Elaeagnifolium* Plant against Animal and Microbial Rennet in Manufacturing White Soft Cheese

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IN this study, white soft cheese (WSC) was made from buffalo's milk using different coagulants. Three treatments were made. All cheese treatments were made with traditional cheese starters (*Lactococcus lactis* spp *lactis* and *Lactococcus lactis* spp *cremoris*, 1:1). The first (T0) cheese was made with veal rennet (control). The other treatments were made by using microbial rennet (T1) and the purified milk clotting enzyme (MCE) from *Solanum elaeagnifolium* (T2). All cheeses were stored at 7ºC for 30 days for ripening and were periodically examined chemically, rheologically, organoleptically and for ripening indices. Results indicated that white soft cheese (control) contained high moisture content. The titratable acidity, total protein and soluble nitrogen (SN) ratio examined was slightly lower compared to other treatments. Regarding the rate of accumulation of total volatile fatty acids (TVFA), it was increased during storage in the three cheese treatments. Regarding the sensory evaluation the highest number of purified enzyme was present in cheese treatment (T2). In all treatments, cheese acceptability increased during different intervals of storage periods (0, 15 and 30 days). However, the improvement was slow in the control sample, while faster in purified enzyme samples. It could be concluded that white soft cheeses, with good characteristics can be produced from heat treated buffalo's milk using the purified coagulant from fruit seeds of the plant *Solanum elaeagnifolium* addition as a traditional starter.

Keywords: Soft Cheese, Coagulation, Milk clotting enzymes, Microbial, Veal rennet.

Introduction

International cheese produce increased by about 3.6 million tons (FAO, 2010), while veal rennet supply decreased because of the shortage of calves. There is need to search for other alternatives from different sources. Veal rennet contains chymosin (EC 3.4.23.4) and has been widely used as a MCE. High increased international cheese production and reduced supply of veal rennet, have led to systematic investigation for new alternatives to MCE from other different sources. Much research working has directed towards discovering MCE (Cavalcanti et al., 2004) which would satisfy replacing veal rennet in cheese production.

Moreover, people stipulation the use of MCE from the plant sources have led to new research field recently. Microbial rennet produced by bacteria and fungi has proven as a suitable substitutes for veal rennet, but increasing attention has been on MCE extracted from plants (Tavaria et al., 2001) such as chymopapain, papain, endoprotease papaya (*cysteine protease*) and carican, exoprotease which has been purified from commercial Carica papaya, Bromelain from pineapple (*Ananas comosus* L.), (*Bromelia plumieri*) and (*Cynara cardunculus*) (Goodenough & Owen, 1987; Maksimenko et al., 1990; Monates et al., 1990, Zimacheve et al.,1994). Plant proteases are interesting in food usages because they can be easily extracted by hydrolysis and at low cost (Silva & Malcata, 2005).

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Different substitutes for MCE from different sources were proposed over time for cheese production but only a few were accepted due to the sensory and chemical characteristics of different types of cheeses obtained. Traditionally, rennet is obtained from the abomasum of animals such as veal, pig, goat etc. The veal rennet was the main rennet used for a long time. Due to the increase in cheese manufacture, a problem arose to supply veal rennet. Therefore, it was necessary to find alternatives from MCE to replace veal rennet without reducing product quality (Alihanoğlu et al. 2018; Akbar et al., 2019; Kiss et al., 2019).

Cheese manufacturing should be expanded to improve consumers nutritional status at low cost, empower small-scale farmers and stimulate the local economy as it can be an ideal way of preserving milk and helps fight malnutrition in poor communities (Nyamakwere et al., (2021). MCE used must be of very high in clotting activity (CA) and low in proteolytic activity (PA) (ratio: CA/PA). This depends on the activity of the enzyme to hydrolyze specifically on -casein (Jacob et al., 2011). Most MCEs from plants are able to hydrolyse the κ-casein, whey proteins and other casein. Consequently, only few MCE obtained from plants are suitable for the production of cheese.

In cheese production, the MCE from the plants has been used in West African, Mediterranean and southern European countries**.** Currently in Portugal and Spain more types of cheeses are being made using *Cynara sp.* as an MCE from plant. In Nigeria, MCE extracts from *Calotropisprocero* are used in cheese manufacture **(**Shah et al., 2014). The MCE extract from seeds of *Solanum dubium* is used in the making of cheese in Sudan (Guiama et al., 2010). In Mexico, the berries are used as MCE in the formation of artisanal soft asadero cheese (Gutiérrez-Méndez et al., 2012).

Solanum elaeagnifolium is a wild herb in the El-Arish area, North Sinai Governorate, Egypt. Its MCE was separated and purified in previous work (Kholif et al., 2016). The main objective of this work was to use MCE *Solanum elaeagnifolium* in manufacturing white soft cheese and comparison by microbial and animal rennet in terms of chemical, sensory and rheological quality.

Materials and Methods

Materials

Fresh buffaloes' milk was obtained from the animal farm managed at Faculty of Agriculture, Cairo University, Giza, Egypt. Rennet: Liquid rennet was obtained from Dairy Science and Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt at the rate of 3 mL/100 L. Microbial rennet powder Chy-Max from Chr. Hansen., Holding A/S, Boege, 2970 Hoersholm, Danmark, was used for cheese production at the rate of 1g /100 L. Commercial sodium chloride was obtained from El-Nasr Company, Alexandria, Egypt.

Plant extraction

The *Solanum elaeagnifolium* plant seed used in this study were collected from the Faculty of Environmental Agricultural Sciences farm, El-Arish, North Sinai Governorate, during the month of May when the fruits was yellow, with black and completely dry seeds. The *Solanum elaeagnifolium* whole berries were dried at (30ºC) for 7 days carefully cleaned and the outer shell of the fruits was removed. The seeds were powdered using an electric grinder. The dry powder (5 g/30 mL) was macerated with different buffers (Cheded, 1975).

Methods

Extraction of crude enzyme extracts

Five grams of seed powder were soaked in a conical flask for 24 h at 5ºC using 0.1 M sodium phosphate pH 5.9 (30 mL) with frequent shaking for the first 3 hours and solutions were then centrifuged at 8000/g at 4ºC for 20 min, then filtered through filter paper (Whatman No.1). The aqueous filtrate was used for testing milk clotting activity (Abdalla et al., 2011).

Determination of milk clotting activity

One milk-clotting unit is defined as the amount of enzyme that clots 10 ml of the substrate within 40 min. Milk-clotting activity was determined according to the method described by Arima & Iwasaki (1970) with a slight modification. The substrate (10% skim milk in 0.01 M $CaCl_2$) was prepared and the pH was adjusted to 6.5. The substrate (2.0 mL) was pre incubated for 5 min at 37°C, and then 0.2 ml of MCE was added. The curd formation was observed at 37°C while manually rotating the test tube from time to time. The end point was recorded when discrete particles were discernible (Ahmed et al., 2010; Guiama et al., 2010; Gutiérrez et al., 2012).

Experimental procedure of manufacture of white soft cheese made from heat treated milk

White soft cheese was manufactured from standardized raw and heat-treated buffaloes'

milk (72°C/2 min) according to Fahmi & Sharara (1950). Cheese milk (5.5-6.5% fat, 3.5–3.9% protein and 15.5-15.84% TS) was salted with 4% NaCl, 0.7 mL/L veal rennet (control), 0.66 mL/L microbial rennet (T1) and 1 mL/L from purified MCE isolated from *Solanum elaeagnifolium* (T2). The resultant cheese was cut into equal pieces and packaged in plastic bags then covered with heat-treated whey (72°C/2 min). The bags were sealed then stored at refrigerator (~ 7±3°C) for 15 days. Cheese samples were analyzed periodically when fresh, 15 and 30 days for its chemical quality.

Physico-chemical and chemical analysis

The pH values were measured by using a digital pH meter (Jenway 3305, Jenway Limited, Essex, England). Titratable acidity (TA), ash, protein contents computed as total nitrogen (TN) and soluble nitrogen (SN) (determined through Kjeldahl (Gerhardt Vapotest VAP 30 distillation systems, Gerhardt Turbotherm and Turbosog digestion systems) were measured. The fat was determined by using centrifuge at 60°C for 3-5 min at 1300 rpm in a Centrifuge Gerber Compact (Gerber Instruments AG, Effretikon, Switzerland). The total volatile fatty acids (TVFA) were determined in cheese samples according to the method described by Kosikowski (1982). The value of TVFA was expressed as mL of 0.1 N NaOH/100 g cheese. All of these were conducted using the official methods (AOAC, 2012). All trials were conducted in triplicate.

Disc-PAGE electrophoresis

For preparing the separating gel 15%, 2.4 mL DH_2O, 5 ml (29.2% acrylamide and 0.8% bis-acrylamid), 2.5 mL 1.5M tris pH8.8, 100 uL 10% SDS, 100 uL 10%APS and 100 uL TEMED were used. Then, for preparation of the Stacking gel 4%, 6.1 ml dH2O, 1.3 mL (29.2% acrylamide and 0.8% bis-acrylamid), 2.5 mL 0.5M tris pH6.8, 100 uL 10% SDS, 100 uL10% APS and 100ul TEMED were mixed. Then, in preparing running buffer, 25 mM Tris-Hcl, 200 mM Glycine and 0.1% (W/V) SDS Start run (80V for 4 hr) were added. After run SDS the gel in staining solution (50% DH_2O, 40% methanol, 10% glacial acetic acid and 0.1% coomassie brilliant blue) for 20 min with gentle agitation was used. Destain gel in destaining solution (50% dH2O, 40 % methanol and 10% glacial acetic acid) (Laemmli, 1970).

Textural profile analysis

Textural profile analysis was performed on the soft cheese sample according to the method of Glibowski et al. (2008) using the double compression test (TA-XT2i texture analyzer (Stable Microsystems, Godalming, UK); Experiments were carried out by compression tests that generated plot of force (N) versus time (s). A 25 mm diameter perplex conical- shaped probe was used to measure the TPA of the soft cheese sample in their cups performing five repetitions. In the first stage the samples were compressed by 30% of their original depth. The speed of the probe was 2 cm min during the pretest.

Sensory evaluation

Cheese samples were organoleptically scored for flavor (50 points), body and texture (40 points) and appearance (10 points) according to the score card suggested by Davis (1965). Samples were judged by the staff members of the Dairy Science Department, National Research Center

Statistical analysis

Statistical analysis was performed according to (SAS Institute 1990), using General Linear Model (GLM) with the main effect of treatments. Duncan's multiple range was used to separate among of three replicates at $p \leq 0.05$.

Results and Discussion

Activity of plant extracted enzyme under different buffering conditions

In a previous study by Kholif et al. (2016) For the extraction of the coagulant, fruit seeds of Solanum elaeagnifolium plant in four extractions buffers (distilled water pH 6.8, 0.1 M acetate pH 5.6, 0.1 M sodium phosphate pH 5.9 and 0.1 M citrate pH 5.6), were tried to select the one with a reasonably high activity. The results showed extraction in buffer 0.1 M sodium phosphate pH 5.9 gave the highest MCA (19.21 IU/mL), Specific activity (960.50), while the distilled water, acetate and citrate buffers gave low activity. And was obtained purification results of the MCE using different purification means resulted in 4.21 rate purification with a yield of 4.008 % and specific activity of 3850.

Yield and chemical analysis of cheese treatments

Veal rennet is most well-suited for clotting milk, offering high in yields after cheese manufacture (Jacob et al., 2011). Also the impact of different coagulant types on yield percentage of Domiati cheese (Darwish, 2016). The yield (%) in all cheeses decreased significantly ($P < 0.05$) during pickling as presented in Table 1. There were differences in yield when using three types of MCE. The highest cheeses yield in veal rennet (control) was significantly ($P < 0.05$) when

compared to microbial (T1) and plant coagulants (T2) which was not significantly different ($P < 0.05$). Cheese yield from also depends on other factors such as the chemical composition of milk, the processing methods used and the type of cheese. Cheese gives yield about 20%, for soft cheese has been reported (Lobato-Calleros et al. 2002; Lobato-Calleros et al. 2007).

In this study, cheese yield of 32.1% was given when using veal rennet as the coagulant while 31.8% yield for plant extract rennet. Microbial rennet showed the lowest yields 29.5% (Table 1).

Aworh and Muller (1987) reported that in the manufacture of West African cheese by using MCE extracted from Sodom apple (*Calostropis procera*) plant, the cheese obtained 0.73% higher yield compared to that of veal rennet. The moisture contents (%, w/w) of cheese produced using animal rennet, microbial or plant extract were 65.11%, 63.03% and 64.65%, respectively for fresh cheese at day1. The cheese obtained with microbial rennet showed the lowest moisture content compared with the other treatments, although this is still in the range of the moisture reported for soft cheese (Lobato Calleros., 2006; Torres-Llanez et al., 2006). The study results by Mamo et al. (2020) found lower moisture content in salted stored Danbo Cheeses. The fat content as well as total protein content were slightly low in all treatments of cheese during refrigerated storage.

TABLE 1. Curd yield and chemical composition of soft cheese using animal & microbial rennet and plant extract.

Parameter	Treatments	Storage period (days)			
		Fresh	15	30	SE
pH	Control	$6.83^{a}_{\pm 0.07}$	$6.31^{b}_{\pm 0.06}$	$6.08^{c}_{\pm 0.06}$	0.04
	T1	$6.65^{a}_{\pm 0.07}$	$6.10^{b}_{\pm 0.06}$	$5.70^{c}_{\pm 0.06}$	0.09
	T2	$6.88^{a}_{\pm 0.07}$	$6.27^{b}_{\pm 0.06}$	$6.05^{b}_{\pm 0.06}$	0.006
Acidity	Control	$0.18^{a}_{\pm 0.03}$	$0.20^{b}_{\pm 0.04}$	$0.22^{c}_{\pm 0.05}$	0.011
	T1	$0.17^{a}_{\pm 0.01}$	$0.21^{b}_{\pm 0.04}$	$0.24^{c}_{\pm 0.05}$	0.014
	T2	$0.18_{\pm 0.03}$	$0.22_{\pm 0.04}$	$0.26_{\pm 0.03}$	0.020
Moisture	Control	$65.11^{a}_{\pm 0.65}$	$65.00^{a}_{\pm 0.65}$	$64.90^{a}_{\pm 0.65}$	0.13
	T1	$63.03^{a}_{\pm 0.63}$	$63.12^{a}_{\pm 0.63}$	$63.30^{a}_{\pm 0.63}$	0.012
	T2	$64.65^{a}_{\pm 0.65}$	$64.68^{a}_{\pm 0.65}$	$64.55^{a}_{\pm 0.65}$	0.012
Total Protein	Control	$12.2^{a}_{\pm 0.12}$	$12.2^{ab}_{\pm 0.13}$	$12.3^{b}_{\pm 0.10}$	0.005
	T1	$11.9^{a}_{\pm 0.13}$	$12.2^{ab}_{\pm 0.13}$	$12.4^{b}_{\pm 0.12}$	0.004
	T2	$12.1^{a}_{\pm 0.11}$	$12.4^{ab}_{\pm 0.12}$	$12.5^{b}_{\pm 0.10}$	0.004
Fat %	Control	$18.40^{a}_{\pm 0.18}$	$18.20^{ab}_{\pm 0.18}$	$18.00^{b}_{\pm 0.18}$	0.006
	T1	$18.60^{a}_{\pm 0.19}$	$18.20^{a}_{\pm 0.18}$	$18.00^{a}_{\pm 0.18}$	0.033
	T2	$20.40^{a}_{\pm 0.20}$	$20.10^{a}_{\pm 0.20}$	$20.00^{a}_{\pm 0.20}$	0.057
Fat/DM	Control	$52.76^{a}_{\pm 1.51}$	$52.02^{a}_{\pm 1.49}$	$51.30^{a}_{\pm 1.46}$	0.029
	T1	$50.33^{a}_{\pm 1.36}$	$49.36^{a}_{\pm 1.34}$	$49.06^{a}_{\pm 1.34}$	0.031
	T2	$57.73^{a}_{\pm 1.63}$	$56.93^{a}_{\pm 1.61}$	$56.44^{a}_{\pm 1.59}$	0.024
Ash	Control	$0.77^{b}_{\pm 0.02}$	$0.76^{ab}_{\pm 0.0.3}$	$0.78^{a}_{\pm 0.012}$	0.0044
	T1	$0.77^{c}_{\pm 0.02}$	$0.77^{bc}_{\pm 0.041}$	$0.79^{a}_{\pm 0.021}$	0.009
	T2	$0.77^{b}_{\pm 0.024}$	$0.78^{b}_{\pm 0.024}$	$0.80^{ab}_{\pm 0.021}$	0.004
Yield	Control	$32.10_{\pm 0.031}$	$32.42_{\pm 0.013}$	$31.78_{\pm 0.011}$	0.004
	T1	$29.50_{\pm 0.021}$	$29.80_{\pm 0.012}$	$29.21_{\pm 0.011}$	0.009
	T2	$31.80_{\pm 0.031}$	$32.12_{\pm 0.011}$	$31.48_{\pm 0.011}$	0.004

C: Animal rennet. T1: Microbial rennet. T2: Plant extract.

Regarding the acidity and pH values shown in Table 1, the decrease in pH and increase in acidity during storage indicate significant relation ($p \leq 0.05$) between treatments and time. Acidity in the cheese samples on zero time were 0.18, 0.17 and 0.18, while at 30 days were 0.22, 0.24 and 0.26 for control, T1 and T2 ,respectively, and using averages and corresponding 95% confidence intervals, it is concluded that the MCE had no significant effect at moisture, fat, total protein and ash contents, where there were no significant differences between the treatments (see Table 1). However, pH at the surface after 30 days was significantly ($P < 0.05$) higher in cheese manufactured by MCE from the plant compared to other treatments. Mamo et al. (2020) reported that by adding *mesophilic S. lactis* (LAB) in raw milk reduced the pH of the milk. Renneting with the crude fungal and the commercial enzymes slightly decreased the pH (0.8–1.0), the corresponding values of the pH after 30 days of storage were 4.14 and 3.94 for the same treatments, in the same order. The pH at the surface of soft cheeses produced from MCE extracts of C. *Cardunculusor* using veal rennet was slightly lower to that of raw milk (results not shown). It may occur due to the absence of the starter cultures. The decline in pH with whey drainage at the surface and the center of the curd cheese during coagulates may be due to high activity of metabolic bacteria of such groups that show high growth rates in the interior of the cheese as *Lactococcus, Enterobacteria* and *Lactobacillus*. (Fernandez del Pozo et al., 1988) found that, the pH increased highly after 28 days of storage at the surface of treatments, particularly in cheeses manufactured with MCE from extracts of C. *cardunculus*. This observation is probably due to metabolism by lactic acid-utilizing yeasts.

Total protein percentage with different coagulants during storage

Results of TN in white cheeses made from mix of buffalo's milk and treated with three different coagulants during storage are recorded in Table 1. These results showed TN in all experimented white cheeses with the advancement of the storage period. TN at zero time in white cheese samples were 11.83, 11.88 and 12.03 for control, T1 and T2 cheeses, respectively. The corresponding values after 30 days of refrigerated storage were 12.25, 12.31 and 12.45 for the same treatments, in the same order. White cheese with T2 exhibited obviously higher TN than that in control and T1 cheese. El- Kholy (2015) and Abd El-Salam et al. (2017) reported that, the TN content was increased pronouncedly ($p \leq 0.05$) during storage period. Also, there is a marked ($p \leq 0.05$) variation between treatments. Hattem & Hassabo (2015) also reported increase in TN during storage. This may be due to the corresponding decrease in water content.

Soluble nitrogen percentage with different coagulants during storage

In Fig. 1 The SN results of control and other white cheese treatments with the different coagulants and the effect during the ripening period at 15ºC for 30 days are included. It is that, rate of SN has been increased during ripening period. This was attributed to the rate of PA, throughout the storage period. The SN contents of white cheeses samples were 0, 11, 0.108 and 0.134% for control, TI and T2 cheeses at zero time respectively. These results indicated that treatment T2 had the highest SN content compared to control and T1 treatments after 30 days. Moreover, T1 treatment had the lowest SN content. From the afore mentioned results, it could be seen that the use of different coagulants with starter culture in the manufacture of white cheeses results in an increase in SN content. This increase could be due to the activity of protease and peptidases released from different coagulants and starters culture, hence increasing proteolysis in cheese. These results are similar with Abd El-Salam et al. (2017). They found that SN values in cheese made with purified enzyme from Artichoke (*Cynara cardunculus* L.) flowers were higher than veal rennet.

Total volatile fatty acids (TVFA) contents during storage

Results in Figure 2 showed the TVFA in white cheese treatments with three coagulants during the storage period at 7 ºC for 30 days. The obtained results indicated that, the TVFA has been increased ($p \leq 0.05$) with the increase of the ripening period in all cheese treatments. Values of TVFA in the cheese samples on zero time were 12.14, 13.2 and 14.15 ml of 0.1 N Na OH/100g cheese for control, TI and T2 cheeses, orderrespectively. The corresponding values for ripening cheese after 30 days were 52.5, 55.15 and 79.85 of 0.1 N Na OH/100 g cheese for treatments in the same order. From these results, it could be noticed that among samples, T2 possessed the highest value ($p \leq 0.05$) of TVFA while control (C) had the lowest ($p \leq 0.05$). These observations could be due to the difference between samples

in activity of lipolytic activity. Abou-Zeid (2015) reported that, the MCE was prepared from the composite weed *Cichorium pumilum*. Cichorium enzymes purified extract (CEPE) was added at concentrations range of 0.1 to 0.3 % (V /V) to milk (M samples) or to cheese curd (P samples). They illustrated that the level of TVFA in the control sample were significantly decreased (p 0.05) than those of the corresponding treated cheeses. El-Kholy (2015) reported that obtained data of TVFA showed that cheese made with vegetable coagulants (*Cynara scolymus*) contained more volatile acids (p ≤ 0.05) compared to control cheese at each period of ripening. TVFA were significantly high (p ≤ 0.05) throughout cheese ripening. However, this increase was noticeable in (pineapple, bcarica papaya, Husk tomato) cheese followed by control (El-Hawary et al., 2015). Shehata et al. (2004) found that the cheese treated with cheese slurries had higher TVFA contents, and the values increased the ripening period for all cheese treatments. Hattem and Hassabo (2015) found that, the TVFA content gradually increased (p ≤ 0.05) with nearly the same rate in all samples with the prolongation of the ripening period.

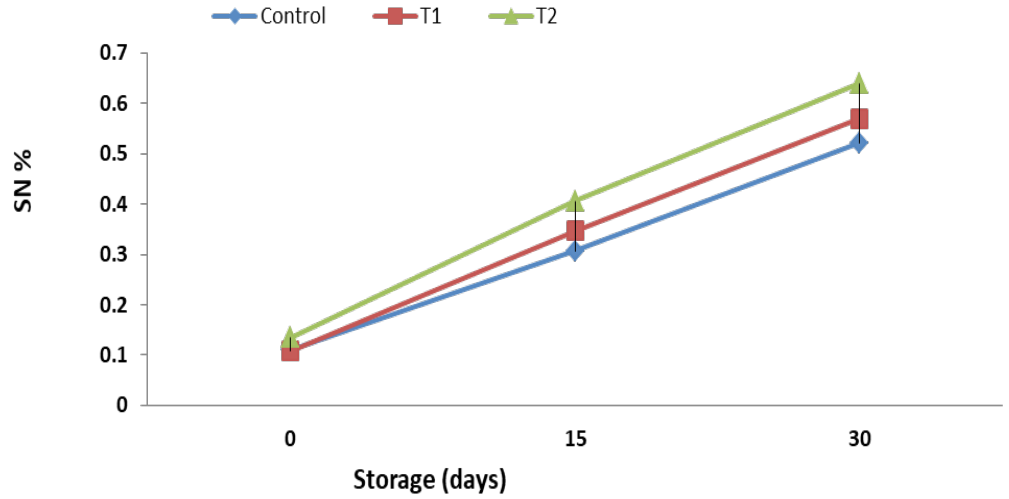

Fig. 1. Soluble nitrogen contents of soft cheese treated with three different coagulants during storage.

C: Animal rennet. T1: Microbial rennet. T2: Plant extraction.

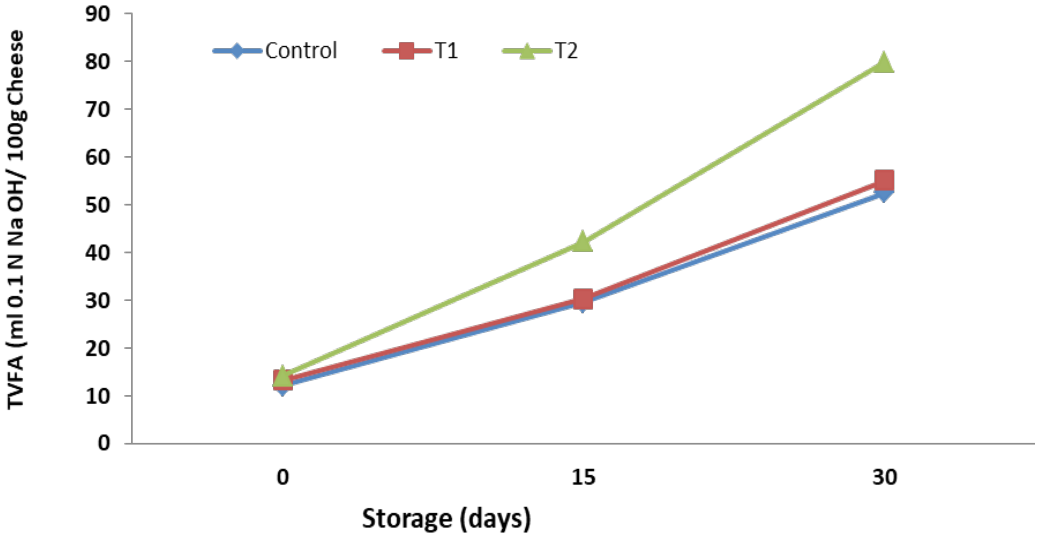

Fig. 2. Total volatile fatty acids contents of soft cheese treated with three different coagulants during storage.

C: Animal rennet. T1: Microbial rennet. T2: Plant extraction.

Textural profile analysis

Textural properties are one of the most essential attributes that determine the acceptance and quality of soft cheese. The results showed different textural properties in Table 2. The hardness of soft cheese was affected by three different coagulants during ripening period. In general, the sample with microbial rennet (T1) exhibited higher hardness than control followed by T2 treatment with plant extract at 15 and 30 days of storage. While On the fresh day, cheese samples with MCE (T2) exhibited higher hardness. During ripening the hardness in cheese samples increased significantly ($P \leq 0.05$) and gradually till the end of storage. (Awad, 2016) Found that an increase in the hardness in soft cheese during ripening period is in similar with the present results. Changes in hardness can be attributed mainly to the reduction in the moisture content of soft cheese as close relation was apparent between total solids and hardness.(Abd El-Salam et al. 2017) reported that the hardness in all cheese treatment increased with progressing storage period, and observed hardness to cheese with purified enzyme from Artichoke (*Cynara cardunculus* L.) was higher than cheese with veal rennet. The gumminess was affected by different coagulants and ripening period. The significant increase in gumminess ($P \leq 0.05$) was found amongst all treatments at the end of storage. The cheese with purified MCE (T2) exhibited lower gumminess than two other treatments i.e., with veal (control) and microbial rennet (T1). Chewiness of soft cheeses in the three treatments increased during storage. Although, cheese with veal rennet (control) has higher chewiness than other treatments, but found no significant difference ($P \leq 0.05$) between treatments. From the foregoing results it can be concluded that the most obvious changes in the TPA of soft cheese as affected by different coagulating agents causing in hardness.

TABLE 2. TPA of soft cheese treated with three different MCE during storage.

Treatments			Storage Period (days)	Textural properties
T2	**T1**	**Control**		
$7.6^{a}_{\pm 0.04}$	$4.6^{a}_{\pm 0.006}$	$6.3^{a}_{\pm 0.003}$	fresh	**Hardness N**
$4.8^{b}_{\pm 0.04}$	$9.0^{b}_{\pm 0.006}$	$5.9^{b}_{\pm 0.003}$	15	
$6.4^{c}_{\pm 0.04}$	$9.6^{b}_{\pm 0.006}$	$7.6^{c}_{\pm 0.003}$	30	
$0.534^{a}_{\pm 0.04}$	$0.549^{a}_{\pm 0.06}$	$0.536^{c}_{\pm 0.04}$	fresh	**Cohesiveness (B/A area)**
$0.555^{a}_{\pm 0.03}$	$0.515^{a}_{\pm 0.06}$	$1.772^{a}_{\pm 0.04}$	15	
$0.668^{b}_{\pm 0.05}$	$0.573^{a}_{\pm 0.06}$	$0.833^{b}_{\pm 0.04}$	30	
$0.705^{b}_{\pm 0.01}$	$0.676^{c}_{\pm 0.02}$	$0.664^{c}_{\pm 0.11}$	fresh	**Springiness Mm**
$0.791^{b}_{\pm 0.01}$	$0.723^{a}_{\pm 0.02}$	$0.773^{b}_{\pm 0.01}$	15	
$0.944^{a}_{\pm 0.01}$	$0.684^{b}_{\pm 0.02}$	$0.895^{a}_{\pm 0.011}$	30	
$4.058^{a}_{\pm 0.008}$	$2.525^{c}_{\pm 0.007}$	$3.377^{c}_{\pm 0.015}$	fresh	**Gumminess N**
$2.664^{b}_{\pm 0.008}$	$4.635^{b}_{\pm 0.007}$	$10.455^{a}_{\pm 0.015}$	15	
$4.275^{a}_{\pm 0.008}$	$5.501^{a}_{\pm 0.007}$	$6.331^{b}_{\pm 0.015}$	30	
$2.861^{b}_{\pm 0.007}$	$1.707^{c}_{\pm 0.003}$	$2.242^{c}_{\pm 0.04}$	fresh	**Chewiness N/mm**
$2.107^{b}_{\pm 0.007}$	$3.351^{b}_{\pm 0.003}$	$8.082^{a}_{\pm 0.04}$	15	
$4.036^{a}_{\pm 0.007}$	$3.763^{a}_{\pm 0.003}$	$5.666^{b}_{\pm 0.04}$	30	

C: Animal rennet. T1: Microbial rennet. T2: Plant extraction

Electrophoretic patterns of soft cheese

Milk was used for comparison with in the three treatments consisting of animal, microbial rennet and milk clotting enzyme from fruit seeds of *Solanum elaeagnifolium* plant in the presence of a column of standard protein of multimolecular weights in a range from14.4 to 94 KDa. The results showed that there is a significant similarity between microbial rennet (T1) and MCE from plant (T2) in terms of molecular weight, which was about 34 KDa, while animal rennet was of molecular weight about 36 KDa.

Proteolysis in cheese samples that lowers the intensity of protein bands was examined using Disc-PAGE in the three cheese treatments was investigated the proteolysis in cheese sample. Data obtained showed changes in the K- casein and β-casein bands. Figure 3 showed considerable decrease in β-casein and K- casein in the treatments, and the increase in the intensity of the band corresponding to αs1 and αs 2 casein as shown in the same Fig. 3. It may be due to the activity of the proteases present in all three coagulants. These results are in similar with the results of Farkye et al. (1991) and Grudden et al. (2005). They found that the hydrolysis of β-casein is attributable due to the activity of plasmin enzyme which hydrolysis -casein to protease peptones and γ-casein. On the other hand, Grudden et al. (2005) reported that, the composition of the casein micelles may effected by of temperature during storage.

Sensory properties of white soft cheese evaluation

Sensory properties of soft cheese treatments made with three different coagulants during the ripening period at 7ºC for 30 days are presented in Fig. 4 (A-D). As shown in Fig. 4 (A) appearance of cheese made with veal rennet (control) scored the highest after 30 days ripening period than T1 sample made with microbial rennet and T2 cheese made with plant extract. Appearance scores of 8.1, 7.9 and 7.73 points were achieved after 30 days storage in control, T1, and T2 in white soft cheese treatments, respectively. These data means depict that T1 showed no difference in appearance than control and T2. After the 30 days, cheeses with control and T2 treatment were smoother and softer than T1 treatment. White soft cheese treatments kept the same appearance up to 1 month of ripening period. In Fig. 4 (B) the body and texture scores of samples at 30 days were 35.9, 34.6 and 36.8 points for control, TI and T2 cheeses, respectively. These findings exhibit that the use of different coagulants improved body and texture white soft cheese during 30 days storage interval. There was a little difference between treatments with different coagulants. The highest score for T2 with MCE was achieved after 1 month followed by control and T1. The body and texture scores slightly increased for all treatments including control with increasing of storage period. The results in Fig. 4 (C) also indicated that there was a remarkable difference in flavor scores between control and all other cheese treatments. The flavor scores were

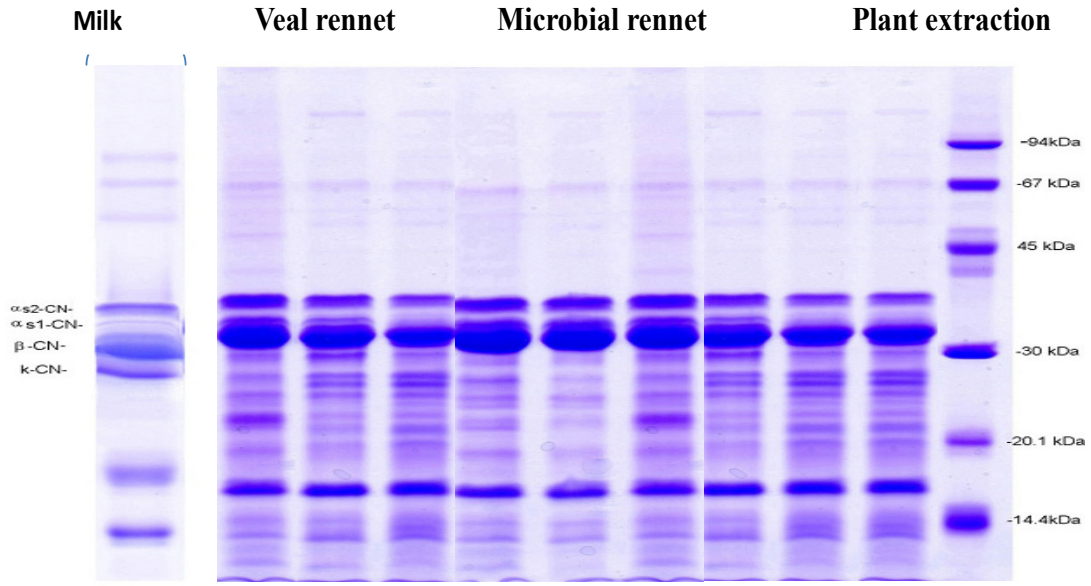

Fig. 3. Electrophoretic patterns of white soft cheese treated with different coagulants during storage.

44.66, 43.3 and 46.7 points out of 50 after 1 month in control; TI and T2 white soft cheeses, respectively. Cheese made with MCE scored the highest after 30 days of ripening period followed by control and T1 respectively, whereas, the T1 scored the lowest. Flavor continued to improve till the end of storage in all of cheese treatments. The flavor enhancement in white soft cheeses with different coagulants is due to the role of different coagulants and starter culture traditional to hydrolyze the milk components involved in cheese flavor such as proteins, fats, lactose, citrates and phosphates. In Fig. 4 (D) general acceptability of white soft cheeses scored 88.67, 85.8 and 91.2 points out of 100 for 1 month in control, TI and T2, respectively. The data showed that T2 gained the significantly (P 0.05) highest acceptability followed by control treatment. On the other hand, the T1 treatment gained fewer score than other treatments with different coagulants. Storage has affected the total acceptability of cheese properties. However, the acceptability increased by extending the storage period. The acceptability was lower in T1 treatment while, its improvement was faster in T2 treatment with plant extraction. Stored cheese samples of T2 gained the highest score at the 30 days to all properties than control treatment of cold storage Furthermore, it could be concluded that white soft cheeses using plant extraction from *S. elaeagnifolium* fruits seeds, can be recommended for consumption. El-Kholy (2015) found that the flavor intensity of cheese produced with MCE from plant was higher than with veal rennet on day 60. The cheeses manufactured with 1.6 g/L of artichoke flower extract had more flavor intensity and acidic characteristics in ripened cheese. No difference was observed in respect of white soft cheese appearance among all treatments.

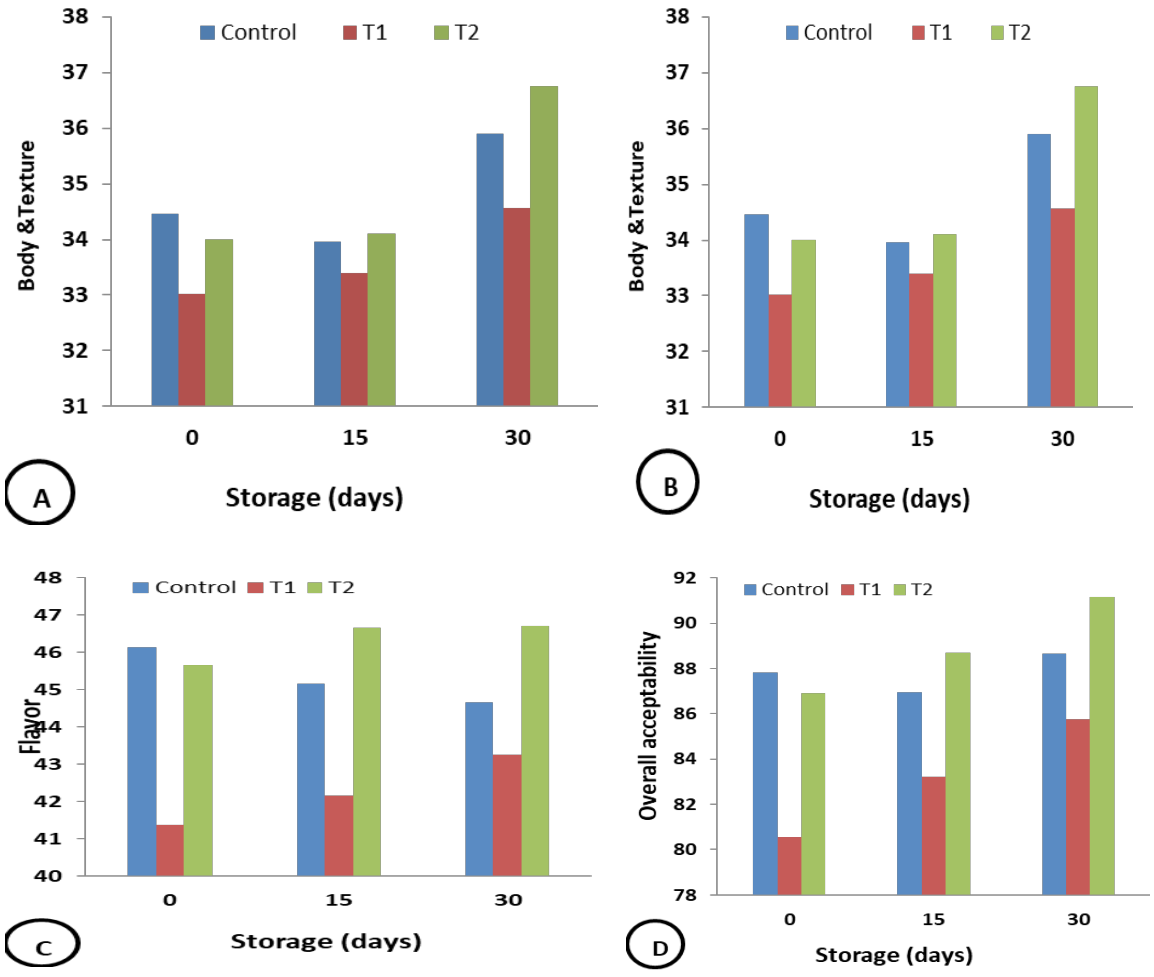

Fig. 4 (A-D): Sensory evaluation of whit soft cheese manufactured with different coagulants during storage. (Averages of three replicates)

Conclusion

It is deduced from the present study that milk clotting enzyme from *Solanum elaeagnifolium* plant yielded soft cheese with higher proteolysis and higher flavor intensity, lower pH, as well as increased acceptability of body and texture of resultant cheese compared with other commercial rennet. Further work is in progress to create the possible use of extract from fruit seeds of *Solanum elaeagnifolium* plant for production of other types of cheeses as rennet replacer**.**

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