U. of K. J. Vet. Med. Anim. Prod., Vol.6, Issue 1 (2015) p 27-37

ISSN: 243456

UNIVERSITY OF KHARTOUM
Journal of Veterinary Medicine and Animal Production

Chemical Composition of *Mudaffara* Cheese Manufactured by *Solanum dubium* Coat Extract and Chymosin

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Abstract

This study was conducted to determine the chemical composition of *Mudaffara* cheese manufactured by *Solanum dubium* coat extract and chymosin as coagulant enzymes. *Mudaffara* cheese was manufactured from warmed (35ºC) raw cow milk to which a starter culture (2% w/w of 1:1 combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was added. In the first treatment, chymosin extract (20 ml /10 L milk) was added, while in the second treatment *Solanum dubium* coat extract (20 ml / 10 L milk) was added. After coagulation, whey was drained and curd was cut and placed in an incubator at 40ºC for 3 hours, followed by cooking at 75ºC for 5 min. Black cumin (0.5% w/w) was added to the curd and braided into the final form. The curd was preserved in the salted whey (10% w/w) for 24 hours and then the cheese was preserved at 4ºC for 28 days. Chemical analysis was carried out at day 1, 7, 14, 21 and 28.

The results showed that all chemical components under study were not significantly affected by the type of coagulant except fat content which was significantly ($P<0.001$) highest in cheese made with chymosin (17.2%). During the storage period of cheese made by two coagulants, fat content fluctuated showing the highest content (18.46%) at day 21. The protein content gradually decreased till day 21 (19.29%) before increasing towards the end of the period (21.61%). The total solids content steadily increased to 53.61% at day 14 before decreasing at day 21, and then increased at the end (58.45%). The ash content increased to the highest level (15.59%) at day 14, followed by a decrease at day 21 (9.01%) and then increased at day 28 (17.18%). The titratable acidity increased reaching the maximum at day 21 (0.57%) then decreased at the end of the storage period to 0.50%. The fat content of cheese made with chymosin and *Solanum dubium* coat extract showed a peak at day 21, while protein, total solids and ash contents had the peak at day 28. The acidity fluctuated during the storage period being high at day 7 for cheese made with chymosin and at day 21 for cheese made with *Solanum dubium* coat extract.

Keywords: *Mudaffara* cheese, chemical, chymosin, *Solanum dubium*, storage period

المستخلص

أجريت هذه الدراسة لتحديد التركيب الكيميائي للجبن المضفر المصنع باستخدام مستخلص قشرة الجبين والكايموسين كمخثرات انزيمية. صنع الجبن المضفر من لبن بقري خام دافئ (35°م) واضيف البادئ (2٪ وزن/وزن من بكتيريا الاستربتوكوكس ثيرموفيلس و اللاكتوباسيلس بولغاريكس بنسبة 1:1). أضيف في المعاملة الأولى الكايموسين (20 مل/10 لتر لبن)، بينما أضيف في المعاملة الثانية مستخلص قشرة الجبين (20 مل/10 لتر لبن). بعد التخثر تم تصفية الشرش وقطعت الخثرة ووضعت في الحضان عند درجة حرارة 40°م لمدة 3 ساعات، ثم طبخت الخثرة عند درجة حرارة 75°م لمدة 5 دقائق. أضيف الكمون الأسود الى الخثرة (0.5٪ وزن/وزن) و ضفرت في شكلها النهائي. حفظت الخثرة في الشرش المملح (10٪ وزن/وزن) لمدة 24 ساعة، ومن ثم حفظ الجبن في درجة حرارة 4°م لمدة 28 يوما. أجري التحليل الكيميائي عند اليوم الاول والسابع والرابع عشر والحادي والعشرين والثامن والعشرين . أظهرت النتائج أن جميع المكونات الكيميائيه قيد الدراسة لم تتأثر معنويا بنوع المخثر ماعدا محتوى الدهن الذي كان أعلى معنويا ($P<0.0001$) في الجبن المصنع من الكايمو سين (17.2٪). أثناء فترة تخزين الجبن المصنع بالمخثرين، كان محتوى الدهن متذبذبا مع أعلى محتوى (18.46٪) في اليوم الحادي والعشرين. انخفض محتوى البروتين تدريجيا حتى اليوم الحادي والعشرين قبل الزيادة بنهاية الفترة (21.61٪). ازداد محتوى الجوامد الكليه بشكل ثابت الى 53.61٪ في اليوم الرابع عشرقبل ان ينخفض في اليوم الحادي والعشرين، ومن ثم ازداد عند نهاية فترة التخزين (8.45٪). ازداد محتوى الرماد الى الحد الأعلى (15.59٪) في اليوم الرابع عشر ثم تلاه نقصان في اليوم الحادي والعشرين (9.01٪)، ومن ثم ازداد في اليوم الثامن والعشرين (17.18٪). ازدادت الحموضة العيارية حتى وصلت الى الحد الأقصى في اليوم الحادي والعشرين (0.57٪)، ثم نقصت في نهاية فترة التخزين الى 0.50٪. محتوى دهن الجبن المصنع بالكايموسين ومستخلص قشرة الجبين بلغ القمة عند اليوم الحادي والعشرين، بينما بلغ محتوى البروتين والجوامد الكليه والرماد القمة عند اليوم الثامن والعشرين. تذبذبت الحموضة خلال فترة التخزين، مع الزيادة عند اليوم السابع للجبن المصنع بالكايموسين وعند اليوم الحادي والعشرين للجبن المصنع باستخدام مستخلص قشرة الجبين.

Introduction

Milk is an extremely nutritious food, being an aqueous colloidal suspension of proteins, fat, sugar, numerous vitamins and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Sangoyomi *et al*., 2010). Although milk and its various derivatives such as butter, yoghurt and cheese are vital human foods, they provide an excellent medium for the growth of many kinds of microorganisms (Adesokan *et al.*, 2008). Cheese is an important dairy product and integral part of a healthful diet due to its substantial contribution to human health. It is primarily a concentrated form of milk with the benefit of a prolonged shelf life and is a rich source of essential nutrients such as proteins, bioactive peptides, amino acids, fat, fatty acids, vitamins and minerals (Walther *et al*., 2008). Traditional cheeses represent a heritage and are the result of accumulated empirical knowledge passed on from generation to generation (Chanidis and Chroniadou, 2008). In Sudan, there are two main types of cheeses namely white cheese (*Gibna Bayda*), braided semi hard cheese (*Mudaffara*) and cheeses introduced recently to the market Mozzarella and Roumi (Nour El Daim and El Zubeir, 2007). These cheeses vary in their chemical composition and organoleptic characteristics due to composition of milk, production methods, microbial flora, type of package, microbial activity during ripening and ripening conditions (**Mustafa** *et al.*, 2013). *Mudaffara* cheese is a semi-hard cheese originated from the Middle East and now is widely produced in Sudan (Nour El Daim and El Zubeir, 2007).

In Sudan, *Mudaffara* cheese is usually made from raw cow milk but a mixture of cow and sheep or goat milk are also used (Ahmed, 1995). Cheese is usually made by enzymatic coagulation using the enzyme chymosin which is an aspartic protease produced in the abomasum of suckling calves (Kumar *et al.*, 2010). The increase in cheese production coupled with a

diminishing supply of natural animal rennet is responsible for increases in the demand for alternative milk coagulating sources like microbial coagulants and coagulants extracted from plants (Chazarra *et al.*, 2007). Both chymosin and plant coagulants cleave the Phel05-Metl06 peptide bond of κ-casein, but plant coagulants are more proteolytic and have broader specificity on αsl- and β-caseins than chymosin (Esteves *et al.*, 2003). Plant coagulants are available for milk coagulation and their excessive proteolytic nature reduces cheese yield and increases the bitter taste (Lo Piero *et al.*, 2002). Some plants of the family Solanaceae such as *Solanum elaeagnifolium*, *Solanum torvum*, *Solanum innacum* and *Solanum dubium* have been tried for the extraction of milk clotting enzymes. Some research showed positive results using *Solanum torvum* and *Solanum dubium* for the manufacture of white cheese (Ahmed *et al.*, 2009). *Solanum dubium* Fresen is an indigenous plant wildly grown in central, northern and western Sudan that flourishes during the rainy season, it is a woody herb, unripe fruits are green color, while the ripe ones are yellow, fruits usually dry on the stem. The seed is dark brown in color, and due to its bitter taste animals do not eat it. Dairy farmers in some parts of the Sudan use the berries of *Solanum dubium* to make white soft cheese known as *Gibna Bayda* from goat and sheep milk (Yousif *et al.*, 1996). The resultant cheese has a slight bitter taste and a fragile crumbly texture, and the bitterness being caused by the presence of some alkaloids or non specific proteolytic activity of enzymes that is obtained from the berries of *Solanum dubium* (Ahmed *et al.*, 2009). No attempt has been made in Sudan to investigate the production of *Mudaffara* cheese from plant enzyme. This study was carried out to use *Solanum dubium* coat extract for the manufacture of *Mudaffara* cheese and to compare the resultant cheese with that manufactured with chymosin physicochemically

Materials and Methods

Collection and preparation of milk samples

This study was carried out in the Department of Dairy Production, Faculty of Animal Production, University of Khartoum during the period January to March, 2014.

Materials

The starter culture, black cumin and salt were obtained from the local market, while commercial rennet powder was obtained from Chris Hansen's laboratories, Denmark. Fresh cow's milk (20 L) was obtained from the University of Khartoum dairy farm, transported to the laboratory at 4ºC and analyzed for fat, protein, total solids, ash and titratable acidity.

Solanum dubium plant

The plants were collected from Shambat area, Khartoum North. The coats and seeds were separated and carefully cleaned, washed several times with distilled water and the coats were then coarsely powdered using electric grinder. The coats were analyzed to determine dry matter, ether extract, crude protein, crude fiber and ash.

Preparation *Solanum dubium* coat extract

Twenty grams of the crushed coats were shaken with 100 ml distilled water and then the mixture was blended by agitator for 15 min until the mixture became homogeneous. Coat extract was placed in the refrigerator at 4ºC for 24 hr, filtered and 20 ml of the extract was used for coagulation.

Manufacture of *Mudaffara* cheese

Warm milk (35ºC) was divided evenly into two equal batches (treatments), the starter culture (2%) was added to both treatments, and chymosin (20 ml/10 L milk) was added to the first treatment, while *Solanum*

dubium coat extract (20 ml/10 L milk) was added to the second treatment. In the first treatment milk was thoroughly stirred and left to develop a curd, the curd was then cut by a sterile knife for whey drainage and placed in an incubator (40ºC) for 3 hr until the curd was ready for cooking in water (75ºC) for 5 min to encourage enough elasticity, and the same procedure was followed in the second treatment. The curd in both treatments was formed into balls and stretched on the clean table, the black cumin (0.5% w⁄w) was mixed with the curd and braided into the final form, and the braided curd was preserved in salted whey (10% w⁄w) for 24 hr, after which cheese was preserved in the whey at 4ºC for 28 days. Chemical analysis was carried out at 1, 7, 14, 21 and 28 days.

Chemical analysis of milk and cheese

The fat content (Gerber method), the protein content (Kjeldahl method), total solids content, ash content and the titratable acidity were determined according to the AOAC (2000).

Statistical analysis

Data were analyzed by Statistical Analysis Systems (SAS, ver.9). General linear model (GLM) was used to determine the effect of treatment and storage period on the chemical composition of *Mudaffara* cheese. Mean separation was carried out by Duncan multiple range test at $P \leq 0.05$.

Results and discussion

The chemical composition of raw milk used in cheese manufacture was as follows: fat 3.5%, protein 3.6%, total solids 12.04%, ash 0.7%, titratable acidity 0.19%. The chemical composition of *Solanum dubium* coat was as follows: crude fiber 58.9%, crude protein 11%, dry matter 96.09%, ash 8.1%, nitrogen free extract 11.79%, fat 6.34%. The chemical composition of black cumin was as follows: crude fiber 26.32%, crude protein dry matter 94.45%, ash 2.70%, fat 19.12%, nitrogen free extract 14.97%. The net weight of *Mudaffara* cheese was as follows: cheese produced by *Solanum dubium* 11%; cheese produced by chymosin 12.5%.

Effect of type of coagulant on the chemical composition of *Mudaffara* cheese

The effect of type of coagulant on the chemical composition of *Mudaffara* cheese was shown in Table (1). Type of coagulant had no significant effect on all chemical components under study except fat which was significantly (P<0.001) higher in cheese made with chymosin (17.2±2.13%).

Table 1: Effect of type of coagulant on the chemical composition of *Mudaffara* cheese (mean±SD)

Chemical Composition	Type of coagulant		LS
	Chymosin	*Solaum dubium*	
Fat%	17.2±2.13 [a]	15.98±1.46 [b]	***
Protein%	21.49±2.53 [a]	20.47±2.56 [a]	NS
Total solids%	51.89±12.25 [a]	49.37±9.96 [a]	NS
Ash%	10.96±9.32 [a]	11.92±9.2 [a]	NS
Acidity%	0.48±0.06 [a]	0.49±0.16 [a]	NS

Means in the same row bearing similar superscripts are not significantly different (P>0.05).
***=P<0.0001
NS=Not significant
LS=Level of significance
SD = Standard deviation

Protein and total solids contents were slightly higher but statistically insignificant in cheese made with chymosin (21.49±2.53% and 51.89±12.25% respectively), while ash content and acidity were slightly higher but statistically insignificant in cheese made with *Solanum dubium* coat extract (11.92±92% and 0.49±0.16% respectively). This may be due to the fact that enzyme extract from *Solanum dubium* was too proteolytic compared to

chymosin. The results of fat content are incompatible with Kheir *et al.* (2011) who reported a higher fat content in cheese made by *Solanum dubium* fruit extract compared to that made by chymosin. Nunez *et al*. (1991) and Abu-Zeid (1994) reported that the fat content was higher in cheese from vegetable rennet compared to that from animal rennet. Hashim *et al.* (2011) found that the type of coagulant (animal or plant) had no significant effect on the fat content. The results of protein content are in line with Pezeshki *et al.* (2011) who reported values of 11.27% and 12.25% in cheese made by *Withania* coagulant and animal coagulant respectively, Kheir *et al*. (2011) who reported that there was a small difference in the protein content of cheese made with two coagulants (*Solanum dubium* fruit extract and chymosin). However, the results are in disagreement with those of Nunez *et al*. (1991) who reported protein content slightly higher (14%) in cheese made with vegetable rennet compared to that made with animal rennet (13.3%). The results of total solids content are in disagreement with Kheir *et al*. (2011) who reported that the total solids content was higher in cheese made with *Solanum dubium* fruit extract compared to cheese made with chymosin. The ash content in cheese prepared by *Solanum dubium* extract was higher (11.92±9.2%) but statistically insignificant than the other cheese (10.96±9.32. The findings of ash content agreed with those of Kheir *et al.* (2011) who reported slightly higher ash content in cheese made by *Solanum dubium* compared with that made with chymosin. A slightly but insignificantly higher acidity (0.49±0.16%) was noticed in cheese made by *Solanum dubium* coat extract compared to that made by chymosin (0.48±0.06%). Similar results were reported by Kheir *et al.* (2011) who reported a slight increase in titratable acidity of white soft cheese made by *Solanum dubium* fruit extract (0.96%) compared to that made by chymosin (0.95%).

Effect of the storage period on the chemical composition of M*udaffara* cheese made with *Solanum dubium* coat extract and chymosin

Effect of storage period on the chemical composition of *Mudaffara* cheese is shown in Table 2. The fat content fluctuated throughout the storage period decreasing at day 7 (15.08±1.88%), followed by an increase at day 21 (18.46±1.2%), before decreasing to 16.16±1.90% at the end. The increase in fat content till day 21 might be due to high moisture loss during the storage, but the decrease in fat content at end of storage period may be attributed to breakdown of fat by microorganisms and loss from cheese. These results are in accordance with those of El Owni and Hamid (2008), Abdalla *et al.* (2011), Hamid and Abdelrahman (2012) and Hamid (2014) who reported an increase in fat content during the storage period in Sudanese white soft cheese. The results are not in line with Altahir *et al.* (2014) who reported a decrease in fat content of the *Mudaffara* cheese during the storage period. The protein content significantly ($P<0.01$) decreased to the minimum at day 21 (19.29±1.49%), then increased towards the end of storage period (21.61±3.57%). The reduction in protein content till day 21 was possibly due to the activity of proteolytic microorganisms leading to protein degradation. Abdalla and Mohamed (2009) noted the protein degradation leading to formation of water compounds. These results agreed with the findings of Abd El-Wahab (2008), Bakheit (2010) and Altahir *et al.* (2014) who reported a decrease in protein content of *Mudaffara* cheese during the storage period. Osman (2005), Mustafa (2006),

Table 2: Effect of the storage period on the chemical composition of *Mudaffara* cheese

Chemical composition	Storage period (days)					LS
	1	7	14	21	28	
Fat	16.67±1.66^{b}	15.08±1.88^{c}	16.58±1.31^{b}	18.46±1.2^{a}	16.16±1.90^{b}	***
Protein	22.30±1.47^{a}	22.22±1.65^{a}	19.49±2.47^{b}	19.29±1.49^{b}	21.61±3.57^{a}	**
Total solids	45.102±3.73^{b}	48.20±8.83^{b}	53.61±12.72^{ab}	47.8±6.38^{b}	58.45±15.70^{a}	*
Ash	5.53±0.51^{c}	9.88 ±5.71 ^{abc}	15.59±11.15^{ab}	9.01±5.06^{ab}	17.18±12.98^{a}	*
Acidity	0.42±0.11^{b}	0.47±0.11^{b}	0.49±0.07^{ab}	0.57±0.16^{a}	0.50±0.09^{ab}	*

Means in the same row bearing similar superscripts are not significantly different (P>0.05).
*=P<0.05
**= P<0.01
***= P<0.001
LS=Level of significance
SD = Standard deviation

Nour El Daim and El Zubeir (2007) and Abdalla and Mohamed (2009) found that the protein content of white cheese decreased during storage at cold temperature. The results are in disagreement with the findings of Tarakci and Kucukoner (2006), El Owni and Hamid (2008) and Hamid (2014) who reported an increase in protein content of white soft cheese during ripening. The total solids content was significantly (P<0.05) affected by the storage period. It increased gradually at day 14 (53.61±12.72%), decreased at day 21 (47.8±6.38%), and then increased at the end of storage period (58.45±15.70%). The increase in total solids content possibly could be explained by the expulsion of moisture from cheese curd. These results are in accord with El Owni and Hamid (2008) and Abdel Razig and Babiker (2009) who reported increasing total solids content with the advancement of the storage period, and in disagreement with Nour El Daim and El Zubeir (2007) and El Owni and Hamid (2009) who reported decreasing total solids content with the advancement of the storage period. Talib *et al.* (2009) indicated that the period might have resulted in contraction of cheese curd as a result of acidity development throughout the pickling period, which helped to expel the whey from cheese curd, and the effect of osmotic pressure on the brine solution. Abd El-Wahab (2008) reported that the total solids content of *Mudaffara* cheese stored at cold temperature decreased from 60.06% in the first day to 52.2% after 2 months of the storage. The ash content gradually increased to 15.59±11.15% at day 14, then decreased to the minimum at day 21 (9.01±5.06%), followed by an increase at the end of storage period (17.18±12.98%). The increase at the end of storage period might be attributed to decrease in moisture content. These results are in accordance with those of Abdel Razig and Babiker (2009) and El Owni and Hamid (2009) who reported an increase in ash content during the storage period, and in disagreement with those of Osman (2005) and Nour El Daim and El Zubeir (2007) who reported the decrease in ash content of cheese stored at cold temperature. The titratable acidity is significantly (P<0.05) affected by the storage period, increasing gradually until the day 21 (0.57±0.16%), then decreased at the end of the storage period (0.50±0.09%). The increase in titratable acidity may be due to growth of lactic acid bacteria leading to increased level of lactic acid. These results are in line with those of Abd El Razig *et al.* (2002) and Abd El-Wahab (2008) who reported that the titratable acidity

of *mudaffara* cheese ripened in 10% salt whey increased gradually from 0.43% in the first day to 2.10% after 2 months of storage. Osman (2005), Abdalla and Mohamed (2009), El Owni and Hamid (2009), Hashim *et al.* (2011) and Hamid (2014) reported increase in acidity towards the end of storage period of white soft cheese. The decrease in titratable acidity at the end of storage period might be attributed to increased level of lactic acid which had an antagonistic effect on lactic acid bacteria or depletion of lactose sugar.

Effect of type of coagulant and the storage period on the chemical composition of *Mudaffara* cheese

The fat content fluctuated during the storage period in both cheeses (Fig. 1). The change in fat content may be attributed to the fact that salt (sodium chloride) was not distributed evenly in cheese causing loss of fat from cheese. The results are not in line with Talib *et al.* (2009) and Kheir *et al.* (2011)

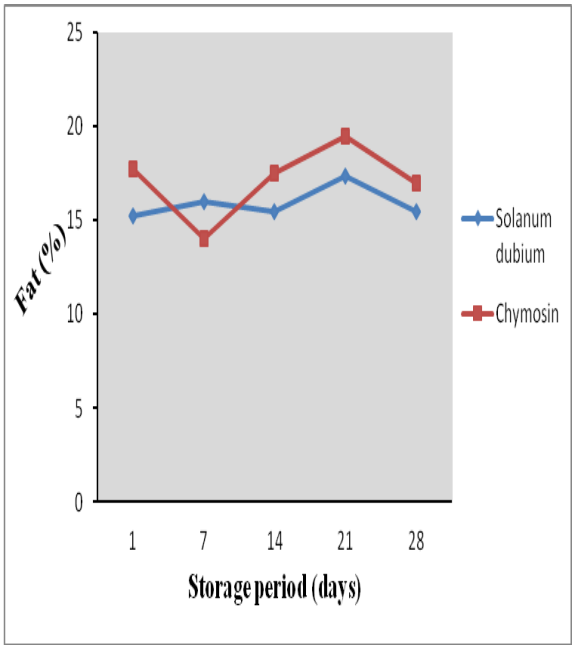

Figure 1: Effect of type of coagulant and the storage period on fat content of *Mudaffara* cheese

who reported no significant effect of storage period on the fat content of cheese made by *Solanum dubium* fruit extract and chymosin. The protein content increased at day 7 in cheese made by chymosin, then decreased at day 21 before increasing at day 28, while in cheese made by *Solanum dubium* the protein content decreased at day 14 followed by an increase at the end of storage period (Fig. 2).

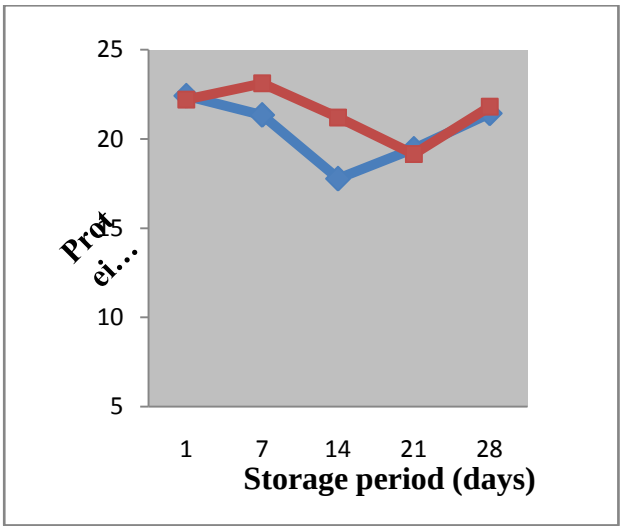

Figure 2: Effect of type of coagulant and the storage period on protein content of *Mudaffara* cheese

The reason is that proteolysis by microorganism's caused leakage of protein content during the storage. The results are not in line with those of Talib *et al.* (2009) and Kheir *et al.* (2011) who reported a decrease in protein content of soft white cheese manufactured by *Solanum dubium* seed and fruit extracts during pickling. The total solids content followed the same pattern during the storage period of cheese made by *Solanum dubium* coat extract and chymosin increasing to the maximum at day 14, followed by a decrease at day 21 before increasing again at day 28 (Fig. 3).

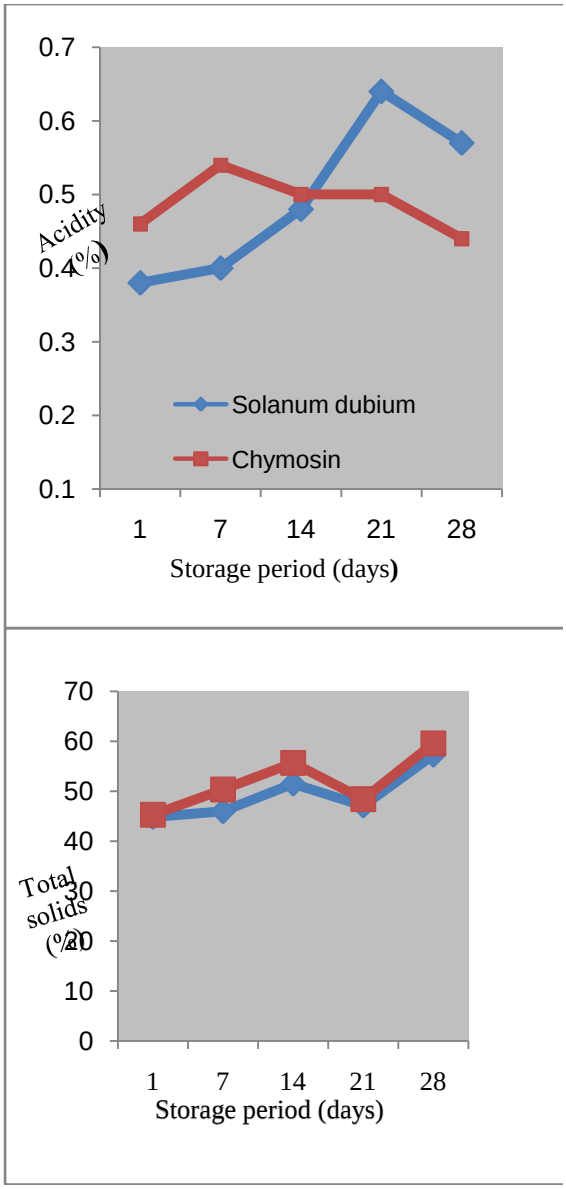

Figure 3: Effect of type of coagulant and the storage period on total solidscontent of *Mudaffara* cheese

. These findings are in disagreement with Kheir *et al.* (2011) who reported no significant effect of storage period on total solids content of cheese made with *Solanum dubium* and chymosin. The ash content of cheese made by *Solanum dubium* coat extract and chymosin reached the maximum content at day 14, and then decreased at day 21 before increasing again to the maximum at the end of the storage period (Fig. 4). These results are in accordance with Kheir *et al.* (2011) who reported that the ash content gradually decreased to the lowest at day 75 in both cheeses (chymosin and *Solanum dubium* fruit extract) before increasing at end of the storage period. The titratable acidity of cheese made with chymosin increased to the maximum at day 7 then decreased gradually towards the end, while for cheese made with *Solanum dubium* coat extract it increased to the maximum at day 21 before decreasing at the end of storage period (Fig. 5).

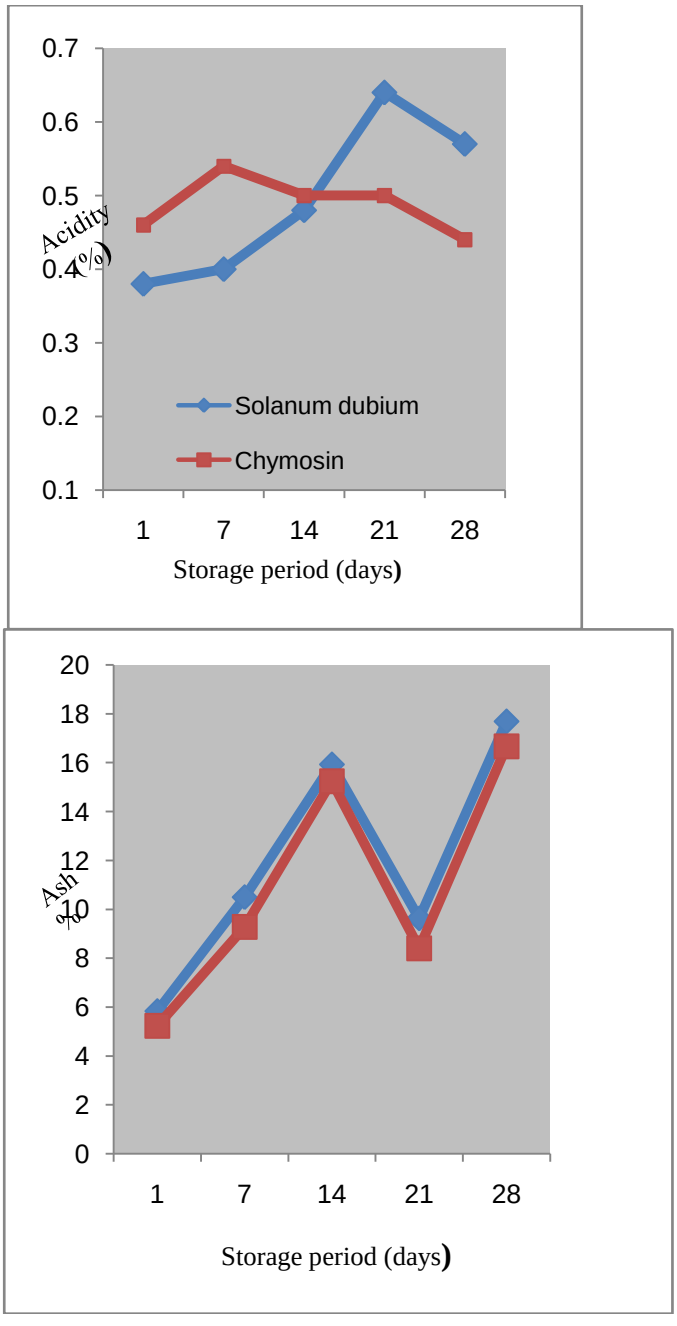

Figure 4: Effect of type of coagulant and the storage period on ash content of *Mudaffara* cheese

These results are in line with those of Talib *et al.* (2009), Hashim *et al.* (2011) and Kheir *et al.* (2011)

who reported the development of acidity by plant proteases in cheese during the storage period

Conclusion

Type of coagulant only significantly affected the fat content of *Mudaffara* cheese, while the storage period significantly affected all chemical components under study.

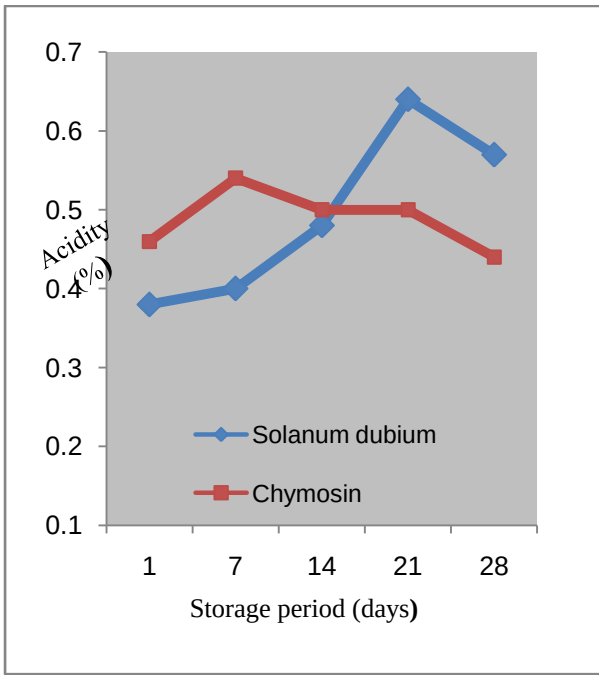

Figure 5: Effect of type of coagulant and the storage period on acidity of *Mudaffara* cheese

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