

## Influence of Plant Based Coagulant (Enzyme Extracts from Albizia and Sunflower Seeds) on Quality of Domiati Cheese.

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### ABSTRACT

The effects of protein extract of albizia (*Abizia lebeck*) and sunflower (*Helianthus annuus*) seeds on milk-clotting activity, lipolysis, proteolysis, textural characteristics and flavour development in Domiati cheese over 60 days pickled during 60 days were studied. Changes in the chemical composition of experimental Domiati cheese samples seemed to be not affected by plant based coagulant (albizia and sunflower seeds). On the other hand, cheese was within legal requirements for Domiati cheese in Egypt. Lipolysis and proteolysis of pickled cheese were higher when coagulated with protein extract of albizia and sunflower seeds, compare with control, while hardness, adhesiveness, gumminess and chewiness were lower in cheese made using plant based coagulant than control. A significant variation in cohesiveness and springiness were found among the plant based coagulant used in Domiati cheese making. The cheese made using protein extract of albizia (*A.lebeck*) received high scores in flavour acceptability and texture, compared with that made by mixing protein extract of sunflower (*H.annuus*) and control.

**Keywords:** Domiati cheese, Albizia, sunflower, Texture profile analysis

### INTRODUCTION

Domiati cheese is the most common and popular In Egypt. It is a different compare with other pickled types by the fact that a high percentage of salt (Up to 15%) is put directly to the cheese –milk (Abou-Donia, 2007), rather than at the process end to the cheese curd. Domiati cheese was consumed fresh or after pickling period for several months (Ayad 2009). Domiati cheese has been made from buffalo's milk, cow's milk or a mixture of both. It is made by coagulating the milk with calf rennet.

Chymosin is a aspartic protease found in calf rennet and used for enzymatic coagulation of milk in cheese-making. The essential cleavage occurs at Phe105-Met106 bond of *k*-casein, resulting form the cheese curd (Jolles, *et al.*, 1963).

Enzymatic coagulation may be achieved by a several types of proteolytic enzymes from different sources, such as microbial species (*Cryphonectria parasitica*, *R. pusillus* and *Rhizomucor miechei*) or plant coagulatnts. The use of animal rennet may be restricted for several reason, such as vegetarian or consumer interest regarding genetically modified food (e.g., France, Germany and Netherlands forbid the application of recombinant calf rennet in food industries). Moreover, the infection of animal with bovine spongiform encephalopathy has decreased both supply and demand for animal rennet (Roseiro *et al.*, 2003). Plant coagulant has been identified from *Calotropis procera* (Sanni *et al.*, 1999), *Ananas comosus* (Cattaneo *et al.*, 1994), *Ficus bengalensis*, *Opuntia phylloclades*, *Cereustriangularis*, *Euphorbia caducifolia*, *F. elastica*, *E. hista* (Umar, *et al.*, 1990), *Lactuca sativa* (Lo, *et al.*, 2002). Unfortunately, almost of these plant coagulants have been reported to be unsuitable because of production of extremely bitter cheeses. However the aqueous extracts and protein extracts of *Cyanara cardunculus*, *Helianthus annuus* and *Abizia lebeck* have been found to possess proteolytic enzymes which clotted milk readily, without developing any desirable flavour or bitterness in cheese after pickled period (3 months) (Otani *et al.*, 1991;

Cordeiro *et al.*, 1994; Park *et al.*, 2000; Sidrach *et al.*, 2005).

The objective of the present work was to study the potential ability of protein extracts from *Helianthus annuus* and *Abizia lebeck* seeds as calf rennet replacer for production of Domaiti cheese.

### MATERIALS AND METHODS

#### Preparation of crude enzyme extract

Ten grams of albizia seeds and sunflower seeds were crushed, followed by soaking the mill seeds in distilled water (100 ml) containing NaCl (1%) and sodium azide (0.02%) for 24 hours at 5°C with agitation. The mixtures were then filtered to give crude aqueous extracts. The precipitation of proteins from crude aqueous extracts were performed by using ammonium sulfate at 40% saturation, the mixture was kept for 45 min at 4°C. The mixture was centrifuged (16,000xg at 4°C for 15 min), and the pellets were removed and added gradually to the supernatant up to 60% saturation in the case of *Helianthus annuus* or 70% in the case of *Abizia lebeck* and were kept for 45 min at 4°C, followed by centrifugation (15,000x g at 4°C for 10 min). The supernatants were discarded and the pellets were dissolved in 20 ml of distilled water, the mixture was put in dialysis tubing at 4°C for 48h to remove salts and finally dried to give the protein extracts of *H. annuus* and *A. Lebeck* (Egito *et al.*, 2007).

#### Cheese making procedure:

Domiati cheese was made as described by Abou-Donia (2008), the milk was heated to 50°C and salted using sodium chloride to give final concentration of 12%. Each quantity of milk was divided into three parts. A suitable amount of commercial rennet and 20mg ml<sup>-1</sup> of *H. annuus* and *A. lebeck* protein extracts were added to coagulate milk cheese within 2-3h. The cheese curds were placed in the wooden frames lined with cheese cloth. After 3 to 4 h, and cheese curds were pressed using weights.. The cheese was then cut into blocks of about 10 x 10 x10 cm. The cheese blocks were transferred into cans and filled with brine (12% salt). The cans were stored at room temperature (20 to 30°C) for 60 d.

### Cheese Composition analysis

Total protein was determined by the Kjeldahl method (AOAC, 2000) and content of fat by Gerber method (AOAC, 2000), the moisture content was measured according to AOAC, (2005), pH meter (CG710, West Germany) was used to measure pH values of cheese samples according to Ling, (1963).

### Microbiological analysis

Ten grams of each cheese sample were mixed for 5 min with 90 ml of sterilized 2% sodium citrate solution and serially diluted using sterilized saline (0.85% NaCl). Appropriate dilutions of sodium citrate solution of cheese samples were plated on tryptone soya agar (TSA) at 35°C for 48 h, MacConkey agar for enumerating coliform bacteria at 37°C for 48 h, Potato dextrose agar for enumerating the molds and yeasts at 20°C for 5 d, and staphylococcus medium 110 for enumeration of staphylococci at 37°C for 48 h (Difco's Manual, 1985).

### Evaluation of proteolysis and lipolysis

The water –soluble extract (WSE) was determined according to Kuchroo and Fox (1982), and were estimated in water soluble extract by using the Cd-ninhydrin method (Foldertsma and Fox, 1992). Free fatty acids were estimated by the method of Deeth *et al.*, (1975).

### Evaluation of textural properties of cheese

Determination of Textural characteristics of cheese samples were carried out by texture analyzer (TA 1000, Lab Pro), USA). Samples of cheese were divided into 50 mm<sup>3</sup>. A two-bite penetration test was implemented with the TA 60 degree cone, Perpex probe for Domiati cheese operated at a crosshead speed 50 mm/sec. Hardness, Adhesiveness, cohesiveness, springiness and gumminess and chewiness were estimated in triplicate as reported by Szczesniak *et al.*, (1963) and Bourne, (1978).

### Sensory Assessment

Evaluation of sensory properties of cheese samples was carried out at the Dairy Department, Mansoura University by 10 panelists, including staff members, consumers and cheese producers. Each one of panelists was given 3 cheese blocks (7 x 3 x 3 cm) per sample. Cheese samples were placed in identical plastic sample cups identified by a random three digit number. The coded cheese samples were randomly presented to panelists. The Panelists were asked to award the cheese a total grade out of 100, to assess whether each sample was closed to Domiati cheese and to give additional comments. Cheeses were evaluated at the end of pickled period and the following scale was used: 0 – 30 = unacceptable; 31-60=poor; 61-85=acceptable; 86-100=good.

### Statistical Analysis

Data were statistically analyzed using SAS software package (SAS institute, 2004) was used ANOVA.

## RESULTS AND DISCUSSION

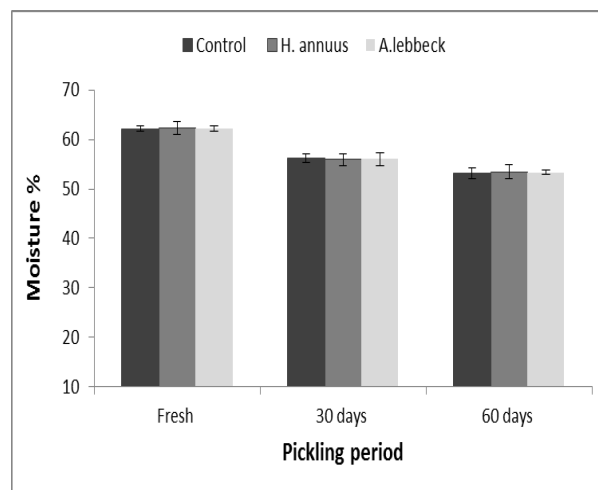
### Chemical composition of Domiati cheese

The total solid in all chesses decreased significantly ( $P < 0.05$ ) over pickling (Fig.1). The total

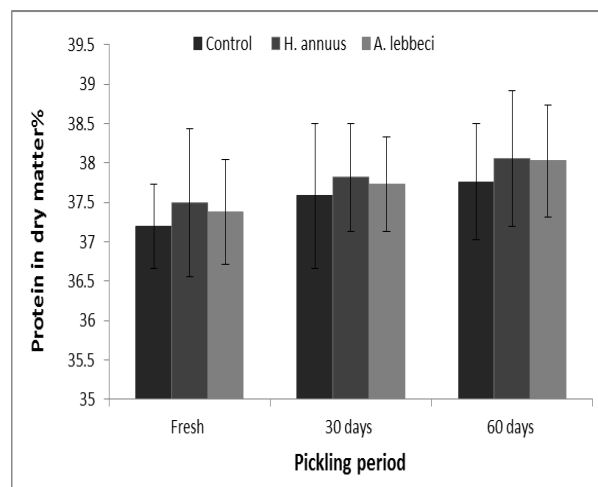
solid increased over the first month of pickling (Fig.1). Treating cheese with different coagulants in the present study, had no significant influence on the total solid of Domiati cheese (Fig.1). The average content of total solid of fresh Domiati cheeses is in agreement with that reported by Awad *et al.* (2001).

The contents of protein and fat in cheeses were found to be dependent on the total solid content in cheeses over pickling (Fig. 2 & Fig.3). There was no significant difference ( $P < 0.05$ ) of protein and fat content in all cheese treatments (Fig. 2 & Fig.3). The salt in moisture content increased insignificantly ( $P < 0.05$ ) during the period of pickling (Fig. 4). Similar results were found by Awad *et al.* (2001).

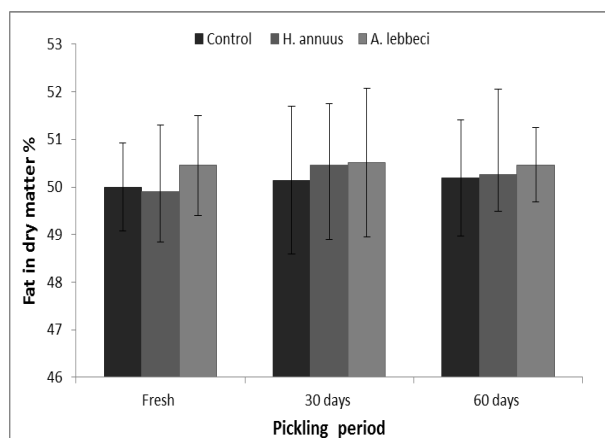
The overall aged Domiati chemical composition was consistent with the typical composition of Domiati cheese (Abd El-salam and ALichanidis, 2004) and maintaining the legal limit for Domiati cheese in Egypt (Egyptian Standards, 2000).



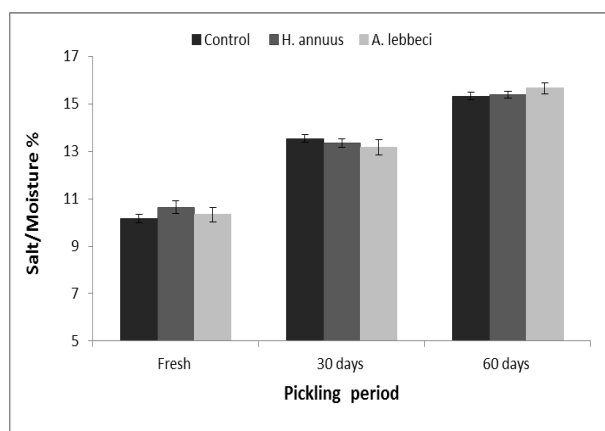
**Figure 1. The moisture content of Domiati cheese made with different coagulant types. Values are means of at least three separate determinations, and error bars represent  $\pm$ SD.**



**Figure 2. The effect of coagulant types on protein content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**



**Figure 3. The influence of coagulant types on fat content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**



**Figure 4. The effect of coagulant types on salt content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**

#### Yield percentage of Domiati cheese

The impact of different coagulant types on yield percentage of Domiati cheese were presented in Figure (5). The yield (%) in all cheeses decreased significantly ( $P < 0.05$ ) during pickling (Fig.5). The treatments used in this study had no significant ( $P < 0.05$ ) influence on the yield percentage of Domiati cheese.

#### pH values of cheese over Pickling

The pH values of cheese samples measured during pickling are listed in Table (1). The pH values decreased gradually during pickling period (table1). The pH values of fresh Domiata cheese made using albizia seed protein extract was the lowest compare with control and sunflower seed protein. This might be attributed the albizia seed protein extract had greater protein degradation ability by activation of non starter lactic acid bacteria.

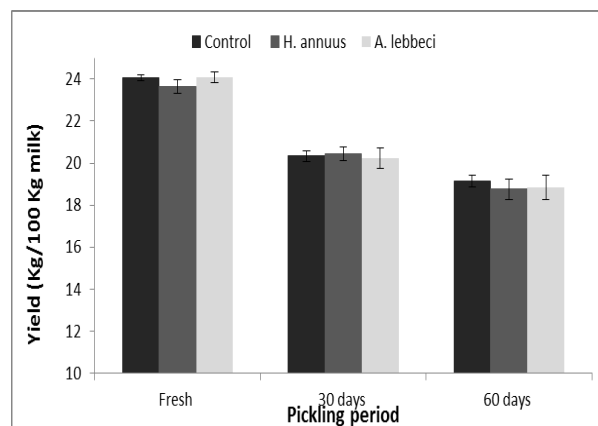
#### Lipolysis of Domiati cheese

The lipolysis occurred in picketed Domiati cheese was determined in terms of total free fatty acids (FFA) (Table1). There was significant increasing ( $P < 0.05$ ) in FFA associated with progressing period of pickling (Table 1). Cheese made by protein extract of Albizia seed showed higher FFA values over pickling

than cheese made by rennet, followed by using protein extract of sunflower seed as rennet replacer, compare with control. High FFA values in cheeses containing protein extract of sunflower seed and albizia seed might be attributed to the activation of microflora in cheeses to the release lipases and intracellular esterases.

#### Proteolysis of Domiati cheese

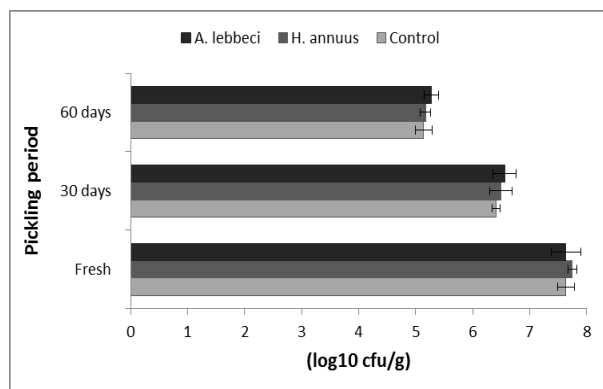
Free amino acids release (m M leucine equivalents) in WSE of cheese samples at different stages of pickling are presented in Table 1. The values of free amino acid (FAA) were the highest in treatment made using protein extract of *A. lebbeci*, compare with other treatments throughout the period pickling. This finding is imputed to several factors such as low pH values, high content of moisture, high content of viable bacteria, and high residual proteolytic enzymes. FAA were raised significantly ( $P < 0.05$ ) as storage pickling progressed in all treatments. These results are consistent with Egito, *et al.*, (2007), who reported that protein extract of Albizia and sunflower seeds could be a potentially animal rennet replacer, the protein extract of albizia seed was higher than protein extract of sunflower, the both of protein extracts were exhibited good milk-clotting and caseinolytic ability required for ripening of cheese.



**Figure 5. The effect of coagulant types on yield percentage of Domiati cheese. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**

#### Assessment of microbial profile of Domiati cheese

The total viable bacterial count in cheese samples throughout the period of pickling is shown in (Fig. 6). There was no significant difference ( $P < 0.05$ ) in total viable bacterial count of cheese samples. A gradual decrease in total viable bacterial count was detected over pickling in all cheese samples, resulting in a bout a 2 log reduction after 2 months. The present results were similar to those reported by El-Koussy *et al.*, (1976); Ahmad *et al.*, (1978); Abou-Donia, (1981) and Abd-El-Khalek *et al.*, (2008), who investigated a decrease in the total viable counts over pickling of Domiati cheese. Staphylococci, coliform bacteria, yeasts and molds were not detected in any samples of Domiati cheese over pickling period. The results were in agreement with those found by Awad *et al.*, (2010).



**Figure 6. Viability of bacterial populations in Domiati cheese using protein extract of *A. lebbeck* and *H. annuus* seeds. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**

#### Texture Profile analysis of experimental Domiati cheese

Texture profile analysis parameters of experimental cheese samples were determined (Table 2). Texture properties of cheese samples were significantly ( $P < 0.05$ ) affected by using substitution of calf rennet by protein extract of *A. lebbeck* and *H. annuus* seeds. Hardness, Adhesiveness, gumminess and chewiness were significantly lower ( $P < 0.05$ ) in experimental cheese samples made by using protein

extract of *A. lebbeck* compare with other treatment and control (Table 2), followed by cheese made using protein extract of *H. annuus*. However the cohesiveness and springiness were significantly higher in cheese coagulated with protein extract of *A. lebbeck* seed. A gradual decline in cohesiveness, springiness, gumminess and chewiness were detected during the progress of pickling period in all cheeses, in contrast to hardness and adhesiveness were significantly higher in all samples of cheese over pickling (Table 2). This results were in perfect harmony with those presented by Koca and Metin (2004); Volikakis *et al.*, (2004); Korish and abd Elhamid (2012). The lower values of hardness, gumminess, chewiness and adhesiveness in cheese coagulated with protein extract of *H. annuus* and *A. lebbeck* seeds might be related to high ability of these protein extracts for hydrolysis of casein particles compare with chymosin (Egito *et al.*, 2007). The Matrix of cheese is formed by interconnected casein particles and the solubilization of colloidal calcium phosphate (CCP) and hydrolysis of these molecules will decrease hardness (De Jong, 1976; Creamer and Olson, 1982; Creamer *et al.*, 1982).

**Table 1. The effect of coagulant types on pH, free amino acid (FAA) and Free fatty acids.**

Treatment	pH			FAA			FFA		
	1 d	30 d	60 d	1 d	30 d	60 d	1 d	30 d	60 d
Control (Chymosin)	6.49 <sup>a</sup> $\pm$ 0.08	5.63 <sup>a</sup> $\pm$ 0.18	4.79 <sup>a</sup> $\pm$ 0.04	0.022 <sup>c</sup> $\pm$ 0.004	0.096 <sup>c</sup> $\pm$ 0.007	0.220 <sup>c</sup> $\pm$ 0.035	0.51 <sup>b</sup> $\pm$ 0.04	1.12 <sup>b</sup> $\pm$ 0.06	1.76 <sup>b</sup> $\pm$ 0.04
Protein extract of <i>H. annuus</i>	6.26 <sup>b</sup> $\pm$ 0.09	5.34 <sup>b</sup> $\pm$ 0.04	4.45 <sup>b</sup> $\pm$ 0.07	0.033 <sup>b</sup> $\pm$ 0.002	0.197 <sup>b</sup> $\pm$ 0.047	0.283 <sup>b</sup> $\pm$ 0.038	0.63 <sup>a</sup> $\pm$ 0.07	1.25 <sup>a</sup> $\pm$ 0.11	1.81 <sup>a</sup> $\pm$ 0.07
Protein extract of <i>A. Lebbeck</i>	6.08 <sup>c</sup> $\pm$ 0.11	5.12 <sup>c</sup> $\pm$ 0.12	4.24 <sup>c</sup> $\pm$ 0.08	0.045 <sup>a</sup> $\pm$ 0.007	0.273 <sup>a</sup> $\pm$ 0.027	0.363 <sup>a</sup> $\pm$ 0.035	0.67 <sup>a</sup> $\pm$ 0.04	1.38 <sup>a</sup> $\pm$ 0.10	1.87 <sup>a</sup> $\pm$ 0.09

Data are means  $\pm$ S.E.,  $N=3$ ; mean values in the same column with different letters in the superscript are indicated a significantly different ( $p < 0.05$ ).

**Table 2. The influence of plant based coagulant on texture profile analysis of experimental Domiati cheese.**

Treatment	Pickling period (days)	Hardness (Newton)	Adhesiveness (Jole)	Springiness (Millimeter)	Cohesiveness (Dimensionless)	Gumminess (Newton)	Chewiness (Jole)
Control	1	7.38 $\pm$ 0.17	37.44 $\pm$ 0.26	0.75 $\pm$ 0.06	0.40 $\pm$ 0.04	2.92 $\pm$ 0.26	2.23 $\pm$ 0.12
	30	8.67 $\pm$ 0.54	38.55 $\pm$ 0.58	0.55 $\pm$ 0.05	0.28 $\pm$ 0.03	2.42 $\pm$ 0.25	1.56 $\pm$ 0.18
	60	9.33 $\pm$ 0.27	40.32 $\pm$ 0.56	0.42 $\pm$ 0.08	0.24 $\pm$ 0.05	2.20 $\pm$ 0.51	1.03 $\pm$ 0.31
	Mean	8.46 <sup>a</sup>	38.77 <sup>a</sup>	0.57 <sup>a</sup>	0.31 <sup>a</sup>	2.51 <sup>a</sup>	1.61 <sup>a</sup>
protein extract of <i>H. annuus</i>	1	6.20 $\pm$ 0.40	34.80 $\pm$ 0.37	0.75 $\pm$ 0.03	0.43 $\pm$ 0.03	2.72 $\pm$ 0.41	2.03 $\pm$ 0.30
	30	7.21 $\pm$ 0.32	35.93 $\pm$ 0.24	0.58 $\pm$ 0.02	0.33 $\pm$ 0.04	2.35 $\pm$ 0.16	1.36 $\pm$ 0.10
	60	8.49 $\pm$ 0.24	38.05 $\pm$ 0.34	0.43 $\pm$ 0.05	0.25 $\pm$ 0.03	2.16 $\pm$ 0.17	0.96 $\pm$ 0.19
	Mean	7.30 <sup>b</sup>	36.26 <sup>b</sup>	0.59 <sup>a</sup>	0.34 <sup>b</sup>	2.41 <sup>b</sup>	1.45 <sup>b</sup>
protein extract of <i>A. lebbeck</i>	1	5.73 $\pm$ 0.15	33.69 $\pm$ 0.25	0.77 $\pm$ 0.03	0.45 $\pm$ 0.03	2.57 $\pm$ 0.11	1.93 $\pm$ 0.12
	30	6.44 $\pm$ 0.18	34.66 $\pm$ 0.33	0.65 $\pm$ 0.04	0.34 $\pm$ 0.05	2.19 $\pm$ 0.28	1.81 $\pm$ 0.11
	60	7.06 $\pm$ 0.14	36.13 $\pm$ 0.14	0.45 $\pm$ 0.04	0.31 $\pm$ 0.03	2.17 $\pm$ 0.21	0.94 $\pm$ 0.25
	Mean	6.41 <sup>c</sup>	34.83 <sup>c</sup>	0.62 <sup>b</sup>	0.37 <sup>c</sup>	2.31 <sup>c</sup>	1.35 <sup>c</sup>

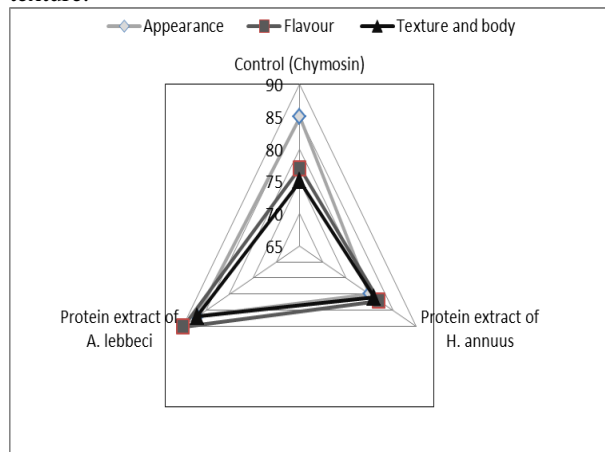
Data are means  $\pm$ S.E.,  $N=3$ ; mean values in the same column with different letters in the superscript are indicated a significantly different ( $p < 0.05$ ).

#### Sensory evaluation of cheese

The mean scores for appearance, intensity of flavour and acceptability of body and texture of cheese samples at 60 days of pickling are presented in Fig. 6. Generally, the Domiati cheese made with protein extract of *A. lebbeck* was highest scores for flavour of Domiati

cheeses, followed by cheese made using protein extract of *H. annuus*. This finding might because of their high levels of FFA and FAA. Small peptides and free amino acids have significant influence on the flavour of cheese (El Soda, 1993; Awad, 2006). Moreover, the free fatty acids concentration, particularly Short-chain free fatty

acids, is answerable for the characteristic cheese flavour (Kanawjia *et al.*, 1995). Panelists found differences ( $P < 0.05$ ) in body and texture between cheese made using protein extract of *A. lebbeck* and control. The making of Domiati cheese using protein extract *A. lebbeck* seed received the highest grades in acceptability of body and texture.



**Figure 6. Sensory evaluation of Domiati cheese using different coagulant types. Values are means of at least three separate determinations, and error bars represent  $\pm$ SE.**

## CONCLUSION

It is deduced from the present study that protein extract of *A. lebbeck* and *H. annuus* seeds yielded Domiati cheeses with higher proteolysis and lipolysis, higher flavour intensity, lower pH, as well as increased acceptability of body and texture of resultant cheese compared with control. Further work is in progress to create to possible use protein extract of *A. lebbeck* and *H. annuus* seeds for production soft cheese as rennet replacer.

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## تأثير المنفعة النباتية (المستخلصات الإنزيمية لبذور نبات عباد الشمس و نبات الألبيزيا) على جودة الجبن الدميطي.

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تهدف هذه الدراسة إلى دراسة تأثير المستخلص البروتيني لكلاً من بذور نبات الألبيزيا ونبات عباد الشمس على تجبن اللبن والتحلل الدهني والتحلل البروتيني والخواص الريولوجية وتطور النكهة في الجبن الدميطي المخزن لمدة شهرين. لم يتأثر التركيب الكيماوي للجبن الدميطي الناتج عند استخدام المستخلص البروتيني لبذور نبات الألبيزيا وبذور نبات عباد الشمس كبديل للمنفعة، وكان التركيب الكيماوي للجبن في الحدود القانونية المنصوص عليها في المواصفات القياسية المصرية للجبن الدميطي. تم ملاحظة زيادة مقدار الأحماض الدهنية المنفردة والأحماض الأمينية المنفردة في الجبن المصنع بالمستخلص البروتيني لبذور الألبيزيا وبذور نبات عباد الشمس مقارنة بالمنفعة. انخفاض مقدار كل Hardness و Adhesiveness و Gumminess و Chewiness في الجبن الناتج من تجبن المستخلصات النباتية مقارنة بالكنترول (المنفعة)، على النقيض من ذلك حدث ارتفاع ملحوظ في Springiness و Cohesiveness في الجبن المعامل بالمستخلصات النباتية مقارنة بالكنترول. حصلت عينات الجبن التي تم تصنيعها باستخدام المستخلص البروتيني لبذور نبات الألبيزيا على أعلى درجات التقييم الحسي من حيث النكهة والقوام مقارنة بالجبن الناتج من المستخلص البروتيني لبذور نبات عباد الشمس والكنترول (المنفعة).