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RIPENING INDICES OF UF DOMIATI CHEESE MADE USING CHEESE SLURRY FORTIFIED WITH PROTEOLYTIC AND LIPOLYTIC ENZYMES

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ABSTRACT:-

This study aimed to evaluate the effect of adding cheese slurries fortified with protease and lipase enzymes on the ripening process of cheese made from ultrafiltered (UF) cow's milk. Three cheese slurries were prepared from cow's milk curd: the first served as a control (slurry A), the second was fortified with protease at 0.02% and lipase at 0.002% (slurry B), and the third was fortified with protease at 0.04% and lipase at 0.005% (slurry C) The slurries were added to three types of milk with different total solids content (10%, 20%, and 30%), with 10% of each slurry added to each milk type. The samples were ripened at (6 ± 1 °C) for 60 days, and ripening indices were measured at regular intervals throughout the ripening period. During the ripening period, formol values, Shilovich values, and soluble tyrosine and tryptophan contents were determined.

The results showed a gradual and significant increase in these values, indicating improvements in the ripening process due to the use of enzyme-fortified slurries. Notably, slurry C, which contained the highest concentration of enzymes, had a more pronounced effect on enhancing the quality of the resulting cheese.

***Conclusively,** these findings suggest that the addition of enzyme-fortified cheese slurries significantly enhances the ripening process and sensory properties of cheese made from UF concentrated milk, potentially leading to improve the quality in the final cheese product.*

Keywords: Lipase , protease ,Domiati cheese , cheese slurry, Shilovich value, Formol value.

INTRODUCTION

Kristoffersen *et al* (1967) developed a rapid method for the production of cheese flavor by the incubation of cheese curd slurries containing about 40% moisture at 30-35°C for a period of 4–5 days.

Akhgar *et al* (2016) accelerated soft cheese ripening made from ultrafiltered milk when they added 25 g of the ripened cheese slurry to each one Kg of the milk retentate. **Hamdy *et al* (2016)** added 0-3% of ripened cheddar cheese slurry to the milk retentate. The resultant Cheddar cheese had higher score points for flavor intensity, texture, and overall quality.

Because the ultrafiltered cheese has slow protein breakdown, slow flavor development, and hard texture, the present study aimed to accelerate Domiati cheese ripening made from milk retentate by addition of ripened cheese slurry fortified with proteolytic and lipolytic enzymes.

Therefore, we used protease and lipase enzymes for increasing the proteolysis and lipolysis in cheese slurry. Domiati cheese enhancing the flavor and texture in the present study via the addition of protease and lipase to the cheese curd that called cheese slurry.

MATERIALS AND METHODS:-

Raw cow's milk from a private farm in Zagazig, Sharkia Governorate, Egypt, was used for making cheese slurry and control Domiati cheese. Milk retentate was supplied by the research milk unit of the Dairy Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Microbial rennet powder (CHY-MAX, 22580 IMCU/g, Chr. Hansen's Lab, Denmark).

Lipase and protease enzymes were obtained from Chemic Company in El-Maadi, Egypt.

The chemicals that used in the study were of analytical grade and were obtained from El-Nasr and Sigma Companies. The commercial fine salt was acquired from the local market.

1. *Preparation of cheese slurry :-*

The method described by **Fahmi and Sharara (1950)** was followed for cheese production. Initially, fresh cow's milk was heated to 72°C for 15 seconds and then cooled to 45°C. After that, 4% of table salt (NaCl) was added, along with rennet at a rate of 0.02 g/kg of milk. Once coagulation was complete, the curd was cut, and the whey was drained using cheese cloth.

Then, the resulting curd was divided into three parts, each part is 500 grams. Three cheese slurries (Slurry A, Slurry B, Slurry C) were prepared. Based on these slurries, 12 treatments were manufactured, which are detailed in Table 1.

Table (1): Slurry type ,enzyme supplement and milk type used for manufacture of Domiati cheese from ultra filtered milk

Treatments	Slurry type	Supplements	Milk used
T1	Non -Supplements		Fresh milk 10% TS
T2	slurry (A)	Supplements Slurry with no	Fresh milk 10% TS
T3	slurry (B)	Slurry with 0.02% protease + 0.002% lipase	Fresh milk 10% TS
T4	slurry (C)	Slurry with 0.04% protease + 0.005% lipase	Fresh milk 10% TS
T5	no Supplements		2x UF-20%TS
T6	slurry (A)	Supplements Slurry with no	2x UF-20%TS
T7	slurry (B)	Slurry with 0.02% protease + 0.002% lipase	2x UF-20%TS
T8	slurry (C)	Slurry with 0.04% protease + 0.005% lipase	2x UF-20%TS
T9	no Supplements		3x UF-30%TS
T10	slurry (A)	Supplements Slurry with no	3x UF-30%TS
T11	slurry (B)	Slurry with 0.02% protease + 0.002% lipase	3x UF-30%TS
T12	slurry (C)	Slurry with 0.04% protease + 0.005% lipase	3x UF-30%TS

Ripening Indices of Domiati Cheese

To assess the biochemical changes during ripening, the ripening indices were measured, including:

1. Soluble tyrosine and tryptophan.
2. Formol and Shilovich indices.

Determination of Soluble Tyrosine and Tryptophan Content

The content of tyrosine and tryptophan in the cheese was determined according to **Vakaleris and Price (1959)**. Ten grams of the cheese sample were mixed with 40 ml of 0.5 M sodium citrate and 80 ml of distilled water. The mixture was homogenized for 7 minutes using an auto-mixer. The homogenized cheese suspension was then quantitatively transferred to a 200 ml volumetric flask and filled up to volume with distilled water. A 100 ml portion of this suspension was treated with 10 ml of 1.41 N hydrochloric acid solution, filtered through Whatman No. 42 filter paper, and the

absorbance of the filtrate was measured at 270 nm and 290 nm using UV spectrophotometer.

The tyrosine and tryptophane contents was calculated using the following equations:

- Tyrosine content (mg/100g cheese) = (0.95 × E270 - 1.31 × E290) × 906

-Tryptophane content (mg/100g cheese) = (0.0307 × E290 – 0.02 × E270) × 1021

Where E270 and E290 represent the absorbance readings at 270 nm and 290 nm, respectively.

Determination of Formaol and Shilovich Indices

Five grams of cheese were added to 50 ml of warm distilled water (45-50°C) and ground into a paste. The mixture was quantitively decanted and filtered through a Buchner funnel with two layers of Whatman No. 40 filter paper. The filtrate was divided into two portions, and each portion was titrated with 0.1N sodium hydroxide, using different indicators (thymolphthalein for one and phenolphthalein for the another). After adding neutral formolin to the second portion, the titration continued until the endpoint was reached. The ripening indices were calculated as follows:

- Schilovich Ripening Index (S.R.I.) = (A - B) × 100.
- Formol Ripening Index (F.R.I.) = (C) × 100.

Where A and B are the sodium hydroxide volumes used in titration before and after formalin addition, respectively.

Statistical Analysis

All the data generated in the experiment were statistically analyzed as completely random design according to **Snedecor and Cochran, (1982)**, using the Linear Model Program of SPSS (2014) as The following model

$$Yijk = \mu + Ti + eij.$$

Where: Yijk = Observation for each dependent variable, μ = Overall mean, Ti = Treatments effect (i = 1, 2,… and 12), eij = Random error.

The differences among treatments means were compared using Duncan's Multiple-Range Test Procedure **Duncan, (1955).**

RESULTS AND DISCUSSION

Soluble tyrosine content of Domiati cheese (mg/100g):-

The results in Table (2) represent the changes in soluble tyrosine contents of UF-Domiati cheese manufactured by addition of cheese slurry fortified with proteolytic and lipolytic inzymes during 60 days of ripening under 6±1°C.

The results obtained showed that the tyrosine content of cheese increased gradually by increasing period of ripening up to 60 days.

Table (2): Changes in soluble tyrosine content in Domiati cheese made from concentrated milk by during ripening for 60 days as affected by added proteolytic and lipolytic enzymes at 6±1°C.

Milk Type	Treatments	Fresh	30 Days	60 Days
Fresh milk 10% TS	T1	12.35 ± 0.02^{e}	32.45 ± 0.18^{h}	51.02 ± 0.34^{k}
	T2	12.76 ± 0.02^{c}	42.53 ± 0.23^{e}	54.76 ± 0.25^{h}
	T3	12.84 ± 0.025^{b}	49.19 ± 0.52^{d}	58.41 ± 0.26^{f}
	T4	12.94 ± 0.025^{a}	51.34 ± 0.32^{c}	60.56 ± 0.25^{d}
2x UF-milk 20%TS	T5	12.16 ± 0.018^{g}	34.58 ± 0.17^{g}	52.04 ± 0.35^{j}
	T6	12.25 ± 0.02^{f}	48.88 ± 0.04^{d}	56.62 ± 0.25^{g}
	T7	12.46 ± 0.025^{d}	51.33 ± 0.83^{c}	59.43 ± 0.21^{e}
	T8	12.94 ± 0.021^{a}	55.44 ± 0.29^{b}	62.89 ± 0.52^{b}
3x UF-milk 30%TS	T9	12.32 ± 0.008^{e}	36.45 ± 0.17^{f}	53.55 ± 0.19^{i}
	T10	12.37 ± 0.01^{e}	49.46 ± 0.19^{d}	57.60 ± 0.30^{f}
	T11	12.76 ± 0.021^{c}	54.46 ± 0.24^{b}	61.53 ± 0.17^{c}
	T12	12.93 ± 0.026^{a}	59.57 ± 0.22^{a}	66.44 ± 0.20^{a}
Sig		**	**	**

[a ,b,c] Means within the same column with different superscripts, differ significantly at ($P < 0.05$). .** = Highly significant ($P < .01$) .

T1-T4: Fresh milk with 10% total solids. **T1:** Without additives. **T2:** With 10% Slurry A. **T3:** With 10% Slurry B. **T4:** With 10% Slurry C. **T5-T8:** Filtered milk with 20% total solids. **T5:** Without additives. **T6:** With 10% Slurry A. **T7:** With 10% Slurry B. **T8:** With 10% Slurry C. **T9-T12:** Filtered milk with 30% total solids. **T9:** Without additives. **T10:** With 10% Slurry A. **T11:** With 10% Slurry B. **T12:** With 10% Slurry C.

Results also, showed an enhancing role of slurry fortified with proteolytic and lipolytic enzymes. Increasing the addition percent of these enzymes to the slurry increased the tyrosine content of the resultant cheese made from UF-MILK than the control. These results are agreement with those obtained by **El Shafie (1979)** in Domiati cheese using enzymatically hydrolysates.

Soluble tryptophane content in Domiati cheese (mg/100g):

Results of tryptophane content of Domiati cheese made from cow's milk and that made using UF-milk fortified with cheese slurrry with proteolytic and lipolytic enzymes are presented in Table (3). It was found that the content of tryptophane in fresh Domiati cheese of all treatments was very low but it was increased gradually with the progress of the ripening

period. Also, addition of cheese slurry fortified with proteolytic and lipolytic enzymes caused an increase in tryptophane content in Domiati cheese by increasing the percent of added enzymes to the cheese slurry.

The results are in agreement with the results obtained by **El Shafie (1979)** in Domiati cheese made with addition of enzymatically hydrolysates.

It could be suggested that addition of cheese slurry which was fortified with proteolytic and lipolytic enzyme inhanced the ripening process and improved the cheese quality of UF-Domiati cheese.

Table (3): Changes in soluble tryptophane content of Domiati cheese made using concentrated milk during ripening for 60 days at 6±1°C as affected by added proteolytic and lipolytic enzymes.

Milk Type	Treatments	Fresh	30 Days	60 Days
Fresh milk 10% TS	T1	2.16 ± 0.02^{h}	10.44 ± 0.20^{h}	30.50 ± 0.21^{k}
	T2	2.45 ± 0.02^{e}	12.50 ± 0.13^{f}	32.56 ± 0.16^{j}
	T3	2.56 ± 0.03^{d}	14.80 ± 0.13^{d}	34.85 ± 0.12^{i}
	T4	2.95 ± 0.02^{c}	16.51 ± 0.21^{c}	40.61 ± 0.25^{e}
2x UF-milk 20%TS	T5	2.35 ± 0.02^{g}	11.45 ± 0.10^{g}	35.90 ± 0.50^{h}
	T6	2.38 ± 0.01^{g}	13.75 ± 0.17^{e}	39.37 ± 0.11^{f}
	T7	2.93 ± 0.02^{c}	16.70 ± 0.20^{c}	41.51 ± 0.22^{d}
	T8	3.16 ± 0.03^{b}	17.36 ± 0.07^{b}	46.72 ± 0.19^{b}
3x UF-milk 30%TS	T9	2.48 ± 0.01^{de}	12.61 ± 0.18^{f}	38.44 ± 0.25^{g}
	T10	2.95 ± 0.02^{c}	14.58 ± 0.22^{d}	40.60 ± 0.24^{e}
	T11	3.00 ± 0.01^{c}	16.52 ± 0.17^{c}	44.77 ± 0.20^{c}
	T12	3.58 ± 0.09^{a}	19.86 ± 0.07^{a}	49.50 ± 0.26^{a}
Sig		**	**	**

^{a,b,c} Means within the same column with different superscripts, differ significantly at ($P < 0.05$).
.** = Highly significant ($P < .01$) , See abbreviation in Table 2

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Formol ripening index of Domiati cheese:-

The formol titration value in ml/100g cheese of all treatments during ripening period of 60 days at 6°C is presented in Table (4). The formol value of fresh cheese of all treatments ranged between 10 ml/100g for Damiati cheese made from fresh cow's milk to 14 ml/100g in Domiati cheese made from 3x UF-30% TS milk. Formol values increased gradually during ripening and this agrees the finding of **Taha (1978)** in Domiati cheese. It was found that the use of cheese slurry fortified with proteolytic and lipalytic enzymes increased formol ripening value during the ripening

period. These results are in agreement with these of **El Shafie (1979)** when enzymatic hydrolysates were used in Domiati cheese. Also, increasing the percent of added proteolytic and lipolytic enzymes to the cheese slurry

Table (4): Changes in formol titration value of Domiati cheese made using concentrated milk and cheese slurry fortified with proteolytic and Lipolytic enzymes during ripening for 60 days at 6±1°C.

Milk Type	Treatments	Fresh	30 Days	60 Days
Fresh milk 10% TS	T1	10.05 ± 0.05^{f}	16.47 ± 0.25^{j}	32.40 ± 0.20^{k}
	T2	10.20 ± 0.11^{f}	19.34 ± 0.19^{h}	36.51 ± 0.26^{i}
	T3	10.48 ± 0.25^{ef}	22.34 ± 0.20^{e}	37.40 ± 0.28^{f}
	T4	10.55 ± 0.29^{def}	23.36 ± 0.19^{d}	41.35 ± 0.20^{d}
2x UF-milk 20%TS	T5	11.14 ± 0.07^{cde}	17.25 ± 0.21^{i}	35.46 ± 0.23^{j}
	T6	11.25 ± 0.13^{cd}	20.45 ± 0.22^{g}	38.44 ± 0.28^{e}
	T7	11.40 ± 0.20^{c}	25.54 ± 0.28^{b}	41.25 ± 0.13^{d}
	T8	11.59 ± 0.29^{c}	27.36 ± 0.20^{a}	55.42 ± 0.29^{b}
3x UF-milk 30%TS	T9	11.63 ± 0.31^{c}	19.42 ± 0.21^{h}	37.65 ± 0.33^{f}
	T10	12.62 ± 0.31^{b}	21.59 ± 0.30^{f}	40.63 ± 0.32^{d}
	T11	14.27 ± 0.16^{a}	24.36 ± 0.18^{c}	48.40 ± 0.24^{c}
	T12	14.63 ± 0.32^{a}	27.40 ± 0.24^{a}	59.35 ± 0.20^{a}
Sig		**	**	**

[a ,b,c] Means within the same column with different superscripts, differ significantly at ($P < 0.05$). .** = Highly significant ($P < .01$), See abbreviation in Table 2

increased the formol value of the cheese resulted from the use of UF-milk for making Domiati cheese.

Shilovich ripening index:

The shilovich-ripening index of UF-Domiati cheese with added cheese slurry fortified with proteolytic and lipolytic enzymes during 60 days of ripening at 6±1°C was presented in- Table (5).It was found that shilovich index of fresh Domiati cheese made from fresh cow's milk was 20 ml/100 g cheese and it was 21 and 22 ml/ 100 g cheese for Domiati cheese with added cheese slurry fortified with proteolytic and lipolytic enzymes. When Domiati cheese was made from 2x UF 20% TS milk the Shilovich index rised to 25 in cheese with added cheese slurry fortified with proteolytic and lipolytic enzymes and increased to 26 for the cheese made from 3x UF-30% TS milk with cheese slurry fortified with proteolytic and lipolytic enzymes.

These values were increased gradually with the progress of the ripening period showing values of 35 and 39 for cheese made from UF_milk of 30% TS after 30 days and 56 and 58 after 60 days for cheese made from UF-milk 30% TS.

Table (5): Changes of Shilovich value of Domiati cheese made from ultrafiltered milk during ripening for 60 days at 6±1°C as affected by addition of proteolytic and lipolytic enzymes.

Milk Type	Treatments	Fresh	30 Days	60 Days
Fresh milk 10% TS	T1	20.10 ± 0.06^{g}	29.23 ± 0.12^{h}	42.44 ± 0.28^{k}
	T2	20.26 ± 0.13^{fg}	31.25 ± 0.21^{g}	45.43 ± 0.22^{j}
	T3	21.01 ± 0.19^{ef}	34.37 ± 0.22^{e}	47.24 ± 0.12^{i}
	T4	21.58 ± 0.66^{e}	36.23 ± 0.13^{c}	49.34 ± 0.19^{e}
2x UF-milk 20%TS	T5	22.63 ± 0.32^{d}	29.40 ± 0.24^{h}	45.43 ± 0.27^{j}
	T6	24.31 ± 0.17^{c}	33.47 ± 0.25^{f}	48.22 ± 0.17^{f}
	T7	25.19 ± 0.10^{b}	34.56 ± 0.29^{e}	49.24 ± 0.12^{e}
	T8	25.46 ± 0.27^{b}	38.38 ± 0.19^{b}	52.24 ± 0.12^{d}
3x UF-milk 30%TS	T9	23.37 ± 0.19^{d}	30.62 ± 0.31^{g}	48.24 ± 0.12^{h}
	T10	25.26 ± 0.14^{b}	33.44 ± 0.22^{f}	53.56 ± 0.29^{c}
	T11	26.29 ± 0.15^{a}	35.54 ± 0.28^{d}	56.25 ± 0.12^{b}
	T12	26.65 ± 0.32^{a}	39.39 ± 0.24^{a}	58.33 ± 0.19^{a}
Sig		**	**	**

[a ,b,c] Means within the same column with different superscripts, differ significantly at ($P < 0.05$). .** = Highly significant ($P < .01$) , See abbreviation in Table 2

Conclusions

It could be concluded that using proteolytic and lipolytic enzymes in the fortification of cheese slurry, when added to cheese curd made from milk with increasing total solids (TS) through ultrafiltration to 20% and 30% TS, has a highly significant effect.

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دلائل التسوية للجبن الدمياطي المصنع باستخدام معلق الجبن المدعم بالإنزيمات المحللة للبروتين والدهن

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هدفت هذه الدراسة إلى دراسة تأثير إضافة معلقات جبن مدعمة بإنزيمات البروتييز والليبيز على عملية تسوية الجبن المصنعة من لبن الأبقار المرشح بالترشيح الفائق (UF). تم تحضير ثلاث معلقات جبن الأول كان بمثابة عينة كنترول (Slurry A)، والثاني دُعم بإنزيم البروتييز بنسبة ٠.٠٢% وإنزيم الليبيز بنسبة ٠.٠٠٢%

(Slurry B)، والثالث دُعم بإنزيم البروتييز بنسبة ٠.٠٤% وإنزيم الليبيز بنسبة ٠.٠٠٥% (Slurry C). أُضيفت هذه المعلقات إلى ثلاثة أنواع من اللبن مختلفة في الجوامد الصلبة الكلية (١٠%، ٢٠%، و٣٠%)، حيث أُضيف ١٠% من كل معلق إلى كل نوع من اللبن. تم تخزين جميع المعاملات عند درجة حرارة ٦ ± ١ درجة مئوية لمدة ٦٠ يومًا، وتم قياس مؤشرات النضج خلال فترة التسوية (٦٠ يوم). تم تحديد قيم الفورمول، قيم شيلوفيتش، ومحتوى التيروسين والتريبتوفان الذائب.

التوصية: من هذه النتائج وجد أن إضافة معلق الجبن المضاف إليه إنزيم بروتييز وإنزيم ليبيز أدي إلى تحسن مقاييس التسوية المذكورة وشجع على تسوية الجبن.