CHARACTERIZATION OF MILK CLOTTING PROPERTIES OF ADULT CATTLE RENNET MODIFIED WITH Moringa oleifera SEEDS AND ITS SUITABILITY AS CALF RENNET ALTERNATIVE IN CHEESE MAKING

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ABSTRACT: The present study focused on the mixing of adult cattle rennet with Moringa seeds during the extraction process to improve its strength and use it as an alternative for the calf rennet in cheese production. The Moringa oleifera seeds powder were mixed with adult cattle rennet at a ratio of 2%. Crude and partially purified extracts (using ammonium sulphate 60% concentration) were used. The study include six different milk coagulants; adult cattle rennet extract (ARE), mix of adult cattle rennet with Moringa seeds (AME), partially purified adult cattle rennet extract (ARP), partially purified adult rennet mixed with Moringa seeds (AMP), commercial rennet (RRE) and calf rennet extract (CRE). The flocculation time, clotting time, clotting activity, proteolytic activity, the optimum pH and temperature for clotting activity, curdling quality (RCQ) and protein profile of curd formed were determined (SDS-PAGE). The physical properties include water release (WR) and water holding capacity (WHC), yield and sensory attributes of Domiati cheese made by using different coagulant extracts were estimated. The results showed that CRE has shorter flocculation and clotting time and higher milk clotting activity but ARE had the opposite, in the midst were treatments AME, ARP and AMP. The pH range of 5.5 to 6.5 and temperature between 50 - 60°C were suitable for milk clotting activity of milk coagulants. The best (RCQ) was found to be for the curd formatted by CRE, AMP, ARP and RRE. Physical properties gave the lowest (WR) and highest (WHC) in CRE curd cheese, followed by AMP, RRE, ARP and AME cheese, whilst it was observed the contrary in ARE. Electrophoretic patterns show that the levels of degradation αs1-casein, κ-casein and β-casein differed with milk coagulant types. Using Moringa seeds and partially purified by ammonium sulphate improved yield and cheese sensory with characteristic; coherent texture and creamy taste. In conclusion mixing of adult cattle rennet with Moringa seeds powder during the extraction process and/or partially purified gave an opportunity to be used as an alternative to calf rennet in cheese making.

Key words: Moringa oleifera seeds, adult cattle rennet, calf rennet; ammonium sulphate, commercial rennet, curd properties, Domiati cheese, sensory evaluation.

INTRODUCTION

Coagulation of milk is a basic step in the manufacture of most cheeses and can be achieved by a number of proteolytic enzymes from various sources, such as different animal rennet, microbial proteinases and proteinases extracted from fruits and plants (Vioque et al., 2000). Animal rennet is traditionally manufactured by extraction from the fourth stomach abomasum. The enzymes extracted from abomasum consist mainly of two enzymes maxing chymosin and pepsin. The relative proportion of the two enzymes varies with the age of the animal at slaughtering time. Calf rennet, originally obtained from the abomasum of recent born ruminant calves contains chymosin as the major, milk-clotting components of 88 to 94%, and pepsin to a lesser extent but adult cattle rennet may contain 90 to 94% of pepsin and only 6 to 10% of chymosin (Broome and Limswotin, 1998). Chymosin has been extensively used in

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the cheese making to produce a stable curd with
good flavour due to its high specificity for
cleaving the k-casein Phe 105- Met 106 bond
(Rao et al., 1998). Whereas pepsin is much less
specific and hydrolyses casein coupled with
certain beneficial effect during ripening of
cheese (Papoff et al. 2004). Compared to
chymosin, pepsin has number of difficulties
such as longer clotting time, forming a soft curd,
and an undesirable taste. The clotting activity
affects the properties of the curd, such as
firmness or softness, during processing
(Walstra et al., 2005). However, the worldwide
increase in cheese production, coupled with
reduced supply and increasing prices of calf
rennet, some religious factors (Islam and
Judaism) and others related to vegetarianism of
some consumers, have encouraged the search for
alternatives sources of milk-coagulants to be
used as appropriate rennet substitutes. Research
effects have been directed for new proteases
with high specific milk-clotting activity and low
general proteolytic activity to resemble animal
rennet properties (Shah et al., 2014). Accordingly, some plant proteases similar
chymosin are able to cleave few sites at αs- and
β caseins, which may occur in maintaining the
micelles stability (Silva et al., 2002). Pontual et
al. (2012) confirmed that Moringa oleifera is a
ew source of proteases with potential use for
cheese production on account of promoting
extensive hydrolysis of k-casein and low
degradation of αs- and β-caseins compared with
other plant coagulants. It has been concluded that
Moringa oleifera plant is an exciting source of
milk clotting enzymes such as aspartic
enzyme in different parts (Pontual et al., 2012).
Agreeing to Tajalsir et al. (2014), the milk
clotting activity of M. oleifera was only found in
the seeds extract while the other parts were
either deficient or has very low milk clotting
activity. Moringa oleifera seeds extract are rich
in proteases having specific clotting activity 200
times higher than that of flower extract.
Approving to these results, it is concluded that
the seed extract of M. oleifera generates suitable
milk clotting activity for cheese making
(Sánchez-Muñoz et al., 2017). Mahami et al.
(2012) proved that Moringa oleifera seed extracts used to prepare cottage cheese with
increasing in the yield of cheese. On the same
line, sensorial properties including texture,
appearance, flavour, taste and colour of the
produced cheeses were improved by used
Moringa oleifera seeds as coagulant (Orhevba
and Taiwo, 2016).

Precipitation with ammonium sulphate is
extensively used in processing of biological
products, such as proteins. According to Kent
(1999) protein purification using ammonium
sulphate has three main advantages; it is a rapid
and inexpensive method, it does not affect the
structure and function of proteins and the salt
can be easily removed from the protein solution
by dialysis. Sedimentation with ammonium
sulphate could be an effective way to produce
substantial amounts of active purified proteases,
and concentrated the enzyme to a workable
volume that could efficiently be used for milk
coaugulation in cheese making industry (Tajalsir
et al., 2014).

The present study was undertaken to evaluate
the milk clotting properties of crude and
partially purified adult rennet modified by
mixing with Moringa oleifera seeds during the
process of extraction. The suitability of the
resultant extracts as calf rennet alternatives in
cheese making was given considerable attention

MATERIALS AND METHODS

This study was carried out during November
2017 to August 2018 in the Dairy Technology
laboratory of Dairy Department, Agricultural
Faculty, New Valley University, Egypt.

Materials

Fresh abomasum of adult cattle and calf were
brought from one butcher in El-kharga City,
New Valley Governorate, Egypt. The dried pods
in Moringa oleifera were collected from the
local farm in El-Kharga City, New Valley
Governorate, Egypt. Raw cow’s milk (total solid
12.8%, 3.5% protein, 3.7% fat, 5.0% lactose and
pH 6.7) was obtained from private animal farm
in El-kharga City, New Valley Governorate,
Egypt. Low heat skim milk powder imported
from USA (Dairy America California).
Commercial liquid calf rennet was purchased
from Misfád, Misr Food Additives, Egypt.
Sodium chloride, ammonium sulphate, sodium
acetate, and other chemicals were purchased
from Gomhoria Company, Assiut Governorate,
Egypt.
Experimental Procedure

Preparation of experimental crude rennet extracts

Rennet of adult cattle (ARE) and rennet of calf (CRE) extracts were prepared according to the method of Ahmed et al. (2013), with some modification. After removing the internal contents, abomasum tissues were cleaned with tap water, as well veins and fat contents were external removed. Then they were inflated with air just like a balloon, 20 g sodium chloride were externally sprayed and dried in an open air for about one week followed by storage for one month. The abomasum was then cut into thin pieces. Weight (100g) of dried abomasum was soaked in 1000 ml solution containing 60 g sodium chloride, 2 g sodium benzoate, 0.5 g sorbic acid in distilled water), and adjust the pH to 5.4. The solution was stored at room temperature for one week with daily stirring. The extract was filtered through muslin cloth, clarification by addition 8g aluminum potassium sulphate and free enzyme by addition 16 g disodium phosphate (10% solution) with stirring for 3 min. The extract was again filtered through Whatman filter paper No. 1 and centrifuged at a speed of 5000 rpm for 20 min, then the pellet was discarded while the supernatant was collected and designate as crude enzyme extract.

Preparation of crude adult cattle rennet modified by Moringa seed

Manually the seeds were removed from pods and removed seed coats from seeds to obtain clean seed kernels. The white kernels were then crushed to a powder using an electric grinder to obtain a fine powder. About 20g of seeds powder was blended in 1000 ml crude enzyme of adult cattle rennet. Then it stirred rapidly for sixty minutes, after every interval the samples were allowed to rest for 60 minutes and were filtered through Whatman filter paper No1. Then centrifuge at a speed of 5000 rpm for 20 min. The pellet was discarded and the supernatant was collected designate crude enzyme mix of adult cattle rennet with Moringa seeds (AME).

Partial purification of crude adult cattle rennet extracts

The crude enzyme of adult cattle rennet without and with added Moringa oleifera seeds powder were partially purified by ammonium sulphate precipitation technique. The crude enzyme extract (50 ml) was partially purified by adding of solid ammonium sulphate to 60% concentration at 4°C for 30 min according to Ahmed et al. (2009), with some modifications. The sedimentary protein was collected by centrifugation at 5000 rpm at room temperature for 20 min. The supernatant was discarded while the precipitate was re-dissolved in 5 ml of 0.1 M sodium acetate buffer (pH 5), and then dialyzed overnight at 4°C against the same buffer with several change of the dialysis buffer. The extracts obtained were named ARP and AMP for partially purified for adult cattle rennet without and with added Moringa seeds powder, respectively.

Methods of Analysis

Determination of rennet flocculation and clotting times

The rennet flocculation time (RFT) is the time period between the additions of coagulant enzyme and the appearance of flakes visible to the naked eye (Kappeler et al., 2006). The rennet clotting time (RCT) is the time period between the application of the coagulant enzyme to the milk at 35°C and the aggregation of milk. The rennet curdling quality (RCQ) is evaluated after the curd formed and tipped out into Petri dish to assay the appearance and firmness of the curd and the appearance of the whey as investigated by Kuchtík et al. (2008).

Determination of milk-clotting activity (MCA)

The milk clotting activity of coagulant enzyme was performed as described by Badgujar and Mahajan (2009). The enzyme extract (0.2 ml) was added to 2 ml of substrate solution of reconstitute 10% skim milk powder with 0.01M CaCl₂. The time needed for the formation of curd fragment at 35°C was measured. Milk clotting activity is expressed in term of Soxhlet unit.

Soxlet units (U/ml) = \( \frac{M \times 2400}{E \times t} \)

Where:
M: the volume of substrate (ml).
E: the amount of enzyme extract (ml).
t: the clotting time (sec).
Determination of protein content and proteolytic activity (PA)

Total protein was determined in milk coagulants by the standard micro-Kjeldahl method of the AOAC (1995). A nitrogen conversion factor of 6.25 was used for calculation of protein. Proteolytic activity of coagulant extracts was evaluated by comparing non-protein nitrogen (NPN) contents of raw reconstituted milk and rennet coagulated milk (Ahmed et al., 2013). Briefly, 0.2 ml of enzyme extract was added to 2 ml of 10% (W/W) of reconstituted skim milk powder containing 0.01 M calcium chloride and incubated at 35°C for 40 min. Non-protein nitrogen (NPN) content was determined according to the method of International Dairy Federation (IDF, 1993). The specific activity was determined by dividing the MCA on protein content (mg).

Optimum temperature and pH for milk clotting activity

Aliquots of reconstituted spray-dried skim milk (10 g in 100 ml 0.01 M calcium chloride) were adjusting to different pH values (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0), and incubated at different temperatures (30, 35, 40, 45, 50, 55, 60, 65 and 70°C) . Adjusted to the desired pH with 0.1 M HCl or NaOH and incubated at the desired temperature, according to Corrons et al. (2012).

Electrophoretic Analysis (SDS–PAGE)

Soluble proteins were extracted from curd formed by ARE, AME, ARP, AMP, RRE and CRE coagulants. Crushing 1.0 g of the sample in 1ml of extraction buffer (0.1 M Tris-HCl, 2 mM EDTA, 2% glutathione, pH 7.8). The mixture was centrifuged for 20 min at 13000 rpm and 4°C. A mixture of the supernatant and the sample application buffer (2.5 mM Tris-HCl pH 6.8, 10.0% glycerol, 4.0% SDS, 0.02% bromophenol blue and 10% mercaptoethanol) was prepared with a ratio of (1:1) and then, the mixtures were incubated in water path at 100°C for five min. for protein denaturation, and then loaded in the electrophoresis apparatus. Electrophoresis was carried out according to the method of (Laemmli, 1970). Electrophoresis was performed in a vertical slab gel apparatus "SE 600, vertical slab gel" at 10°C in a 12% polyacrylamide separating gel, 3.5% stacking gel, denaturing conditions using 1% sodium dodecyl sulphate, at 30mA, until the dye band reached the bottom of the separating gel. For the detection of protein bands, the gels were stained with comassie brilliant blue R for one hour. Then, the destining was performed by repeated immersion in a mixture of methanol/ acetic acid/water (1: 1:8, by volume).

Cheese making

Domiati cheese treatments were made according to Abou-Donia (1986) with some modulation. Fresh cow milk (3.7% fat and 3.5% protein) was heated to 72°C for 2 min. and then cooled to 45°C, followed by addition of skim milk powder, CaCl₂ and NaCl at the rate of 5%, 0.02% and 6% respectively. The milk was divided into six equal portions. Milk coagulants; ARE, AME, ARP, AMP and CRE were added at the ratio of 2 ml/l milk and that of RRE concentration was 0.6 ml/l of milk. The resultant cheeses were referred as called ARE, AME, ARP, AMP and CRE and RRE cheese. The milk was mixed and left until coagulation was completed. After coagulation; the curd was cut with a sharp knife. The curd was poured into small stainless steel holes molds overnight. Cheese samples were taken for analyses after one day.

Estimation of cheese curd properties

Water release (WR) analyses were performed using the method reported by Lemes et al. (2016). 10 g of curd cheese were centrifuged at 5000 rpm for 20 min at room temperature. Water release (WR) was defined as follows:

WR (%) = 100 (Initial weight - final weight) / (initial weight).

Water holding capacity (WHC) was calculated according to Farnsworth et al. (2006).

WHC (%) = 100 (curd cheese weight– serum weight) / curd cheese weight

Yield calculation

Cheese yields were calculated as a weight of cheese divided by weight of milk expressed as a percentage (Nelson et al., 2004).
Sensory attributes

Organoleptic properties of fresh Domiati cheese were assessed by taste panel included mainly researchers and graduate students of the Dairy Science Department, Faculty of Agriculture, New Valley University. Fresh cheese samples were evaluated for flavour (50 points), body and texture (40 points) and appearance (10 points) according to Bodyfelt et al. (1988).

Statistical Analysis

SPSS (Version 11.0) software was used for all statically analysis. The effects of crude extract, purified enzyme, commercial rennet and calf rennet on curd properties yield and sensory attributes of Domiati cheese were estimated using analysis of variance (ANOVA). Significant means were compared using Duncan’s test on the level of P ≤ 0.05.

RESULTS AND DISCUSSION

Flocculation and Clotting Times

The coagulation properties of milk are of great importance as they influence cheese yield and quality. Fig. 1 clears that the flocculation time (RFT) of ARE, AME, ARP, AMP, RRE and CRE was found to be 169.67, 133.67, 122.67, 82.33, 56.00 and 28.00 seconds respectively. Clotting time (RCT) was 217, 177.67, 152.00, 119.33, 83.67 and 53.67 seconds, respectively. From these results, it could be concluded that crude and partially purified adult cattle extract containing Moringa oleifera seeds had shorter flocculation and clotting time compared with the corresponding extract prepares without Moringa seeds. Shorter flocculation and clotting time were found to be the partially purified adult rennet extract with added Moringa seeds. Whereas their values were comparable to those of both calf rennet extract and commercial rennet. These results could be explained on the basis that Moringa oleifera seeds contained one or more enzymes with rennet like activity (Sánchez-Muñoz et al., 2017). Partial purification using ammonium sulphate has led to more concentrated milk clotting proteases. This may be due to molecular rearrangements in protein structure can lead to increase of enzyme activity (Purich, 2010). Moreover Castillo et al. (2002) showed that the use of calf rennet as milk coagulant led to faster increase in reflectance due to a higher specific activity to k-casein. Short clotting time is imported in the manufacture of various type of cheeses, such as soft, semi-hard and hard cheese, could be due to increase in rate of aggregation as a result of decrease in volume of the aqueous phase (Mehaia and El-Khadragy 1998).

Milk Clotting Activity (MCA)

Milk clotting activity (MCA) is the most important property of proteases used in cheese production. Accordingly, the highest milk clotting activity (MCA) was recorded for the CRE as illustrated in Table 1. While, ARE had the lowest milk clotting activity. This indicated that the clotting activity is related to the enzyme’s capacity to calving k-casein at the Phe105-Met106 peptide bond, which is the starting point of enzymatic cheese making. Guinee and Wilkinson (1992) showed that the clotting activity of rennet relies on its ability to degrade casein micelles. The action is dependent on the chymosin and pepsin content of the rennet. Therefore, this suggested that the content of pepsin was higher in adult cattle rennet extract (Broome and Limsowtin, 1998), compared with rennet extracted from younger calves containing higher chymosin (Akin, 1996). Normally commercial rennet contains 70% chymosin and 30% pepsin. The standard ratio is approximately 80% chymosin and 20% pepsin (Irigoyen et al., 2001). However, adult cattle rennet may contain up to 90 to 94% of pepsin and only 6 to 10% of chymosin (Broome and Limsowtin, 1998). Good quality rennet should possess a constant clotting activity and contain no other enzymes than chymosin (Çakmakçı and Boroğlu, 2004). It was also observed from Table 1 that the milk clotting activity increased nonetheless decreasing clotting time with mixing adult cattle rennet with Moringa seeds powder during the process of extraction, indicating that M. oleifera is an exciting source of milk clotting enzyme with an excellent clotting property (Sánchez-Muñoz et al., 2017). Partial purification by ammonium
positive linked to yield of cheese (Wedholm et al., 2006).

**Protein Content, Proteolytic Activity and Ratio Milk Clotting Activity (MCA) to Proteolytic Activity (PA)**

Evaluation of enzymatic activities and proteolytic activities (PA) is a crucial step in the selection of an appropriate substitute of calf rennet. Table 1 shows the results of protein content, proteolytic activities (PA) and MCA/
PA for different milk coagulants. The protein contents (g/l) were 1.53, 1.45, 1.31, 1.27, 1.42 and 1.36 for ARE, AME, RRE, CRE, ARP and AMP, respectively. There were significant differences (P≤0.05), between coagulants. However, an increase in protein content decreases milk clotting activity. In addition the results indicated that CRE was lower than ARE in proteolytic activity. This may be due to pepsin naturally present in rennet, which has a lower specific milk-clotting activity with a broader proteolytic activity than chymosin (Slamani et al., 2018). The breakdown depends on synergistic enzymatic action of chymosin and pepsin contained in animal rennet. The low proteolytic activity as shown in trials AMP followed ARP and AME are pre-requisite for an acceptable rennet substitute. The ratio of milk-clotting activity (MCA) to proteolytic activity (PA) of the enzyme is a useful indicator for its quality as a rennet substitute for cheese making (Sánchez-Muñoz et al., 2017). The results clear that ARP and AMP extract had higher ratio (MCA/PA), in agreement with the findings of Barros et al. (2001). Therefore an enzyme with higher ratio of milk-clotting to proteolytic activity is able to form curd, get higher yield and less bitterness development during cheese processing. While, low ratio may developed lower curd recovery, weak curd firmness and release bitter peptides that affect the sensory properties of the final product (Mazorra-Manzano et al., 2013). For this reason, the evaluation of enzyme activities and their comparison with commercial rennet is an important first step in choosing a suitable plant coagulant. From the results found, it can be concluded that, the milk clotting enzymes present in AMP, ARP and AME are promising candidates for application in industrial scale for production of cheese. Tajalsir et al. (2014) showed that partially purified enzyme from Moringa seeds showed higher ratio of milk clotting activity to proteolytic activity compared to that of calf rennet that is extensively used in cheese making industry.

**Optimum pH and Temperature for Milk-Clotting Activity**

The effect of pH and temperature on milk clotting activity is shown in Fig 2. Nearly all milk coagulants preparation; ARE, AME, ARP, AMP, RRE and CRE exhibited almost a linear milk clotting activity with an increased pH from 5.5 up to 6.5. Milk clotting activity significantly enhanced at pH 5.5 but activity was lowered above pH 6.5. Milk clotting activity decreased gradually as pH increased. The milk clotting activity of AME, ARP, AMP, RRE, and CRE was found to be high at pH 6.0 and ARE at pH 6.5. These results agree with those of Tajalsir et al. (2014), who used the same conditions. Furthermore, AME, RRE and CRE reached less activity up to pH 7.0. Also ARP and AMP showed complete inactivation up to pH 7.0. These results are in accordance with Elagamy (2000). Lamas et al. (2001) showed that most plant protease are unstable at alkaline pH value. Calf rennet showed similar conduct, acting better in acid (pH range of 5.5–6.0) than in alkaline reaction medium. The pH of the milk for rapid milk clotting activity is very important during cheese making. Ramet (2001) showed that all clotting cheese enzymes are acid protease, with optimum activity at pH 5.5 and also that the kappa casein presents stability at pH 5.6. Lenoir et al. (1997) found that the effect of pH of milk on flocculation was very sensitive and apparent, thus the flocculation time is further reduced if the renneting pH is below the normal pH of milk. Formaggioni et al. (2001) reported that milk clotting properties were better when associated with decreasing values of pH and increasing values of titratable acidity.

On the other side, the influence of temperature was most pronounced between the 55 - 60°C for milk clotting activity of milk coagulants. Similarly (Kumari et al., 2012) reported that the purified serine protease (clotting enzymes) from Ficus religiosa had optimum temperature around 55 - 60°C, but beyond these values there were a progressive denaturation of the enzyme and at 65°C there were no activity. The highest milk clotting activity was recorded for ARE, AME and ARP at 50 - 60°C, and for AMP at 40 - 50°C, CRE at 50 - 60°C and RRE at 55 - 65°C. The temperature of maximum activity for milk coagulants AMP and CRE was 50°C likewise, milk coagulants ARE, AME and ARP was 55°C furthermore, milk coagulant RRE was 60°C. The clotting activity began to decrease when temperature increased to 70°C, which in the line
Fig 2. Optimum temperature and pH for milk-clotting activity of different milk coagulants

ARE (adult cattle rennet extract)  AMP (partially purified adult rennet mixed with Moringa seeds)
AME (mix of adult cattle rennet with Moringa seeds)  RRE (commercial rennet)
ARP (partially purified of adult cattle rennet extract)  CRE (calf rennet extract)
MCA (milk clotting activity)
to the activity described by Ahmed et al. (2009). It is well known that the reaction rate of enzyme increases with increasing temperature, but after a certain temperature, the rate of reaction decrease due to denaturation and consequently the coagulation time is lengthened (Hashim et al., 2011). Shindo and Arima (1979) reported that cattle chymosin was relatively stable up to 50°C and a relative decline in the milk-clotting activity of almost 55% was observed at 55°C, there being a total loss of activity at 60°C. The optimum temperature of most clotting enzymes are around 40-50°C, but further than these values there are a progressive denaturation of the enzyme and at 65°C there was no activity (Ramet, 1985). On the other hand, Balcones et al. (1996) reported that the decrease in clotting activity as a result of increasing temperature over certain limit could be explained by the changes in salt equilibrium and the complex between κ-casein and β-lactoglobulin.

The Evaluation of the Rennet Curdling Quality

The rennet curdling quality (RCQ) of crude extract, purified enzyme, commercial rennet and calf rennet is shown in Table 2. The curd formatted by CRE was very good and hard without separated whey. AMP had good hard curd, keeping its shape without appearing whey. However ARP had good curd, with little whey of yellow-greenish colour, RRE had hard curd, without appearing whey. AME, had softer creaminess curd, keeping its shape quite perfectly and whey was yellow. ARE, had, weak curd, not keep its shape, whey was milky white. In the same case Slamani et al. (2018), found that curd produced using ovine pepsin was less firm than that made with chymosin. Chymosin, an aspartyl proteinase, is used for coagulating of milk and fabrication of cheese. The results indicated that the best (RCQ) was found in the curd formatted by CRE followed by AMP, ARP and AME. Whilst, the worst (RCQ) was found in the curd shape by ARE coagulant. So, the curd formation from CRE coagulant exhibited the fastest and ARE had the lowest. Results from this study indicated that mix adult cattle rennet with Moringa seeds and its partially purified enzyme demonstrated the highest clotting activity compared with adult cattle rennet. These results are in agreement with those of Tajalsir et al. (2014).

Electrophoretic Profile of the Curd Format by Different Milk Coagulants

Fig. 3 shows SDS-PAGE of curd formed by ARE, AME, ARP, AMP, RRE and CRE extract. The applied electrophoretic method separated curd cheese proteins into multiple components with molecular weights ranged from 10 kDa - 250 kDa, values were similar to those reported by Manzo et al. (2017). There were notable differences in electrophoretic patterns among curds shaped by different coagulant extracts. All coagulant extracts hydrolysis κ-casein but in varying proportions. CRE coagulant characters appeared κ-casein. AME similar CRE had clear hydrolysis of κ-casein. On the other hand, there hydrolysis products (α, β -casein) were found in curd cheeses made with AME coagulant. The results agree with those of Garcia et al. (2012). Electrophoresis profile found that β-casein disappeared from curd formed by ARP curd. Partial purification from M. oleifera seeds (AMP) is a potentially useful tool in cheese production processes, since it did not promote extensive hydrolysis of αs and β-caseins. This is in line with the results reported by Bruno et al. (2010). The result also found in ARE coagulant similar hydrolysis casein to that of AME coagulant. RRE coagulant had higher proteolytic activity against κ-casein than αs and β-casein (Ordiales et al., 2012).

Physical Characteristics of Cheese Curd

Physical characteristics of curd were assessed by determination of water release (WR) and water holding capacity (WHC). Water Release and Water Holding Capacity

The results of water release (WR) and water holding capacity (WHC) of cheese curd are shown in Table 3. The results showed that CRE cheese curd had the lowest water release followed by AMP, RRE, ARP and AME, whilst, in turn, ARE had the highest (p ≤ 0.05), possibly
Table 2. Evaluation of curdling quality (RCQ) clotting by different milk coagulants

<table>
<thead>
<tr>
<th>Coagulant</th>
<th>Appearance, firmness of curd and appearance of whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARE</td>
<td>Weak curd, partly liquefaction and loss of shape, whey milky white colour.</td>
</tr>
<tr>
<td>AME</td>
<td>Well curd but a softer creaminess, keeping its shape quite perfectly.</td>
</tr>
<tr>
<td>ARP</td>
<td>Good curd, a little whey, of yellow-greenish colour.</td>
</tr>
<tr>
<td>AMP</td>
<td>Good curd and hard, keeping its shape without appearing whey</td>
</tr>
<tr>
<td>RRE</td>
<td>Good curd and hard, without appearing whey.</td>
</tr>
<tr>
<td>CRE</td>
<td>Very good curd and hard, without separated whey.</td>
</tr>
</tbody>
</table>

ARE (Adult cattle rennet extract)  AMP (partially purified adult rennet mixed with Moringa seeds)  AME (mix of adult cattle rennet with Moringa seeds)  RRE (commercial rennet)  ARP (partially purified of adult cattle rennet extract)  CRE (calf rennet extract)

Fig. 3. SDS-PAGE profile of curd formed with different milk coagulants

ARE (Adult cattle rennet extract)  AMP (partially purified adult rennet mixed with Moringa seeds)  AME (mix of adult cattle rennet with Moringa seeds)  RRE (commercial rennet)  ARP (partially purified of adult cattle rennet extract)  CRE (calf rennet extract)
Tables 3. Physical properties of Domiati cheese curd formed by different milk coagulants

<table>
<thead>
<tr>
<th>Coagulant</th>
<th>Water release (WR)</th>
<th>Water holding capacity (WHC)</th>
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</thead>
<tbody>
<tr>
<td>ARE</td>
<td>77.10±2.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.43±2.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AME</td>
<td>73.23±6.31&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.27±6.62&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARP</td>
<td>72.93±1.62&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.65±1.35&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMP</td>
<td>69.83±3.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.07±3.15&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>RRE</td>
<td>70.17±3.91&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>30.80±4.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRE</td>
<td>68.97±2.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.87±2.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by different letters are significantly different at P < 0.05.

ARE (Adult cattle rennet extract)       AMP (partially purified adult rennet mixed with Moringa seeds)
AME (mix of adult cattle rennet with Moringa seeds) RRE (commercial rennet)
ARP (partially purified of adult cattle rennet extract) CRE (calf rennet extract)

because of the porous and loose gel in ARE curd. This may be due to that chymosin has high specificity for the k-casein and pepsin has low specificity which affects the properties of the curd, such as firmness or softness (Walstra et al., 2005). Pepsin as a single coagulating agent is not preferred in cheese making because of its high proteolytic activity (Temiz et al., 2008). A decrease in the water release causes the syneresis process, which refers to the serum that is out from the gel structure and accumulates on the surface. An increase in the syneresis process was observed in the ARE, which can be verified by reduction the values of water holding capacity (WHC). This occurrence was more evident when the samples were estimated by centrifugation, indicating a greater water release from gel structure. These results were markedly, and it may depend on the more water release and non-stable structure. Syneresis is one of the most important processes in cheese-making because it directly affects cheese yield and quality through its effect on moisture, mineral and lactose content of curd. It is also imperative to cut the curd at an appropriate firmness so that syneresis takes place properly and loss of milk solids in the whey can be minimized (Bynum and Olson, 1982). There were significant differences (P ≤ 0.05) between the coagulant activities obtained for crude ARE and CRE comparative of other milk coagulants. The results showed that CRE, AMP and RRE curd cheese samples had a higher (WHC). Per cent of (WR) and (WHC) of ARP and AMP gels was similar. The clots formed by milk coagulants; AME and ARP showed the same characteristics; stability and transparent whey exudate. These results showed that both the mix adult cattle rennet with Moringa seeds and partial purified enzyme could be used as alternative of commercial rennet.

Yield and Sensory Evaluation of Fresh Domiati Cheese

Yield of cheese

The casein fraction of milk protein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately affecting cheese quality and yield (Lawrence, 1993). Table 4 shows the yield of Domiati cheese made by different coagulants. It could be noticed that the cheese made by CRE had the highest yield, white cheese made by ARE had the lowest yield compared with cheese made by other coagulants. Furthermore, highly significant differences (p ≤ 0.05) were observed between all fresh cheeses except ARP similar AMP cheese. The speed of hydrolysis of caseins influences in yield, and slow degradation of αs-and β-caseins is guarantee production of a firm curd, which is occurs when chymosin is used (Bruno et al., 2010). Most rennet substitutes are more proteolytic than calf rennet and cause diminished yields of casein and fat. The increase in syneresis was probably due to the decreasing water holding capacity that led
Table 4. Yield and sensory attributes of fresh Domiati cheese made from different milk coagulants

<table>
<thead>
<tr>
<th>Item</th>
<th>ARE</th>
<th>AME</th>
<th>ARP</th>
<th>AMP</th>
<th>RRE</th>
<th>CRE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield</strong></td>
<td>15.86±0.65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.86±0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>17.12±0.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>17.89±0.58&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>18.48±0.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.56±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sensory Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance (10)</td>
<td>5.82±0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.91±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.60±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.73±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.30±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavour (50)</td>
<td>44.27±1.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.87±1.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.30±1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.55±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.55±1.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>46.10±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body and texture (40)</td>
<td>34.45±2.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.36±1.29&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>37.10±5.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>37.40±1.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.91±1.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.30±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall scores (100)</td>
<td>84.54±2.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.14±1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.00±5.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.68±2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.46±2.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.70±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by different letters are significantly different at P < 0.05.

ARE (Adult cattle rennet extract) AMP (partially purified adult rennet mixed with Moringa seeds) AME (mix of adult cattle rennet with Moringa seeds) RRE (commercial rennet) ARP (partially purified of adult cattle rennet extract) CRE (calf rennet extract)

to more release of whey. The whey drainage indicated weakness of the curd, probably decline the yield of cheese. Obviously, this submitted that Moringa seeds may have accounted for the increase in yield. These results are in agreement with the remark of Mahami et al. (2012) who used Moringa seeds in improving the yield and quality of cottage cheese. Results indicated that mixing adult cattle rennet with Moringa seeds or purified by ammonium sulphate resulted in decreasing the coagulation time, decreasing the curd syneresis and increasing the water holding capacity compared with adult cattle rennet only. Differences in the cheese yield among the different types of coagulants might be due to their proteolytic specificity, as highly specific coagulants provide higher cheese yields. Milk with short coagulation time, with lesser water retention and rise water holding capacity is expected to give more cheese yield (Kübarsepp et al., 2005).

Sensory evaluation of cheese

Sensory attributes included appearance, flavour, body and texture as well as overall scores of Domiati cheese made using different rennet sources are illustrated in Table 4. The results show that general acceptability of Domiati cheese as total scores indicated that CRE cheese gained the highest acceptability and that ARE cheeses gained the lowest scores being significantly (p ≤ 0.05) different from other cheese treatments. Chymosin is the major enzyme of calf rennet, and it has been widely used in the dairy industry to produce a stable curd with good flavour due to its high specificity for the Phe105-Met106 bond of k-casein (Rao et al., 1998). Acceptability increased with mixing Moringa seeds and partial purification. Some studies reported that vegetable coagulants lead to a soft and pasty cheese texture, partly to liquefaction and loss of shape (Galán et al., 2008). As well as short peptides produced by its high proteolytic activity affect the flavour, which resulted in an excessively acidic and bitter cheese (Öner and Akar, 1993). On the present study appeared the status contradictory, the cheese made by used mix of adult cattle extract with Moringa seeds and partial purified had better sensory with characteristic; coherent texture and buttery taste. Tajalsir et al. (2014) reported that M. oleifera seed extracts were used as a milk-clotting agent, and the resulting curd was white and firm. The use of a low amount of plant coagulants in milk gelation at low pH, may avoid the negative impact of excessive proteolysis of caseins on the texture and flavour of cheeses (Esteves et al., 2003). Generally cheese made by CRE had the higher overall scores followed AMP, RRE, ARP and AME. The results reveal that mixing Moringa seeds and its partial purification (by ammonium sulphate) are a new source of proteases with...
potential use for cheese making, since it promotes extensive hydrolysis of k-casein and low degradation of αs- and β-caseins resulting. These results agree with Pontual et al. (2012). ARE cheese had the lowest scores; appearance, body and texture as well as flavour. Total sensory parameter of the cheeses clotting by mix Moringa seeds and purified enzyme were close to the RRE and of CRE cheese and both of them had higher sensory scores than ARE cheese.

Conclusion

Clotting studies are important in the manufacture and ripening of cheeses. Good milk-clotting enzyme is characterized by a high specific activity and a low proteolytic activity, later the proteolysis strongly affects the textural and sensory properties of cheese. However, a better understanding of the effect of plant coagulants on rheological properties, sensory characteristics (appearance, flavour, body and texture, taste as well as colour) in addition yield of the cheese is also of great importance when choosing the best substitute of calf rennet. This study focused primarily on maxing adult cattle rennet with Moringa seeds powder during extract processing to increase the rennet strength for milk clotting activity which effect on organoleptic scores and yield of cheeses. The characteristics of the final product could strongly resemble to those of the same cheese made with calf rennet.

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توصيف خصائص تجنيب اللبن بمنحة الماشية الكبيرة وتعديلها بيدور المورونجا لتكون بديلاً مناسباً

منحة العجل الراضية في تصنيف الجبن

هشام عبد الرحمن اسماعيل

قسم الألبان – كلية الزراعة – جامعة الوادى الجديد – مصر

سلتت هذه الدراسة الضوء على خلطة منحة الماشية الكبيرة مع مسحوق بئر المورونجا أثناء الاختلاص لتحسين قوتها التجينية واستخدامها كيديل منحة العجل الرضية في صناعة الجبن، خلط مسحوق بئر المورونجا مع مستخلص منحة الماشية الكبيرة بنسبة 2%، واستخدم المستخلص الخام والمتفق جزئياً باستخدام كرينيبات الأمونيوم تركيز 100%، تضمنت الدراسة ستة أنواع من مجذبات اللبن كالتالي: مستخلص منحة الماشية الكبيرة (ARE)، خليط مستخلص منحة الماشية الكبيرة وبدور المورونجا (AME)، مستخلص منحة الماشية الكبيرة وبدور المورونجا (ARP)، مستخلص منحة الماشية الكبيرة والخليل بئر المورونجا (AMP)، مستخلص المنحة التجارية (RRE) وأخيراً مستخلص العجل الرضية (CRE).

تقدر زمن بداية وتمام التجين والنشاط التجنيبي والتحللي لجميع المجذبات ودرجات الحرارة SDS-RQ والتهيج الريزي لخثرة الخثرة (RQ) (المحوبي السائدة للشراكة التجيني وجودة الخثرة) وسرعة الاحتفظ بالماء (WR)، وأيضاً الخصائص الفيزيائية مثل انسياب الماء (PAGE). جزء من بنسبة الماذاعي المنبع باستخدام مختلف المجذبات التي صدرت الدراسة، أوضح النتائج أن المجذبات احتاج CRE جاهز إلى بداية التجين وتمام التجين قصير وأرتفاع النشاط التجيني بينما المجذبات ARP و CRE وAMP تراوح درجة المرونة ما بين 7-6، ودرجات الحرارة ما بين 32-34 م به، لاحظت خلوية المرونة في الخثرة المختلفة للمجذبات من CRE، AMP، ARP، RRE هي المناسبة للشراكة التجيني، وجودة الخثرة (RQ) (المكونات من الخثرة) كانت أفضل والخصائص الفيزيائية أظهرت انخفاضاً ملحوظاً لانسياب الماء وارتفاع لسهولة الاحتفاظ بالماء في الخثرة المختلفة للمجذبات، بينما经理 في الخثرة المكونة CRE بواسطة المجذبات من المنحة التجارية، كان أعلى في انسياب الماء والقلق في سعة الاحتفاظ به، التغيير الكهربائي للخثرة هم من المجذبات المختلفة أوضحت اختلافات في مستوى تحلل كلاً من منحة الماشية الكبيرة مع بئر المورونجا وكذلك المستخلص من تقنيات جزئياً، حتى الخصائص الفيزيائية للخثرة الناتجة، حيث أعطت القيم المساوكون والمظاهرية، الدراسات أوضحت الدراسة أن المستخلص الناتج من خلطة مستخلص

منحة الماشية الكبيرة مع بئر المورونجا أثناء الاختلاص والمستخلص الناتج من تقنيتها جزئياً مناسب جدًا لاستخدامه كيديل منحة العجل الصغيرة في صناعة الجبن.

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