EFFECT OF LYSOZYME ON SOME PROPERTIES OF TALLAGA CHEESE

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Abstract

The effect of lysozyme on some chemical and microbiological properties of UF Tallaga cheese was studied. Lysozyme concentrations at 75 ppm and 150 ppm were added to the retentate before the manufacture of Tallaga cheese. Manufactured cheese were stored in the refrigerator at 7 ± 1°C for 3 weeks and periodically analysed every week. The results of the chemical analysis showed that there were no differences in acidity % and pH values of both untreated and treated cheese with lysozyme when fresh. But during storage period, it was noticed that the untreated cheese had more acidity % than that of the treated cheese .Also, the results indicated that the total solids and protein contents of the fresh treated cheese with lysozyme were increased.

During stoarge period the total solids increased while the protein content of the treated cheese decreased .Moreover the soluble nitrogen content increased.

The results of bacteriological analysis indicated that the lysozyme reduced the total count and sporeformers counts in fresh and during storage period in treated cheese. While the influence of lysozyme on psychrotrophic counts was not detected in the fresh sample but was detected during the storage period. The results obtained showed that the efficiency of lysozyme in destroying *E.coli* as well as ability to improve the keeping quality of cheese were strongly depended on the concentration of lysozyme . The concentration of lysozyme at 150 was more effective on controlling the survival of *E. coli* in Tallaga cheese than 75 ppm .

Key Words: Lysozyme, Preservation, Tallaga cheese.

INTRODUCTION

Tallaga cheese is a traditional Egyptian cheese and it is a very popular type in the local market. It is stored in the refrigerator at 7 ± 1 °C within few weeks , this may be due to high moisture and relatively low salt contents which making it a good medium for growth of some microorganism and also the uncontrolled hygienic handling which increase microbial contamination (Shehata *et al.* , 1995). Few studies have been performed to improve keeping quality of Tallaga cheese. Mehanna and Rashed (1990) used carbon dioxide to improve the keeping quality of Tallaga cheese. No attempts were made to investigate the effect of lysozyme on keeping quality and certain properties of Tallaga cheese in Egypt. However many efforts have been devoted towards the use of lysozyme as antimicrobial agent in food products (Gill and Holley,

2000). Hughey and Johanson (1987) suggested that lysozyme may be selected on application as food preservation especially when thermophilic sporeformers are problems, and as a safeguard against food poisoning caused by Clostridium botulinum and Listeria monocytogens.

Recently, the use of lysozyme as antimicrobial agent in food products has received increasing attention (Poland and Sheldon , 2001) .

Lysozyme is an enzyme (EC. 3 . 2 . 1 . 17) which cleaves the β (1 – 4) glycosidic linkage between N - acetyl muramic acid and N- acetyl glucose amine found in peptidoglycan layer of the bacteria cell wall (Jolles and Jolles , 1984).

Study has been carried on the level and distribution of lysozyme content in cow and buffalo milk (EL-Aziz , 2006).

Using lysozyme in dairy food has many advantages. Lysozyme has been used in certain cheeses to control late gas production caused by Saccharolytic butyric acid forming Clostridia , particularly Clostridium tyrobutyricum (Wasserfall and Teuber, 1979).

Therefore the aim of this study is to investigate possibilities of improving the quality of Tallaga cheese by using lysozyme as antimicrobial agent and to study the effect of lysozyme on certain chemical and microbiological properties of Tallaga cheese during storage period.

MATERIALS AND METHODS

Buffalo milk retentate produced by Ultra filtration (UF) used in Tallaga cheese in the present study was obtained from Dairy Processing Unit belongs to Animal Production Research Institute. The concentration factor of retentate was 3 (CF =3) with 15% fat content.

Rennet: Hala rennet powder was used as a coagulant which was obtained from CHR - Hansen's luboratorium Denmark.

Lysozyme (Enzyme) : Chicken egg – white lysozyme (EC : 3 . 2 . 1 . 17) was obtained from Sigma Co. and used as antimicrobial.

All chemicals used in this study are analytical grade.

Escherichia *coli (E.coli) 157:H7:* This strain isolated from dairy products was secured from stock culture collection of Serology Unit Animal Health Research, Inst. Doki , Giza.

Cheese making :

Experimental 1

The retentate was divided into two treatments, first treatment depended on pasteurization of milk before concentration by UF and in the second treatment the

retentate was heat treated at 85 °C. Each treatment was divided into three portions, one portion was served as control (I and IV), while the other two portions were treated with lysozyme at concentrations 75ppm (II and V) and 150 ppm (III,andVI).Treated and untreated retentate with lysozyme were salted at the rate of 2 % and 0.02% Ca Cl_2 was added , thenTallaga cheese was made by following the conventional method of Domiati cheese.Cheese samples were stored at 7±1°C for three weeks .This experiment was carried out in doublicat.

The chemical and microbiological analysis of cheese samples were carried out when fresh and after one,two and three weeks of storage.Each analysis was carried out in doublicat and the average of the obtained results was carried.

Experimental 11 (inoculation with *E. coli*)

The retentate was heat treated at 85°C after concentration by UF and then inoculated with *E.coli 157:H7* at 2 x 10^5 C. F. U./ ml .This was divided into three portions, one portion was served as control (VII) and the other two portions were treated with lysozyme at the same concentrations which mentioned in experimental (1) (VIII and IX). All portions were made into Tallaga cheese and storage as described previously.

The cheese samples were analysed microbiologicaly when fresh and after one, two and three weeks of storage.

Chemical analysis:

Tallaga cheese samples were analysed for total solids, titratable acidity, pH value ,total and soluble nitrogen according to the official methods (A.O.A.C 1990)

Microbiological analysis:

Tallaga cheese samples were examined for total count and mould and yeast count as mentioned by A.P.H.A.(1992).E.coli counts were determined according to Oxoid manual (1982) using violet red bil agar. Sporeformers were grown on nutrient agar medium. Psychrotrophic bacteria were examined using the same method of total count and incubated at 7 ±1°C for 10 days.

RESULTS AND DISCUSSION

Effect of lysozyme as a preservative agent on chemical and micrbiological properties of UF Tallaga cheese during storage.

1-Chemical analysis

Acidity % and pH values:

Acidity % and pH values for untreated and treated cheese with lysozyme when fresh and during storage at 7°C for 3 weeks were presented in table (1). The results clearly indicated that, pH values and acidity % of all fresh cheese were nearly the

same. During storage, the changes in acidity % and pH values of all cheese were observed. There was sharply increase in acidity % of untreated cheese made from unheated retentate (control I), that may be due to the higher microbial counts and production of lactic acid. While, the acidity % of untreated cheese made from heated retentate (control IV) was obviously lower than that of unheated one,and slightly increased during storage period. These results were in agreement with those reported by (Mehanna and Rashed , 1990).

The results also showed that the rate of increase in acidity % was lesser in both concentrations of lysozyme than control .

Table 1. Effect of lysozyme on acidity % and pH of UF Tallaga cheese during storage period (in weeks).

Treatments	Acidity (%)				pH value			
	Fresh	1	2	3	Fresh	1	2	3
I	0.15	0.30	0.40	0.50	5.80	5.67	5.56	5.46
II	0.15	0.20	0.30	0.30	5.76	5.72	5.65	5.67
III	0.15	0.20	0.30	0.30	5.76	5.70	5.66	5.65
IV	0.10	0.15	0.15	0.17	6.41	6.30	6.30	6.20
V	0.10	0.13	0.13	0.15	6.41	6.37	6.37	6.32
VI	0.10	0.13	0.13	0.15	6.41	6.38	6.39	6.33

I – Cheese made from unheated retentate (control).
II – Cheese made from unheated retentate with 75 ppm lysozyme.
III – Cheese made from unheated retentate with 150 ppm lysozyme.
IV – Cheese made from heated retentate (control)
V – Cheese made from heated retentate with 75 ppm lysozyme
VI – Cheese made from heated retentate with 150 ppm lysozyme

Total solids (%):

The effect of lysozyme on total solids (T.S), protein and soluble nitrogen% contents of UF Tallaga cheese during storage period were reported in table (2). The results showed that the T.S of fresh treated cheese was higher than those of fresh untreated cheese (I and IV). This may be returned to the addition of lysozyme . A continuous increase in T.S content in all cheese samples was observed along the storage period. It was observed that T.S of control (IV) was lower than those of control (I). The decrease of T.S content of cheese made from heat treated retentate control (IV) may be due to impairing of whey syneresis from curd according to that reported by Walstra *et al.,* (1985). Moreover, the increase of T.S of unheated retentate control (I) during storage may be due to the expulsion of whey from cheese which caused by relatively higher acidity produced by bacteria during storage. On the other hand, the results showed an increase in T.S content of treated cheese than control cheese (I and IV).This my be due to lysozyme promoted syneresis from curd. This was similar to that found by Kamaly *et al.,* (1992) who reported that lysozyme enhanced syneresis of the resultant renneted coagula.

Protein % (total nitrogen x 6.38):

The results of protein content of untreated and treated cheese with lysozyme were recorded in table (2). The results showed that protein content in fresh treated cheese was higher than fresh control cheese. This result is in line with Panfil-Kuncewicz and Kisza (1983) who found that the addition of lysozyme to milk , increased total protein content of milk progressively from 3.22 to 3.42% and casein content increased from 2.5 to 2.56%, micellar casein increased from 2.23to 2.48% and soluble casein decreased correspondingly from 0.27 to 0.08%. Also, they found that hydration and micelle volume decreased with addition of lysozyme. While, during storage the protein content of treated cheese decreased. This result may be due to lysozyme degradation of milk protein during storage. This agreed with data of Kamaly *et al.*, (1992) who indicated that renneted-coagula treated with lysozyme had more protein degradation than those of renneted coagula (controls).

Soluble nitrogen (%):

As shown in table (2) the soluble nitrogen content of all cheese samples was increased during storage. It was clear that soluble nitrogen of the control (IV) was lower than that of control (I). The rate of the increase of soluble nitrogen of control (I) attributed to the higher microbial activity during storage period. While the results showed that the level of soluble nitrogen released from treated cheese with lysozyme was greater than those from untreated cheese (controls and IV) .The increase of soluble nitrogen was proportional to the increase of lysozyme concentration . This results were in agreement with Kamaly *et al.*, (1992) who indicated that lysozyme promoted digestibility of milk protein and liberation of soluble nitrogen.

Table 2. Effect of lysozyme on T.S, protein and soluble nitrogen % contents in UF Tllaga cheese during storage period (in weeks).

2 – Microbiological analysis:

Table (3) shows the effect of lysozyme on microbiological count.

Treatments	T.S (%)				Protein (%)				SN (%)			
	Fresh	1	2	3	Fresh	1	2	3	Fresh	1	2	3
I	32.65	34.20	36.90	38.00	11.08	11.34	11.43	11.79	0.252	0.309	0.502	0.616
II	33.70	36.00	37.00	39.00	12.86	12.41	12.15	11.79	0.392	0.476	0.502	0.516
III	34.14	36.00	38.70	40.10	13.22	13.00	12.86	12.50	0.531	0.560	0.56	0.600
IV	31.80	33.18	36.46	37.50	11.08	11.32	11.43	11.76	0.196	0.305	0.335	0.466
V	32.15	34.00	36.74	38.00	12.50	12.36	12.15	12.15	0.335	0.476	0.56	0.56
VI	33.40	34.60	35.30	36.50	12.85	11.64	11.34	11.08	0.392	0.448	0.504	0.562

- *E. coli* counts:

The results revealed that the *E. coli* counts were detected in all fresh untreated and treated cheese with lysozyme , meanwhile *E. coli* counts were higher in untreated

cheese than the treated cheese. The data showed that the concentration of lysozyme had an effect on the survival of *E. coli*. These results are in agreement with Poland and Sheldon (2001).

Generaly the counts were decreased during the storage period , while the decrease was more slowly in the contaminated treatments (VII, VIII and IX). These results are in agreement with Ismail&Hashem (2007) who used essential oils to inhibit the growth of *E.coli 157:H7*.

Total bacterial counts :

The same table showed that T.C were higher in cheese made with unheated retentate than the other treatments. The counts in all treatments increase until 2 weeks then decreased after 3 weeks.

Sporformer counts:

Sporeformer counts in all treatments were increased until one week then decreased. The counts were higher in the different controls than in the treatments in which lysozyme was added. That might be due to the effect of lysozyme on the bacterial count.

Psychrotrophic bacterial counts:

Psychrotrophic bacterial counts were higher in the treatments of unheated retentate.On the other hand the addition of lysozyme had an effect on the psychrotrophic counts. It was not detected in the fresh samples but it increased during the storage period, at the end of the storage period they decreased, that decrease might be due to the increase of acidity in samples.

Mould and yeast counts:

Mould and yeast were not detected in fresh samples but after two weeks the number increased in all treatments.

According to our results it can be concluded that the efficiency of lysozyme in destroying Escherichia coli 157:H7 as well as ability to improve the keeping quality of cheese were strongly depended on the concentration of lysozyme. However using concentration150 ppm of lysozyme was more effective on controlling the survival *E.coli* 157:H7 in Tallaga cheese than 75 ppm concentration. . Also the heat treatment of retentate before cheese making affected on the survival of *E .coli* 157 : H7.

Table 3. Effect of lysozyme on survival rate of *E.coli 157:H7*, T.C, Sporformers, psychrotrophic, and Mould and yeast during storage Period(in weeks).

Treatment	*E.coli* (10^{-1})				T. C (10^{-5})				Spor (10^{-1})				Psyc. (10^{-1})				Mandy (10^{-1})			
	Fresh	1	2	3	Fresh	1	2	3	Fresh	1	2	3	Fresh	1	2	3	Fresh	1	2	3
I	45	33	21	9	103	120	126	110	12	14	11	8	4	38	63	14	4	38	63	14
II	28	16	10	3	88	96	100	80	8	6	4	2	1	24	42	10	1	24	42	10
III	15	11	8	ND	80	90	98	77	6	5	3	2	1	11	29	ND	1	11	29	ND
IV	23	19	15	6	75	87	92	70	11	12	12	7	ND	12	34	11	ND	12	34	11
V	10	3	ND	ND	64	72	79	61	8	5	4	3	ND	7	16	5	ND	7	16	5
VI	6	ND	ND	ND	59	67	71	58	5	4	4	2	ND	3	10	ND	ND	3	10	ND
VII	70	56	40	25	82	93	96	79	12	13	10	7	ND	11	30	9	ND	11	30	9
VIII	42	39	20	2	71	85	91	68	6	5	4	2	ND	5	15	4	ND	5	15	4
IX	28	15	2	ND	63	74	82	61	4	3	2	2	ND	1	11	ND	ND	1	11	ND

T. C: Total bacterial count C.F.U x 10^5

E.coli : Escherichia coli count C.F.U x 10^1

Spor : Sporformer count C.F.U x 10^1

Psyc : Psychrotrophic count C.F.U x 10^1

Mandy : Mould and yeast count C.F.U x10^1

I,,II,III,IV,V,VI : were defined as previously in table (1).

VII : Cheese made from heat treated retentate and contaminated With *E.coli 157:H7* at 2x10^5 C.F.U/ml (control).

VII I: Cheese made from heat treated retentate and contaminated with *E.coli 157:H7* at 2x10^5C.F.U/ml and added lysozme at 75 ppm.

IX : Cheese made from heat treated retentate and contaminated with *Ecoil 157:H7* at 2x10^5 C.F.U /ml and added lysozyme at 150 ppm

ND: Not detected.

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تأثيراللليزوزيم على بعض خواص جبن الثلاجه

ماجدة عبد العزيز ، ايمان لبيب مصطفى

معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية

تم إستخدام لبن مركز (UF) في تصنيع جبن ثلاجه حيث قسمت الكميه الى قسمين :

- القسم الأول أعتمد على بسترة اللبن قبل عملية التركيز وتم التقسيم كالتالي قبل التصنيع :-

١- لبن مركز غير معامل باللليزوزيم (كنترول I)

٢- لبن مركز معامل بتركيز ٧٥ جزء في المليون ليزوزيم (II) .

٣- لبن مركز معامل بتركيز ١٥٠ جزء في المليون ليزوزيم (III) .

- القسم الثاني تم معاملة اللبن المركز علىدرجةحرارة٨٥مْ قبل التصنيع وقسم أيضاً الى قسمين :

اوّلا :-

١- لبن مركز معامل حراريا غير معامل باللليزوزيم (كنترول IV).

٢- لبن مركز معامل حراريا معامل بتركيز ٧٥ جزء في المليون ليزوزيم (V) .

٣ - لبن مركز معامل حراريامعامل بتركيز ١٥٠ جزء في المليون ليزوزيم(VI) .

ثانياً :-

هذا الجزء تم تلويثه بميكروب إشيريشياكولاي١٥٧:هـ ٧ (*E . coli*) ثم قسم الى ثلاثة أقسام :

١- لبن مركز معامل حراريا ملوث غير معامل باللليزوزيم (كنترول (VII)

٢- لبن مركز معامل حراريا ملوث معامل بتركيز ٧٥ جزء في المليون ليزوزيم (VIII) .

٣ - لبن مركز معامل حراريا ملوث معامل بتركيز ١٥٠ جزء في المليون ليزوزيم (IX) .

تم تحليل العينات التي لم يتم تلويثها كيماوياً وميكروبيولوجياً أما العينات التي تم تلويثها بالـ *E . coli* تم تحليلها ميكروبيولوجياً فقط وذلك خلال فترات التخزين .

أظهرت النتائج مايلي :-

إضافة اللليزوزيم أدى الى إرتفاع في البروتين والمواد الصلبه الكليه في الجبن الطازج ، كما أوضحت النتائج إرتفاع الحموضه وإنخفاض الـ pH بالمقارنة بالجبن المعامل باليزوزيم أثناء التخزين .

كما أوضحت التحاليل الميكروبيولوجية أن إضافة اللليزوزيم يقلل من العدد الكلي للبكتريا والبكتريا المتجرثمه (Sporeformers) في الجبن الطازج وأثناء مراحل التخزين ، كما ظهر تأثير اللليزوزيم على البكتريا المتحمله للبروده أثناء التخزين ، كما أثر إضافة اللليزوزيم على إنخفاض عدد إشيريشيا كولاى (*E. coli*) في الجبن المنتج من لبن مركز السابق تلويثه بنفس الميكروب .