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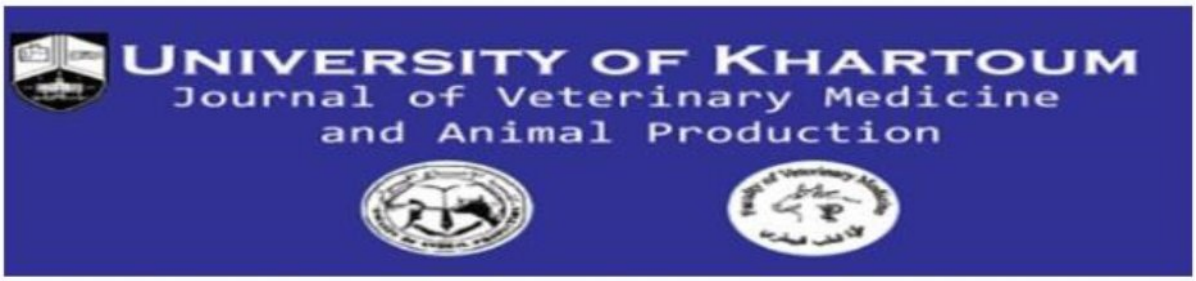

Chemical composition of Mozzarella cheese manufactured by *Solanum dubium* coat extract and chymosin

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Abstract

This study was conducted to evaluate the effect of coagulant type (*Solanum dubium*, coat extract and chymosin) on the chemical composition of Mozzarella cheese. The cheese was manufactured, using *Solanum dubium* extract and chymosin as coagulants, stored for 28 days and the chemical composition (fat, total protein, ash, soluble protein and acidity) was determined at 1, 7, 21 and 28 days- intervals. The results showed that the coagulant type significantly affected fat, total protein, and soluble protein contents, with the highest values being in cheese made with *Solanum dubium* coat extract (20.38, 30.72 and 0.52%, respectively). The storage period significantly affected fat, total protein, acidity and soluble protein contents. The fat and total protein contents slightly decreased at week 2 (17.75% and 25.31%, respectively), before decreasing towards the end of the storage period, while the acidity increased to the maximum at week 2 (1.01%), then gradually decreased towards the end. The soluble protein fluctuated during the storage period, increasing in week 2 (0.33%), followed by a decrease in week 3 (0.30%), then increased at the end. During the storage period, Mozzarella cheese made with *Solanum dubium* and chymosin separately, the fat content slightly increased towards the end (21.00% and 18.00%, respectively), while the total protein content slightly decreased at week 3 (27.55% and 26.77%, respectively), then increased. Total solids content of cheese made with chymosin slightly increased at week 3 (49.49%) then decreased, while that made with *Solanum dubium* coat extract steadily decreased towards the end. The ash content of cheese made with chymosin decreased to minimum at week 3 (1.90%), followed by an increase towards the end, while that of cheese made with *Solanum dubium* slightly increased at week 2 (2.22%), then decreased at week 3(2.05%) before slightly increasing again. The titrable acidity of cheese made with both coagulants followed the same trend, increasing at week 2 (0.74% and 1.28% for cheese made with *Solanum dubium and chymosin*, respectively) followed by a decrease at the end of thevstorage period, The soluble protein of cheese made with both coagulants slightly increased at week 2 (0.35% and 31% for cheese made with *Solanum dubium* and chymosin, respectively), then the solublity of cheese made with *Solanum dubium* increased towards the end, while that made with chymosin slightly decreased.

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

المستخلص

اجريت هذه الدراسة لتقييم تاثير نوع المجبن (مستخلص قشرة الجبين والكايموسين) علي التركيب الكيميائي لجبنة الموزريلا. صنع جبن الموزريلا باستخدام مستخلص قشرة الجبين و الكايموسين كمجبنات، وخزن الجبن لمدة ? اسابيع واجري التحليل الكيميائي (الدهن و البروتين الكلي والجوامد الكلية والرماد والبروتين القابل للذوبان والحموضة) في الاسابيع ? و? و ? و?. اظهرت النتائج ان لنوع المجبن اثر معنويا علي محتوي الدهن والبروتين الكلي والبروتين القابل للذوبان مع اعلي قيم في الجبن المصنع باستخدام مستخلص قشرة الجبين (??M?% و??M?% و??M% علي التوالي). اثرت فترة التخزين معنويا على محتوي الدهن و البروتين الكلي والحموضة والبروتين القابل للذوبان. تناقص محتوي الدهن والبروتين الكلي قليلا في الاسبوع الثاني (????% و ????% علي التوالي) قبل الانخفاض عند نهاية فترة التخزين، بينما زادت نسبة الحموضة لاعلي مستوي لها في الاسبوع الثاني (?%?M) ثم انخفضت تدريجياً عند النهاية. تذبذب محتوي البروتين القابل للذوبان خلال فترة التخزين مع زيادة في الاسبوع الثاني (??M%) متبوعاً بانخفاض في الاسبوع الثالث (?M?M%) ثم انخفض عند نهاية فترة التخزين. خلال فترة التخزين الجبن المصنع من مستخلص قشرة الجبين و الكايموسين كلا علي حدة، زاد محتوي الدهن قليلا عند نهاية فترة التخزين (??MM%) علي التوالي)، بينما انخفض محتوي البروتين الكلي قليلا عند الاسبوع الثالث (????% و????% علي التوالي) ثم زاد بعد ذلك. زاد محتوي الجوامد الكلية للجبن المصنع من الكايموسين قليلا في الاسبوع الثالث (????%) ومن ثم انخفض بعد ذلك، بينما انخفض بثبات بنهاية الفترة للجبن المصنع من مستخلص قشرة الجبين. انخفض محتوي الرماد للجبن المصنع بالكايموسين للحد الادني في الاسبوع الثالث (?%??M) و من ثم زاد عند النهايه، بينما زاد في الجبن المصنع من مستخلص قشرة الجبين في الاسبوع الثاني (????%) ثم انخفض في الاسبوع الثالث (?%?M) قبل الزيادة قليلا مرة ثانية. اتبعت الحموضة نفس اتجاه الرماد بزيادة في الاسبوع الثاني (??M% و????% للجبن المصنع من الجبين والكايموسين علي التوالي) متبوعا بانخفاض عند النهاية. زاد محتوي البروتين القابل للذوبان قليلا في الاسبوع الثاني (??M% و??M % للجبن المصنع من الجبين والكايموسين علي التوالي)، ثم زاد في الجبن المصنع من الجبين بنهاية الفترة، بينما انخفض قليلا في الجبن المصنع بالكايموسين.

Introduction

Cheese making in Sudan is the major preservation method for surplus milk in rural areas especially during the rainy season when plenty of milk is available (El Owni, and Hamid, 2008). White cheese is the major type of cheese beside other cheese varieties produced but in a limited scale, these include *Mudaffara* cheese (Abdel-Razig, 2000), Mozzarella cheese (El Owni and Osman, 2009) and Roumi cheese (FAO, 1990). Such cheeses vary in composition, texture, color, taste and flavor due to the varied composition of milk, production methods, microbial flora and type of microbial activity during ripening conditions (Rotaru *et al*., 2008).

Mozzarella belongs to the "Pasta filata" family of cheeses which involves skillfully stretching the curd

The changes in cheese manufacturing protocols have resulted in a reduction of the manufacturing time and the necessity for consistent and reliable starter activity (Johnson and Lucey, 2006). A required step in cheese manufacture is separating the milk into solid curd (casein and liquid whey) at isoelectric point of casein (pH 4.6), and this is done by acidifying the milk and adding chymosin. The acidification can be accomplished directly by the addition of acid such as vinegar, but usually starter bacteria are employed instead while converting milk sugar into lactic acid. The same bacteria and enzymes play a role in the eventual flavor of aged cheeses (Fox *et al*., 2004).

in hot water to get a smooth textured cheese; the cheese is soft, and may be consumed shortly after manufacture (Sulieman *et al.*, 2013). There are two forms, regular and fresh Mozzarella cheese. Regular Mozzarella is available in low fat having semi soft, elastic texture and is drier than fresh Mozzarella, while the fresh Mozzarella is made from whole milk having softer texture and sweet, delicate flavor and is typically packed in whey. The chemical components in Mozzarella cheese influence Mozzarella meltability and include about 3.17% moisture, 3.8 pH, 5.13% NaCl and 3.8% calcium (Wang *et al.*, 1998). According to Spano *et al.* (2003), Mozzarella cheese is made using traditional procedure by warming whole cow milk at 35−36ºC followed by addition of rennet extract, and after 40 min the curd is cut to the size of hazelnuts, drained and placed on draining table for 6-7 hr. The curd is then cooked in hot water (75-80ºC), hardened in cold water, placed in 23% salt solution for 12 hr and stored at 4ºC for 7 days.

Chymosin (rennin, EC 3.4.23.4) is an aspartic protease produced in the abomasum of suckling calves (Kumar *et al*., 2010). The principal role of chymosin in cheese making is to coagulate milk by specifically hydrolyzing the Phe105−Met106 bond of the micelle stabilizing protein κ−casein which is many times more susceptible to chymosin than any other bond in milk proteins leading to the coagulation of milk (Fox *et al*., 2000).

Publications on new proteases from plant origin for milk coagulation indicated that they are subject with growing interest for dairy technology, and they have been used as milk coagulants in cheese making for centuries either as crude extracts or in purified form (Egito *et al.*, 2007). However, the excessive proteolytic nature of most plant coagulants has limited their use in cheese manufacturing due to lower yields of cheese, bitter flavors and texture defects (Shah *et al*., 2014).

Plant proteases employed for cheese production in various areas of the world include papain, bromelin, ficin, oryzasin, cucumisin and Sodom apple and *Jacartia corumbensis* (Duarte *et al.*, 2009). Solanaceae is a family of flowering plants as well as many toxic plants. The family is also informally known as the nightshade or potato family. The solanaceae is a large varied family of trees, shrubs and herbs including 90 genera and more than 2000 species. The species are distributed throughout the world, but they are more prevalent in tropics and subtropics (Shah *et al*., 2012). *Solanum dubium* Fresen is an indigenous plant wildly grown in Central, Northern and Western Sudan, locally known in Sudan as *Gubbain* because of its milk clotting ability. It is a woody herb with a solid erect stem, green in color and about 30 cm high. Its leaves are alternate, long petiole, simple, ovate, acuminate or obtuse at the apex and pale green in color, while their rootlets are brown and their roots about 5 mm thick and 15 cm long, and its flowers are hermaphrodite, the unripe fruits are green while the ripened ones are yellow (Yousif *et al*., 1996; Ahmed *et al*., 2009; Abdalla *et al*., 2010; Abdalla *et al*., 2011; Ahmed *et al.*, 2011).

Mozzarella cheese production in Sudan started in 1982 in Khartoum Dairy Products Company, but the small scale producers have the ability to produce large quantities of Mozzarella and used as main ingredient in pizza cooking and give the flavor and taste of pizza. Due to large consumption of Mozzarella cheese in Sudan in pizza industry and the scarcity of animal rennet, attempts have been to introduce alternatives for animal rennet of local origin such as *Solanum* dubium. This study was conducted to use *Solanum dubium* coat extract for the manufacture of Mozzarella cheese and to compare the resultant cheese from chemical point of view with that manufactured with chymosin.

Materials and Methods

Collection and preparation of milk samples

This study was carried out at the Department of Dairy Production during the period September-October 2013.

Preparation of *Solanum dubium* coat extract

The plant material used in this study was collected from ElObeid, North Kordfan State, Sudan. *Solanum dubium* fruits were collected during the period May-July 2013, when the fruits were yellow with black and completely dry seeds. The coats were separated from the other parts of the fruits and crushed finely, 20% of the grinded coats were added to distilled water (20 gm/100 ml) in the volumetric flask, left for 5 hr with entire shaking every 30 min, then left at refrigeration temperature for 24 hr followed by filtration by filter paper (Whatman No.43).

Manufacture of Mozzarella cheese

Mozzarella cheese was manufactured according to the method described by Kosikowski (1982). Whole cow's milk (20 L) was heat treated at 73°C for 15 sec, cooled to 40°C and divided evenly into two equal batches (treatments), the starter culture (2% w/w of 1:1 combination of *Streptococcus thermophills and Lactobacillus bulgaricus*) was added to both treatments until the acidity reached 0.35% lactic acid, and chymosin (10 ml/10 L milk) was added to the first treatment, while *Solanum dubium* coat extract (10 ml/10 L milk) was added to the second treatment. The milk was then thoroughly stirred and left to develop a curd, which was then cut by a stainless steel knife for whey drainage and placed in an incubator (40ºC) for 3 hr until the curd was ready for cooking in water (80ºC) for 5 min to encourage enough elasticity. The curd was formed into balls and kept at refrigerator temperature (4°C) for 28 days.

Chemical analysis was carried out at 1, 7, 14, 21 and 28 days intervals.

Chemical analysis

The fat content (Gerber method), protein content (Kjeldahl method), total solids content, ash content and titratable acidity were determined according to AOAC (2000). Soluble protein content of cheese was determined according to Ling (1963).

Statistical analysis

Statistical analysis was carried out by Statistical Analysis System (SAS, Ver. 9). General linear models (GLM) produce was used to determine the effect of type of coagulant and storage period on fat, protein, ash, total solids, acidity and soluble protein content of cheese.Means were separated by Duncan Multiple Range test at $P \leq 0.05$.

Results and discussion

Effect of coagulant type on the chemical composition of Mozzarella cheese:

The data in Table 1 presents the effect of coagulant type on the chemical composition of Mozzarella cheese. The fat content was significantly ($P<0.001$) affected by the type of coagulant which was higher in cheese made by *Solanum dubium* coat extract (20.38%) compared to that made by chymosin (16.5%). The values obtained in this study are higher than those reported by El-Koussy *et al*. (1995) and Shegdoni *et al.* (1979). The protein content of cheese made with *Solanum dubium* coat extract was significantly ($P<0.001$) higher (30.73%) than that made with chymosin (25.02%). The results in this study are lower than those reported by Osman (2000) and Fernandez and Kosikowski (1986). The total solids content of cheese was not significantly ($P>0.05$) affected by the coagulant type, although cheese made with chymosin was slightly higher (48.79%). Results are similar to the values found by Shegdoni *et al*. (1979), but higher than those stated by Coppola *et al*. (1995), El Koussy *et al.* (1995) and Fernandez and Kosikowski (1986) who reported 46% 47.82%, and (49.5%) respectively. Moreover, they are lower than the average value (56.03%) reported by Talib *et al*. (2010). The ash content was not

significantly (P>0.05) affected by the type of coagulant, although the ash content of cheese made with *Solanum dubium* extract was lower (1.99%) than that made with animal chymosin (2.26%). These results are not in accordance with those reported by Talib *et al*. (2010) who reported ash content of 2.79%. The titratable acidity was not significantly affected (P>0.05) by the coagulant type, the lowest titratable acidity being in cheese made with *Solanum dubium* extract (0.53%), while the highest (0.87%) was in cheese made with chymosin. This result is lower than the value of Shegdoni *et al*. (1979) and El-Koussy *et al.* (1995). Soluble protein content of cheese made with *Solanum dubium* extract was significantly (P<0.05) higher (0.52%) than that made with chymosin (0.24%). The results reported in this study are lower than those reported by Costable (2007)

Effect of storage period on the chemical composition of Mozzarella cheese

Table 2 shows the effect of storage period on the chemical composition of Mozzarella cheese. The fat content significantly (P<0.05) decreased in the second week (17.75%) then gradually increased to the maximum (19.50%) at the end of storage period. These results agreed with those reported by Srbinovska *et al.* (2001) and Suleiman *et al.* (2012). The protein content followed the pattern of fat content decreasing significantly (P<0.001) in the second week (25.31%), followed by a gradual increase at the end of storage period. These results agreed with those of El Owni and Osman (2009. Although the effect of storage period on total solids was not significant, the content slightly increased in the second week (48.76%) before gradually decreasing to the minimum (46.87%) in the fourth week. These findings are in agreement with those of Coppola *et al.* (1995) and El-koussy *et al.* (1995). However these results are not in line with those of Fernadez and Kosikowski (1986) and Srbinovska *et al. (*2001). The ash content showed an irregular pattern during the storage period, increasing in the second week (2.17%), followed by decrease in the third week (1.98%) then increased at the end of storage period. These findings are in agreement with those of El Owni and Osman (2009). The titratable acidity showed a maximum content in the second week (1.01%) than regularly decreased towards the end of storage period (0.44%). The findings are in accordance with those of El Owni and Osman (2009) and Sulieman *et al*. (2012). The soluble protein content significantly showed two peaks at the second week and at fourth week (0.68%). These results agreed with those of Yun *et al.* (1994) and Costable (2007).

Changes in chemical composition of Mozzarella cheese as affected by storage period and coagulant type

A slight decrease in fat content of cheese made with both coagulants was noticed in the second week of storage period (Fig. 1).

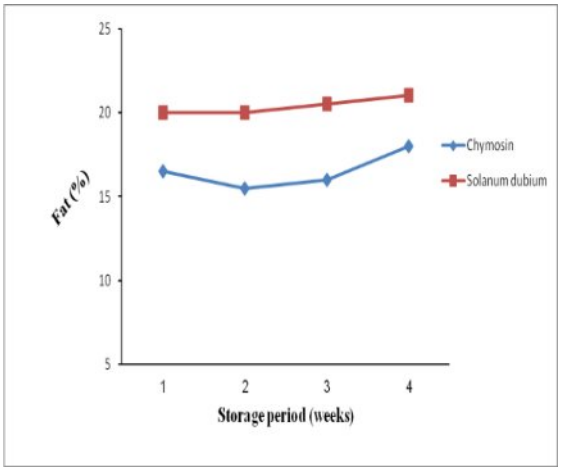

Figure 1: Effect of coagulant type and storage period as fat content of Mozzarella cheese

The decrease in fat content during storage from days 1 to 28 might be due to the lipolytic activity of microorganisms on fat resulting in leakage of some fat from curd into the pickling whey (Khalid, 1991; Abdalla *et al*., 1993; Abdalla and Mohamed, 2009). The protein content of cheese slightly decreased in the second (Fig. 2).

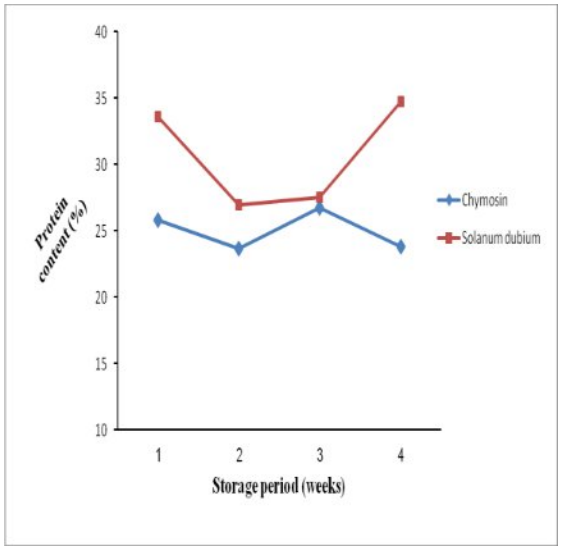

Figure 2: Effect of coagulant type and storage period on protein content of Mozzarella cheese

This may be due to decrease in moisture content. These results agreed with those of Kur (1992), Abdel Razig and Abdalla (1997) and Kim *et al.* (1992). The total solids content of cheese made with chymosin increased to a maximum in the third week before decreasing towards the end, while for cheese made with *Solanum dubium* extract the total solids content showed a gradual decrease to a minimum at the end storage period week in cheese made with both coagulants, then the protein content of cheese made with chymosin slightly increased in the third week before slightly decreasing toward the end. For cheese made with *Solanum dubium* extract the protein content was slightly increased in the third week, then sharply increased to a maximum at the end of storage period (Fig. 3).

The increase could be attributed to the decrease in moisture content (Jelen, 1992; Siber, 1998; El-Sheikh and Abdalla, 2001; Abdel-Razig *et al*., 2002). The ash content of cheese made with *Solanum dubium* extract showed a peak in the second week, while that of cheese made with chymosin decreased to a minimum value in the third week, and the ash content of both cheeses was slightly increased towards the end (Fig. 5).

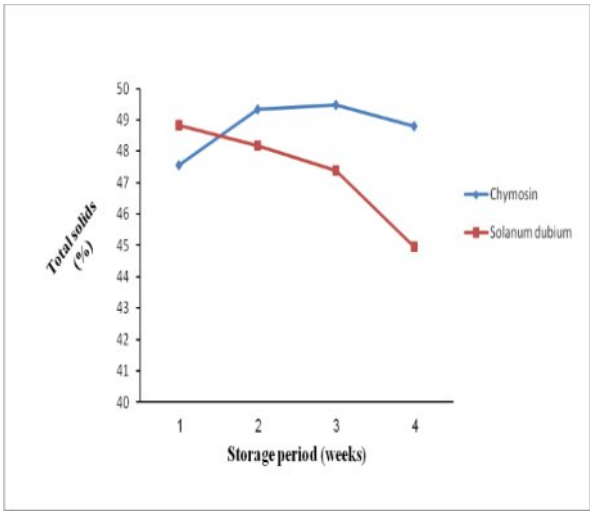

Figure 3: Effect of coagulant type and storage period on total solids content of Mozzarella cheese

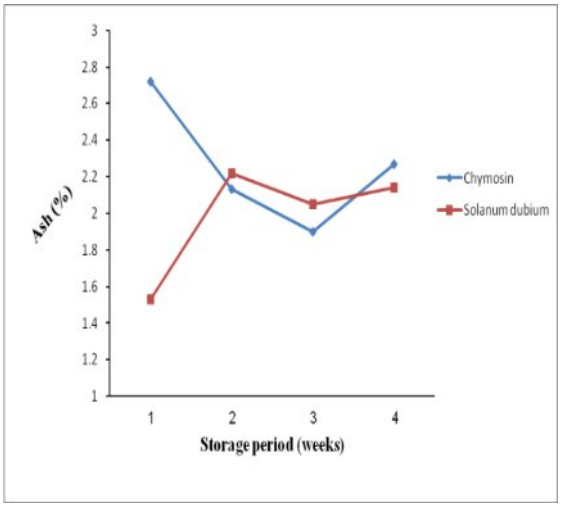

Figure 4: Effect of coagulant type and storage period on ash content of Mozzarella cheese

The development of titratable acidity during storage could be attributed to growth of lactic acid bacteria which increased the level of lactic acid in the cheese (Walstra *et al.*, 1999). The soluble protein content of cheese made with both coagulants followed the same pattern till the third week, then the soluble protein content of cheese made with *Solanum dubium* extract sharply increased, while that of cheese made with chymosin decreased towards the end of storage period Fig. 6). These results are not in accordance with those of Hyaloglo *et al.* (2002)

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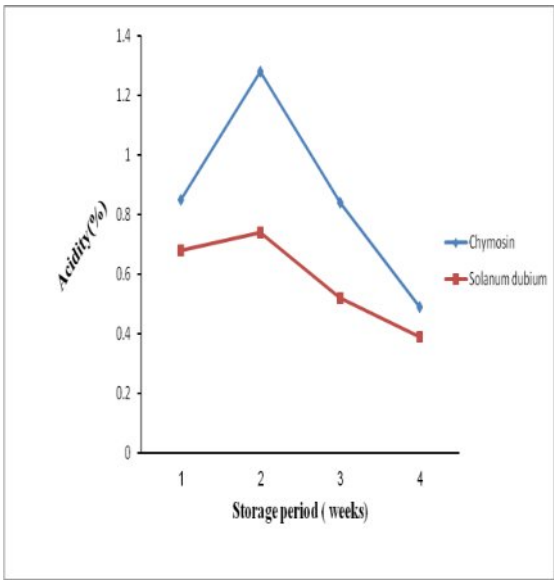

Figure 5: Effect of coagulant type and storage period on acidity content of Mozzarella cheese

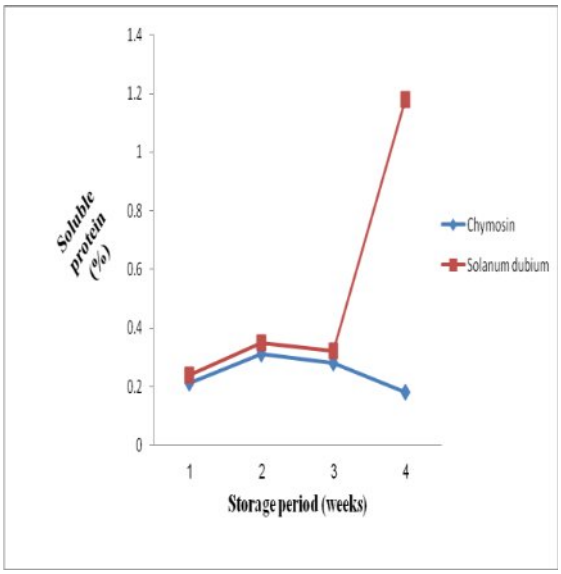

Figure 6: Effect of coagulant type and storage period on soluble protein content of Mozzarella cheese

Table 1: Effect of coagulant type on the chemical composition of Mozzarella cheese

Chemical composition (%)	Type of coagulant		SE	LS
	Chymosin	*Solanum dubium*		
Fat	16.5[b]	20.38[a]	0.326	***
Total protein	25.02[b]	30.72[a]	0337	***
Total solids	48.79[a]	47.34[a]	0.723	N.S
Ash	2.26[a]	1.99[a]	0.103	N.S
Titratable acidity	0.86[a]	0.53[a]	0.118	N.S
Soluble protein	0.24[b]	0.52[a]	0.089	*

Means in the same row bearing similar superscripts are not significantly different (P>0.05)
*** = P<0.001, * = P<0.001, NS = Not significant. SE = Standard error of means
LS = Level of significance

Table 2: Effect of storage period on chemical composition of Mozzarella cheese

Chemical composition (%)	Storage period (weeks)				SE	LS
	1	2	3	4		
Fat	18.25[ab]	17.75[b]	18.25[ab]	19.50[a]	0.45	*
Protein	29.71[a]	25.31[a]	27.16[b]	29.29[a]	0.48	***
Total solids	48.18[a]	48.76[a]	48.44[a]	46.87[a]	1.02	NS
Ash	2.12[a]	2.17[a]	1.98[a]	2.21[a]	0.15	NS
Acidity	0.77[ab]	1.01[a]	0.68[ab]	0.44[b]	0.17	*
Soluble protein	0.23[b]	0.33[ab]	0.30[b]	0.68[a]	0.13	*

Mean values bearing different superscripts within rows are significantly different (P< 0.05)
*** = P<0.001, * = P<0.05, NS = Not significant, SE = Standard error of means
LS = Level of significance

Conclusion

Solanum dubium coat extract showed promising results for the manufacture of Mozzarella cheese. The chemical composition of cheese manufactured by *Solanum dubium* did not show a difference from that manufactured with chymosin. However, more research is needed to evaluate the resultant cheese

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