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CHANGES IN PHYSICO CHEMICAL CHARACTERISTICS, PROTEOLYSIS AND ORGANOLEPTIC PROPERTIES OF EDAM CHEESE MADE WITH ADDED AQUEOUS EXTRACT OF CARDOON (*Cynara cardunculus*) AND GINGER (*Zingiber officinale*) DURING RIPENING

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ABSTRACT: Plant crude aqueous extracts of coagulants obtained from cardoon flowers (*Cynara cardunculus*) or ginger rhizomes (*Zingiber officinale*) were used in Edam cheese making, compared to calf rennet (CR) as control cheese. Effect of replacing calf rennet with crude aqueous extract of cardoon flowers and ginger rhizomes at level of 25, 50, 75 and 100% on yield, chemical composition, proteolysis and organoleptic properties of Edam cheese was evaluated during ripening up to 90 days. Replacing calf rennet with cardoon flowers or ginger rhizomes aqueous extracts, significantly decreased cheese yield and increased weight loss per cent compared with control. This was associated with the increase in the replacing level. Cheese made with ginger rhizomes aqueous extract showed higher weight loss than that made with cardoon flowers aqueous extract. Cheese made with cardoon flowers or ginger rhizomes aqueous extracts showed lower levels in moisture content and total nitrogen on dry matter. However, fat on dry matter and titratable acidity (as % lactic acid) significantly increased in Edam cheese made with ginger rhizomes aqueous extract than that made with cardoon flowers aqueous extracts compared to control. Casein breakdown and the formation of volatile fatty acids (VFA) were more pronounced in cheese made with high replacing rate of calf rennet with ginger rhizomes aqueous extract than cardoon flowers aqueous extract compared to control. When replacing rate was more than 50% of ginger rhizomes and 75% of cardoon flowers aqueous extracts with calf rennet, the resultant cheeses were characterized with pasty texture and slightly flavour defects (especially bitterness) compared to control. The results indicated that, replacing rate of calf rennet with crude aqueous extract of cardoon flowers at level 75% or ginger rhizomes at rate 50% are suitable for the production of Edam cheese with an acceptable quality during ripening.

Key words: Cardoon flowers, ginger rhizomes, calf rennet, proteolysis, total free amino acids, edam cheese.

INTRODUCTION

Plant proteases employed for cheese production in various areas of the world include papain, bromelin, ficin, oryzasin, cucumisin, sodan apple and jacartia corumbesis (Duarte *et al.,* 2009). The use of cardoon flower extracts has received more attention (Agboola 2002; Chen *et al.,* 2003; Agboola *et al.,* 2004; Zhao, *et al.,* 2004). Cheese is excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium. Some properties of cheese such as texture and flavour depend on the origin of milk, animals diet, butter fat content, bacteria, moulds, the processing and aging conditions (Fox *et al.,* 1996 ; Miller *et al.,* 2007). It is well known that calf rennet has been widely utilize as a milk clotting enzymes. Cheese is traditionally produced through the use of commercial calf rennet or rennet substitute as

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microbial enzymes; recombinant proteases metabolized by genetically modified microorganisms and plant proteases (Jacob *et al.*, 2011). Vegetable enzymes from higher plant organs have been extensively investigated as milk clotting enzymes in cheese making.

The milk clotting enzymes extracted from wild cardoons is the most suitable enzymes for the production of cheeses in West European countries (Tejada and Fernandez-Salguero, 2003). Milk coagulation by cysteine protease from the ginger rhizomes extracts was studied by (Hashim *et al.,* 2011). Cardoon (*Cynara cardunculus*) possesses two types of proteolytic enzymes namely cardosin A and cardosin B. The first enzyme has chymosin-like activity and can hydrolyze of the K-casein fraction and the second protease possesses a pepsin-like activity and has specific proteolysis during ripening (Verissimo *et al.,* 1995).

The excessive proteolytic activity of these enzymes lowers cheese yield and produce bitterness in the end products. Among the vegetable enzymes that have been reported to produce satisfactory cheese. Aspartic enzymes extracted from *Cynara scolymus* flower have the specific cleavage of the bovine-K-casein Ph 105-Meth 106 bond (Llorente *et al.,* 2014). However, a little information about the application of aqueous extract of cardoon flowers and ginger rhizomes as milk coagulating enzymes in hard or semi hard cheese manufactured could not be found.

Therefore, the present investigation was carried out to evaluate the changes in physic chemical characteristics, proteolysis and organoleptic properties of Edam cheese made with replacing calf rennet by aqueous extract of cardoon and ginger during ripening

MATERIALS AND METHODS

Milk

Fresh cow's milk was obtained from special Dairy Products Unit at Food Science Department, Faculty of Agriculture, Zagazig University, Egypt. The milk was standardized to 4% fat. Salts (sodium chlorid and calcium chlorid) were obtained from El-Gomhoria Co., Cairo, Egypt.

Calf rennet

Calf rennet powder (Hala) was obtained from Chr. Hansen, Denmark. It was dissolved in distilled water at a concentration of 62.5 mg % (*W/V*), calf rennet served as control (Vieira and Barbosa, 1972).

Crude aqueous extract from cardoon and gingar

Crude aqueous extract from dried cardoon flowers (*Cynara cardunculus*) was prepared as the method described by Chen *et al.* (2003). Crude aqueous extract from ginger rhizomes (*Zingiber officinale*) was prepared as described by Penna *et al.* (2003). The clear aqueous extracts of both cardoon flowers and ginger rhizomes extracts were adjusted to give the same clotting time as that of the calf rennet with milk at 40^{o}C according to the method described by Vieira and Barbosa, (1972) and it is kept at 5^{o}C ± 2 till it was used.

Starter cultures

Mesophilic starter culture containing *Lactococcus lactis* subsp.*cremoris* and *Lactococcus lactis* subsp. *diacetylactis were* obtained from Chr. Hansen's, Copenhagen, Denmark and used in cheese making.

Cheese making

Edam cheese was manufactured as described by Scott (1981). The milk was heated at 80^{o}C/ 5 min., then cooled at 35^{O} C and calcium chloride was added at the rate of 0.02%. Starter culture was added at the rate of 1% and thoroughly mixed with the milk and left at 35^{o}C for one hour. When acidity reached 0.19%, Anato (Chr. Hansen's, Copenhagen, Denmark) was added at the rate of 5 ml /100 kg milk, the milk was divided into 9 portions. The first portion was renneted using calf rennet and it was served as a control. The eight portions were subdivided into tow portions, the first portion was divided into four treatments, these treatments were renneted by replacing calf rennet by cardoon extract at the rate of 25, 50, 75 and 100%. Also, the second portion was also subdivided into four treatments renneted by replacing calf rennet by ginger extract at the same rate as above mentioned. All treatments were left for complete coagulation at 30^{o}C. resultant curds were cutted into large cubes (2 × 2 × 2 cm). The cheese making process was

completed. The resultant cheeses were immersed in a 20 % brine solution for 24 hours at 10°C for salting. After salting, the green cheeses were weighted and placed for 2 days in ripening room for surface drying. The cheeses were carefully coated with wax. Resultant cheeses were kept in the ripening room at 12° C and 80-90 % relative humidity for 90 days.

Cheese analyses

Yield and weight loss of resultant cheese were estimated. The cheese samples were taken when fresh and after 30, 45 and 90 days of ripening. Samples were ground and then stored in the deep freezer at (-18° C) until chemical analyses for moisture, total solids, titratable acidity (as lactic acid), fat, total nitrogen (TN) were determined according to the method described by AOAC (2007). Soluble nitrogen (SN) and non-protein nitrogen (NPN) were determined according to Ling (1963). The total volatile fatty acids (TVFA) were determined by the method of Kosikowski (1986).

Determination of free amino acids

Free amino acids were determined according to Mondino *et al.* (1972) by using amino acids analyzer (Eppendrof – Germany LC3000 Amino acids analyzer).

Sensory evaluation

Cheeses were sensory evaluated for appearance, body and texture and flavour by 10 trained panelists according to Fernandez de Lpozo *et al.* (1988). Results were expressed as a mean of the 10 panelists score.

Statistical analysis

Statistical analysis was done by treating data with SAS (2003) software programs (SAS Institute Inc., Cary, NC) ($P \geq 0.05$).

RESULTS AND DISCUSSION

Yield and Weight Loss of Cheese

Plant coagulant have been considered a possible substitutes for chymosin in cheese making process, but their potential for such use depends on their catalytic properties, stability and specificity, as these factors can affect cheese yield and sensory properties (Jacob *et al*., 2011). Generally, chymosin is considered to have a proteolytic specificity most-well studied for clotting bovine milk offering the best yields during cheese making (Jacob *et al.,* 2011; Vallejo *et al.,* 2012). As shown in Table 1 there were significant ($P \geq 0.05$) differences in curd yield when using different types of coagulants. Calf rennet showed the highest curd yield when compared to all of the plant coagulants. Results present in Table 1 show the effect of replacing calf rennet with cardoon flowers and ginger rhizomes aqueous extracts at different levels on yield and loss of cheese weight during ripening. Cheese yield significantly ($P \geq 0.05$) decreased for all cheeses with ripening progress. Cheese yield significantly ($P \geq 0.05$) decreased in case of replacing calf rennet with cardoon flowers and ginger rhizomes aqueous extracts, particularly at the high replacing rates of both. Also, it is noticed that cheese made with calf rennet and that made with replacing rate up to 75% cardoon flowers or 50% ginger rhizomes aqueous extracts showed relatively similar yield percentage and were higher than all other experimental cheeses. On the other hand, the highest weight loss was noticed by increasing the replacing rate of calf rennet with cardoon flowers and ginger rhizomes aqueous extracts for all experimental cheeses. Also higher weigh loss was found in cheese made by using cardoon flowers and ginger rhizomes aqueous extracts compared to calf rennet (as control cheese). These results agree with that reported by Kheir *et al.,* (2011) and Hashim *et al*., (2011). Also, general trend of these results are in agreement with that reported by Vieira de Sa and Barbosa (1972) who found that the whey from curds by cardoon extracts was more turbid, presumably owing to greater protein breakdown, that reduced the yield.

Compositional Characteristics

Table 2 shows the effect of coagulant type on gross chemical composition (moisture, acidity, fat/ DM and TN/DM %) of Edam cheese made from cows' milk coagulated by calf rennet (control) or with replacing calf rennet with cardoon flowers and ginger rhizomes aqueous extracts at different levels. It could be noticed that, titratable acidity of all cheeses were significantly ($P \geq 0.05$) increased throughout the

Table 1. Effect of replacing rate of calf rennet by crude aqueous extracts of cardoon and ginger on yield and weight loss of Edam cheese during ripening

Properties	Storage period (day)	Control	Replacing rate of calf rennet by vegetable extracts								Significant
			25%		50%		75%		100%		
			Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	
Yield (%)	0	14.15^{a}	14.12^{a}	14.08a^{b}	14.00^{bc}	13.96^{cd}	13.94^{cd}	13.90^{cd}	13.87^{d}	13.85^{d}	***
	30	12.90^{a}	12.78^{b}	12.74^{b}	12.63^{c}	12.59^{cd}	12.56^{cd}	12.50^{de}	12.43^{e}	12.40^{e}	***
	45	12.25^{a}	12.00^{b}	11.96^{b}	11.87^{c}	11.82^{c}	11.75^{d}	11.7^{de}	11.64^{ef}	11.62^{ef}	***
	90	11.96^{a}	11.83^{b}	11.80^{b}	11.73^{bc}	11.65^{cd}	11.60^{de}	11.53^{e}	11.50^{e}	11.48^{e}	***
Weight loss (%)	30	8.83^{g}	9.49^{f}	9.52^{f}	9.79^{e}	9.81^{e}	9.90^{d}	10.07^{c}	10.38^{b}	10.47^{a}	***
	45	13.42^{g}	15.01^{f}	15.06^{f}	15.21^{e}	15.33^{d}	15.71^{c}	15.83^{b}	16.08^{a}	16.10^{a}	***
	90	15.48^{e}	16.22^{d}	16.19^{d}	16.21^{d}	16.55^{c}	16.79^{b}	17.05^{a}	17.09^{a}	17.11^{a}	***

$^{a, b,c,d}$ Means having different letters in the same row significantly differed at $p \geq 0.05$ *** Very high significant

Table 2. Effect of replacing rate of calf rennet by crude aqueous extracts of cardoon and ginger on chemical composition of Edam cheese during ripening

Properties	Storage period (day)	Control	Replacing rate of calf rennet by vegetable extracts								Significant
			25%		50%		75%		100%		
			Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	
Moisture (%)	0	49.92^{a}	49.94^{b}	49.60^{b}	49.35^{c}	49.30^{cd}	49.25^{cd}	49.22^{cd}	49.12^{cd}	49.06^{d}	***
	30	44.86^{a}	44.70^{ab}	44.57^{b}	44.33^{c}	44.30^{cd}	44.21^{cd}	44.18^{cd}	44.10^{cd}	44.04^{d}	***
	45	42.15^{a}	41.98^{b}	41.85^{b}	41.68^{c}	41.60^{cd}	41.46^{de}	41.40^{e}	41.32^{e}	41.25^{e}	***
	90	41.64^{a}	41.4^{8a}	41.30^{b}	41.10^{c}	41.00^{cd}	40.86^{d}	40.80^{de}	40.56^{ef}	40.58^{f}	***
Fat/DM (%)	0	49.92^{f}	50.10^{e}	50.14^{e}	50.41^{d}	50.49^{d}	50.60^{c}	50.65^{bc}	50.73^{ab}	50.79^{a}	***
	30	50.20^{e}	50.31^{d}	50.35^{d}	50.57^{c}	50.63^{c}	50.74^{b}	50.78^{b}	50.86^{ab}	50.93^{a}	***
	45	51.15^{f}	51.28^{e}	51.33^{e}	51.54^{d}	51.59^{d}	51.81^{c}	51.88^{bc}	51.93^{ab}	51.98^{a}	***
	90	51.88^{f}	51.86^{f}	52.11^{e}	52.28^{d}	52.36^{cd}	52.45^{bc}	52.48^{b}	52.60^{a}	52.68^{a}	***
TN/DM (%)	0	6.92^{a}	6.90^{ab}	6.89^{ab}	6.88^{ab}	6.85^{bc}	6.84^{bc}	6.81^{cd}	6.82^{cd}	6.78^{d}	***
	30	6.78^{a}	6.76^{ab}	6.74^{ab}	6.72^{abc}	6.68^{bcd}	6.70^{abc}	6.65^{cd}	6.68^{bcd}	6.61^{d}	***
	45	6.69^{a}	6.66^{ab}	6.63^{abc}	6.64^{ab}	6.60^{bcd}	6.61^{abcd}	6.55^{cd}	6.59^{bcd}	6.53^{d}	***
	90	6.65^{a}	6.61^{ab}	6.59^{abc}	6.58^{abc}	6.55^{bc}	6.54^{bc}	6.51^{bc}	6.52^{bc}	6.50^{c}	**
Acidity (%)	0	0.85^{d}	0.85^{d}	0.86^{d}	0.88^{cd}	0.90^{bc}	0.92^{ab}	0.94^{ab}	0.93^{ab}	0.96^{a}	***
	30	1.08^{c}	1.10^{bc}	1.13^{ab}	1.13^{ab}	1.14^{ab}	1.14^{ab}	1.15^{ab}	1.15^{ab}	1.17^{a}	***
	45	1.19^{d}	1.20^{cd}	1.22^{bcd}	1.23^{bcd}	1.25^{abc}	1.25^{abc}	1.26^{ab}	1.27^{ab}	1.29^{a}	***
	90	1.30^{f}	1.32^{ef}	1.33^{def}	1.34^{cdef}	1.36^{bcde}	1.37^{bcd}	1.39^{ab}	1.38^{abc}	1.42^{a}	***

$^{a, b,c,d}$ Means having different letters in the same row significantly differed at $p \geq 0.05$ ** High significant *** Very high significant

ripening period. This may be attributed to lactose fermentation in the initial period of ripening. Similar results were obtained by Roa *et al.* (1999). Generally, these results show that titratable acidity of all cheeses manufactured using cardoon flowers and ginger rhizomes aqueous extracts were significantly ($P \geq 0.05$) higher than control cheese during ripening period. The highest content of acidity was found to be in cheeses made using 100 % replacing rate of cardoon flowers and ginger rhizomes aqueous extracts. It could be also observed that, cheese made with ginger rhizomes aqueous extracts were the highest in acidity than all experimental cheeses during ripening period. Similar results were reported by Vioque *et al,*. (2000).

It is clear from Table 2 that the moisture content of cheese from all treatments gradually decreased throughout the ripening period especially during the early stages of ripening. Results also indicated that, replacing calf rennet with cardoon flowers or ginger rhizomes aqueous extracts significantly ($P \geq 0.05$) decreased the moisture content of resultant cheese and this was associated with the replacing level. However, the moisture content of Edam cheese obtained with ginger rhizomes aqueous extracts was significantly ($P \geq 0.05$) lower than all experimental cheeses and control cheese. It might be inferred that the structure of the coagulum made using ginger rhizomes aqueous extract retained less liquid during the dewheying stage of cheese (Sanjuan *et al*., 2002). Significant lower moisture levels in cheese manufactured using vegetable coagulant than those produced by calf rennet have been reported by (Sanjuan *et al.,* 2002; Galan *et al.,* 2008). The results in the current research agree with Abdel-Galeel and El-Zawahry (2005) as well as Talib *et al*. (2009) who found that the moisture content of cheese made with *Solanum dubium* seeds extract, was less than that made with calf rennet. Changes in fat on dry matter (fat/DM%) of cheese samples during ripening are presented in Table 2. It is observed that fat/DM (%) increased gradually in all cheeses during ripening period. As indicated in this Table replacing calf rennet with cardoon flowers or ginger rhizomes aqueous extracts significantly ($P \geq 0.05$) increased the fat/DM (%) of the experimental cheeses compared to control cheese during ripening period. This increase was associated with the replacing rate, and the highest increase was noticed in cheese made using ginger rhizomes aqueous extracts. The same Table also, showed marked gradual decrease in total nitrogen on dry matter (TN/DM%) during ripening in cheese of all treatments. This could be attributed to the decrease of moisture during ripening. On the other hand, the obtained results showed that TN/DM (%) values were significantly ($P \geq 0.05$) decreased after 30days of ripening period. Variations in TN/DM (%) values of cheeses prepared with 25, 50, 75 and 100% replacing rate with cardoon flowers or ginger rhizomes aqueous extracts were generally significant ($P \geq 0.05$) compared to control cheese. TN/DM (%) values decreased during ripening with increasing replacing rate. The general trend of these results is in agreement with that reported by Fernandez-Salguero and Sanjuan (1999).

Ripening Indices

Table 3 shows the values of soluble nitrogen as per cent of total nitrogen (SN/TN %), non-protein nitrogenas per cent of total nitrogen (NPN/TN %) and total volatile fatty acids (TVFA) in Edam cheese made using calf rennet replaced by either cardoon or ginger rhizomes aqueous extracts during ripening. As can be seen from this Table, the SN/TN (%) values differed significantly ($P \geq 0.05$) between control cheese and all experimental cheeses. After 30 days of ripening period, the SN/TN (%) values for all experimental cheeses were significantly higher than that made with calf rennet until the end of ripening period. It was observed that, this increase was significantly ($P \geq 0.05$) greater in cheese made with ginger rhizomes aqueous extracts than cheeses made with cardoon flowers aqueous extracts compared to control cheese. This increase of SN/TN (%) was associated with replacing rate with cardoon flowers or ginger rhizomes aqueous extracts. Generally, high levels of SN were observed in cheese made with plant coagulant than that made by using calf rennet (Tejada and Fernandez-Salguero, 2003; Pardo and Fernandez-Salguero, 2007; Agboola *et al*., 2009). They reported that cheese made with cardoon extract showed higher levels of water-soluble nitrogen compared to cheese made by using calf rennet.

Table 3. Effect of replacing rate of calf rennet by crude aqueous extracts of cardoon and ginger on ripening indices of Edam cheese during ripening

Properties	Storage period (day)	Control	Replacing rate of calf rennet by vegetable extracts								Significant
			25%		50%		75%		100%		
			Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	
SN/TN (%)	0	6.68^{f}	6.81^{e}	6.83^{e}	6.86^{de}	6.89^{d}	7.00^{c}	7.08^{b}	7.12^{b}	7.18^{a}	***
	30	9.52^{g}	9.68^{f}	$9.73e^{f}$	9.79^{de}	9.85^{d}	9.95^{c}	10.04^{b}	10.16^{a}	10.22^{a}	***
	45	11.57^{g}	11.80^{f}	$11.86e^{f}$	11.93^{de}	11.98^{d}	12.17^{c}	12.23^{c}	12.44^{b}	12.56^{a}	***
	90	13.92^{h}	14.21^{g}	14.30^{f}	14.32^{f}	14.41^{e}	14.60^{d}	14.77^{c}	14.91^{b}	15.10^{a}	***
NPN/TN (%)	0	3.52^{e}	3.61^{d}	3.64^{d}	3.70^{cd}	3.75^{bc}	3.78^{abc}	3.80^{abc}	3.84^{ab}	3.88^{a}	***
	30	5.57^{e}	5.65^{d}	5.70^{d}	5.73^{d}	5.80^{c}	5.85^{c}	5.94^{b}	5.98^{b}	6.13^{a}	***
	45	6.41^{e}	6.52^{d}	6.55^{d}	6.61^{d}	6.70^{c}	6.76^{c}	6.89^{b}	6.92^{b}	7.06^{a}	***
	90	9.82^{g}	9.95^{f}	9.99^{ef}	10.04^{e}	10.13^{d}	10.23^{c}	10.35^{b}	10.40^{b}	10.52^{a}	***
TVFA (ml Na oH N/10 per 100 gm cheese)	0	8.75^{cd}	$8.69d^{e}$	8.78^{cd}	8.60^{ef}	8.85^{bc}	8.54^{fg}	$8.92a^{b}$	8.48^{g}	8.98^{a}	***
	30	20.20^{b}	20.00^{c}	20.27^{b}	19.86^{d}	20.32^{b}	19.60^{e}	20.43^{a}	19.40^{f}	20.52^{a}	***
	45	30.70^{c}	30.47^{d}	30.75^{c}	30.25^{e}	30.84^{b}	30.12^{f}	30.92^{ab}	30.00^{g}	30.98^{a}	***
	90	38.16^{c}	37.50^{d}	38.23^{bc}	37.12^{e}	38.25^{bc}	36.86^{f}	38.36^{b}	36.00^{g}	38.50^{a}	***

[a, b,c,d] Means having different letters in the same row significantly differed at $p \geq 0.05$ *** Very high significant

Table 3 shows the average values of NPN/ TN (%), as ripening progressed till 90 days. The level of NPN/TN (%) increased significantly in all cheeses. The NPN/TN (%) in cheese made by replacing calf rennet with cardoon flowers or ginger rhizomes aqueous extracts at rate 25, 50, 75 and 100% significantly ($P \geq 0.05$) increased as ripening period progressed. But, the values were significantly ($P \geq 0.05$) higher in cheese made using ginger rhizomes aqueous extracts than cheeses made with cardoon flowers aqueous extracts compared to control cheese. These results are in agreement with some studies carried out on cheese made with ewe's milk were higher levels of NPN have been found in cheese made with plant coagulant than with calf rennet (Tejada and Fernandez-Salguero, 2003; Prado *et al.*, 2007; Galan *et al*., 2008).

From the results in Table 3, it could be seen that replacing calf rennet with cardoon flowers or ginger rhizomes aqueous extracts remarkably affected the total volatile fatty acids (TVFA) content of the experimental cheeses. It was noticed that TVFA of all experimental cheeses significantly ($P \geq 0.05$) decreased by increasing the replacing rate of calf rennet with cardoon flowers or ginger rhizomes aqueous extracts. The results showed that TVFA contents of cheese treatments gradually increased throughout the ripening period. Control cheese showed the highest content of TVFA than all experimental cheeses. Some studies found that cheese produced with vegetable rennet showed less lipolysis than cheese produced with animal rennet (Gaya *et al*., 1990 ; Nunez *et al.*, 1991).

Table 4 presents the evolution of individual and total free amino acids (TFAA) during ripening of Edam cheese made from two different types of aqueous extracts of plant coagulant (cardoon flowers and ginger rhizomes) compared to cheese made with calf rennet. The concentration of TFAA generally increased during ripening up to 90 days in all cheese treatments. However, cheese made with cardoon flowers or ginger rhizomes aqueous extracts contained higher amount of TFAA than control cheese at the end of ripening period. On the other hand, the increasing of amount of TFAA was associated with increasing of replacing rate 25, 50, 75 and 100% with cardoon flowers or ginger rhizomes aqueous extracts (Table 4). Cheese made with ginger rhizomes aqueous extracts showed higher levels of TFAA than cheese made with both cardoon flowers aqueous extracts and control cheese. The major TFAA contents found throughout the whole ripening period in all experimental cheeses and control were Glutamic, Alanine, Isoleucine, Phenylalanine, Lycine and Proline. These amino acids were also the major amino acids found in other cheese varieties during ripening, (Ordanez and Burgos, 1980 ; Garcia-Palmer *et al.,* 1997). It is obvious from the results that increasing TFAA as affected by replacing rate of calf rennet with cardoon flowers or ginger rhizomes aqueous extracts showed a clear tendency to decrease ripening time. The higher levels of TFAA content in cheeses made with cardoon or ginger rhizomes aqueous extracts than the control cheese could be due to the higher proteolytic activity of ginger rhizomes and cardoon flowers aqueous extracts comparing with calf rennet. The general trend for these results is in agreement with that reported by Roa *et al.* (1999).

Sensory Characteristics

As shown in Table 5, significant differences ($P \geq 0.05$) were observed for all sensory attributes between cheeses made by using calf rennet as control or with different replacing rates of calf rennet with cardoon flowers or ginger rhizomes aqueous extracts during ripening period. From these results it could be noticed that replacing calf rennet at a rate higher than 50% of ginger rhizomes aqueous extract or higher than 75% of cardoon flowers aqueous extract significantly ($P \geq 0.05$) decreased the sensory score points for resultant cheese in comparison with the control cheese. The lowest score were shown in case of 75% and 100% of ginger rhizomes aqueous extract replacing level. Increasing replacing rate more than 50% of ginger rhizomes aqueous extract or 75% of cardoon flowers aqueous extracts, the resultant cheeses showed more pronounced pasty texture as well as some flavours defects (especially bitterness) when compared with control cheese. On the other hand, in the present study, cheese made using ginger rhizomes aqueous extract had higher score in flavour than that made with cardoon flowers aqueous extracts after 30 days of storage period. This variation in chesses might be attributed to inherent property of crude

Table 4. Effect of replacing rate of calf rennet by crude aqueous extracts of cardoon and ginger on free amino acids of Edam cheese during ripening

Free amino acids (mg/100g)	Fresh									90 days								
	Control	25%		50%		75%		100%		Control	25%		50%		75%		100%	
		Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger		Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger
Aspartic	22.58	28.73	17.99	19.81	34.09	18.64	25.81	24.61	22.56	39.09	43.86	42.65	59.32	35.24	32.72	33.01	37.22	44.17
Threonine	14.42	5.29	12.67	11.47	5.51	13.10	16.00	15.72	20.52	21.28	21.68	26.43	19.59	20.40	23.06	23.25	33.86	28.00
Serine	17.55	20.87	15.41	16.24	27.99	15.97	18.78	19.13	19.17	36.22	25.65	31.03	48.70	28.89	28.04	28.28	31.63	32.87
Glutamic acid	93.02	84.28	99.20	100.74	87.80	109.01	88.91	101.39	112.05	154.72	130.00	145.90	152.76	184.54	190.26	182.03	184.88	155.59
Glycine	2.77	9.44	-	-	14.21	-	-	3.02	17.78	-	4.01	-	24.41	-	-	-	29.34	-
Alanine	16.63	-	33.14	28.67	-	34.38	33.20	18.13	-	37.27	36.16	54.85	-	51.05	52.74	60.81	-	58.12
Valine	10.69	13.61	13.08	12.75	14.18	13.56	13.75	11.65	14.98	22.26	14.78	22.72	24.67	22.68	23.80	24.00	24.72	24.06
Methionine	11.41	19.07	10.96	12.33	19.86	11.35	16.80	12.44	14.89	17.98	25.32	27.76	34.56	21.93	19.93	20.11	24.57	29.40
Isoleucine	38.06	45.18	42.50	41.22	47.06	44.06	49.60	41.49	39.63	93.05	74.30	81.95	81.88	73.33	77.33	77.99	65.39	86.80
Leucine	14.46	20.21	18.00	16.30	21.06	18.65	20.75	15.76	19.36	39.44	21.89	34.32	36.64	28.99	32.75	33.03	31.94	36.31
Phenylalanine	41.63	55.61	52.09	39.89	57.93	53.97	43.74	34.48	44.46	72.85	45.09	72.26	90.79	70.97	94.77	95.59	73.36	76.55
Tyrosine	3.67	8.98	2.36	5.28	9.35	2.44	4.32	4.00	9.54	5.79	2.37	7.13	10.27	9.40	4.29	4.33	15.74	7.56
Histidine	13.50	5.83	7.42	12.77	6.08	7.69	18.13	14.72	21.77	24.90	9.72	29.95	12.58	22.71	13.44	13.62	35.92	31.73
Lysine	25.16	19.31	21.17	30.47	20.11	21.94	24.79	27.43	32.39	46.08	73.45	40.96	34.99	54.21	38.52	38.85	53.44	43.38
NH_4^+	31.52	22.17	20.81	27.56	23.09	21.56	20.43	34.36	23.73	40.01	96.36	33.75	44.18	49.02	37.86	38.19	39.16	35.75
Arginine	24.31	8.40	26.27	13.69	8.77	27.22	24.08	37.40	31.35	23.01	64.68	39.78	17.25	24.36	47.79	48.21	51.73	42.14
Proline	22.35	39.91	19.57	26.88	26.58	18.21	26.17	24.36	19.11	45.32	43.03	43.25	46.24	47.82	31.97	35.91	31.53	45.80
Total	403.73	406.89	412.64	416.07	423.67	431.73	445.26	440.09	463.29	719.27	732.35	734.69	738.83	745.50	749.27	757.21	764.43	778.23

Table 5. Effect of replacing rate of calf rennet by crude aqueous extracts of cynara and ginger on organoleptic properties of Edam cheese during ripening

Storage period (day)	Properties		Control	Replacing rate of calf rennet by vegetable extracts								Significant
				25%		50%		75%		100%		
				Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	Cardoon	Ginger	
0	Appearance	10	8.00	8.00	8.00	8.00	8.00	8.00	8.00	7.50	7.50	
	Body and texture	40	33.50	33.30	32.00	32.15	33.30	33.40	31.86	33.15	31.65	
	Flavour	50	43.30	41.50	42.95	43.35	41.60	42.25	42.79	41.40	42.85	
	Total	100	84.80^{a}	82.80^{d}	82.95^{d}	83.50^{c}	82.90^{d}	83.65^{b}	82.65^{e}	82.05^{f}	82.00^{f}	***
30	Appearance	10	8.50	8.00	8.00	8.00	8.00	8.50	8.00	8.00	8.00	
	Body and texture	40	34.80	33.87	33.18	34.25	34.45	34.40	33.10	34.70	33.00	
	Flavour	50	44.90	44.10	44.27	43.50	44.05	43.92	44.00	43.15	43.85	
	Total	100	88.20^{a}	85.97^{d}	85.45^{f}	85.75^{e}	86.50^{c}	86.82^{b}	85.10^{g}	85.85^{e}	84.85^{h}	***
45	Appearance	10	9.00	8.50	8.50	8.50	8.50	9.00	8.00	8.00	8.00	
	Body and texture	40	37.20	37.45	34.30	36.40	36.70	37.75	34.15	36.95	33.90	
	Flavour	50	46.75	44.90	45.70	43.90	45.88	44.80	45.50	44.96	45.20	
	Total	100	93.95^{a}	90.85^{d}	88.50^{g}	88.80^{f}	91.08^{c}	91.55^{b}	87.65^{h}	89.91^{e}	87.10^{i}	***
90	Appearance	10	9.50	9.00	9.00	9.00	9.00	9.50	9.00	8.50	8.50	
	Body and texture	40	40.75	38.70	38.00	38.10	39.32	39.35	36.86	38.35	36.50	
	Flavour	50	48.10	47.90	48.15	48.50	47.85	47.40	47.79	47.00	47.60	
	Total	100	98.35^{a}	95.60^{c}	95.15^{d}	95.60^{c}	96.17^{b}	96.25^{b}	93.65^{f}	93.85^{e}	92.60^{g}	***

$^{a, b,c,d}$ Means having different letters in the same row significantly differed at $p \geq 0.05$ *** Very high significant

extract of ginger rhizomes (aromatic plant used as a spice) that provides a pleasant aroma and enhances the flavor of the cheeses (Abdel-Aziz *et al*., 2012). Also, the higher flavour score in cheeses made with ginger extracts may be attributed to a ginger extracts has higher content of essential oil which is a mixture of monoterpenic and sesquiterpenic compounds, contains volatile compounds which is responsible for the characteristic ginger flavour (Zancan *et al.,* 2002).

Conclusion

The results of the current study revealed the possibility of Edam cheese making from the best replacing rate of calf rennet with crude aqueous extracts of cardoon flowers and ginger rhizomes as plant coagulant. So, it could be concluded that an acceptable quality of Edam cheese could be obtained when calf rennet was replaced by 50% or 75% with ginger rhizomes or cardoon flowers aqueous extracts, respectively. No flavour defects were observed in cardoon flowers and ginger rhizomes aqueous extracts cheeses with replacing levels up to 50% of ginger rhizomes or 75% cardoon flowers aqueous extracts. These results show that, cardoon flowers or ginger rhizomes aqueous extracts can be used for the high milk-clotting activity and could be useful in dairy industry both for milk clotting, as an alternative or with calf rennet and for the acceleration of cheese ripening to reduce the time and costs of storage and maturation.

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التغيرات فى الخواص الفيزيوكيماوية والتحلل البروتينى والخواص الحسية فى الجبن الايدام المصنع باستخدام المستخلص المائى لزهور الخرشوف (*Cynara cardunculus*) وريزومات الزنجبيل (*Zigiber officinale*) خلال التسوية

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فى هذه الدراسة تم استخدام المستخلصات المائية الخام لكل من زهور نبات الخرشوف وريزومات الزنجبيل فى صناعة الجبن الايدام ومقارنتها بالمصنعة بالمنفحة الحيوانية، وقد تم دراسة تأثير نسبة استبدال المنفحة الحيوانية بهذه المستخلصات بمعدلات ٢٥، ٥٠ ، ٧٥ ، ١٠٠% على كل من التصافى ونسبة الفقد فى الوزن والتركيب الكيماوى والتحلل البروتينى والخواص الحسية للجبن الايدام خلال مدة التسوية حتى ٩٠ يوم، وقد أوضحت النتائج أن هناك زيادة معنوية ($P\leq0.05$) فى الفقد فى الوزن أثناء التسوية مع انخفاض فى التصافى عند استخدام المستخلصات النباتية محل الدراسة حتى نهاية فترة التسوية وكان هذا الانخفاض فى التصافى وزيادة الفقد فى الوزن مرتبط طرديا بزيادة نسبة الاستبدال. وكان استخدام المستخلص المائى لريزومات الزنجبيل أعلى فى نسبة الفقد فى الوزن للجبن أثناء التسوية عن استخدام المستخلص المائى لزهور الخرشوف عند المقارنة بالجبن الكنترول. كما أوضحت النتائج أن الجبن الناتج من استخدام المستخلصات المائية سواء لزهور الخرشوف أوريزومات الزنجبيل كان منخفض فى محتوى الرطوبة ونسبة النيتروجين الكلى منسوباً للمادة الجافة (TN/DM%) بينما لوحظ زيادة معنوية فى كل من الدهن منسوباً للمادة الجافة (Fat/ DM%) والحموضة (مقدرة كحامض لاكتيك) خلال فترة التسوية مقارنة بالكنترول، وكذلك لوحظ زيادة معنوية فى التحلل البروتينى (SN/TN%)، (NPN/TN%) عند استخدام المستخلصات المائية النباتية وكانت أعلى نسبة عند استخدام مستخلص الجنزبيل مقارنة بالمعاملات الأخرى وكانت هذه الزيادة مرتبطة طرديا مع نسبة الاستبدال، كما أظهرت النتائج أن زيادة نسبة استبدال المنفحة الحيوانية بأكثر من ٥٠% مستخلص ريزومات الزنجبيل و٧٥% مستخلص زهور الخرشوف نتج عنه ظهور القوام العجينى وعيوب فى النكهة (خاصة الطعم المر) فى الجبن الناتج مقارنة بالكنترول، ولذلك يمكن التوصية بامكانية استبدال المنفحة الحيوانية بالمستخلص المائى لريزومات الزنجبيل بنسبة ٥٠% أو بنسبة ٧٥ % فى حالة المستخلص المائى لزهور نبات الخرشوف وذلك لإنتاج جبن ايدام بخواص وجودة مقبولة.

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