SENSORY , CHEMICAL AND BACTERIOLOGICAL EVALUATION OF YOGHURT DURING ITS MANUFACTURE

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ABSTRACT

A total of 90 random samples were taken during yoghurt manufacturing in a dairy plant in Kafr El-Sheikh governorate resembling 30 batches , 30 samples from each of raw cow milk , standardized pasteurized milk and final product . The samples were directly transferred to the laboratory under aseptic condition and subjected to the chemical and microbiological examination , in addition to the sensory evaluation of the final product .

The study revealed that , the chemical properties of the raw milk samples (fat% , protein% , S.N.F% , moisture% , P^H value , acidity% and activity test) lied within the permissible limits stated by ***ES , 154 – 1 (2005)*** *. Moreover , the microbiological criteria comply this standard whereas , the samples contained relatively low counts to total bacterial , Staphylococcus aureus , Mould , and Coliforms and free from Bacillus cereus , Listeria monocytogenes , Clostridium perfringens , Salmonella and E . coli except 3 (10 %) and 9 (30 %) from the samples hading total bacterial and Staphylococcus aureus counts respectively exceeded the limits .*

Concerning the standardized pasteurized milk samples only minor differences could be observed in the chemical composition of the raw and standardized pasteurized milk samples, but microbiologically there is complete reduction to all microorganisms except those resist to pasteurization.

Regarding the final product the chemical properties of the samples (fat% , protein% , S.N.F% , moisture% , P^H value and acidity%) consequently lied within the permissible limits stated by ***ES, 1000 (2005).*** *The microbiological characteristic of the samples met the specification required by this standard except 3 (10 %) samples hading mould and Coliforms counts more than 10 cfu /ml . From sensorial point of view , our study showed that all the samples examined were found in good condition and comply with the requirement of* ***ES , 1000 (2005)*** *except 1 (3.3 %) of these samples due to hading slight wheyed off appearance defect .*

INTRODUCTION

In recent years, there has been increasing demand for a new range of dairy products, including yoghurt which are similar to traditional products but have a low fat content ***(Begona*** and ***Rosario, 2000).***

Yoghurt is one of the most popular fermented dairy product widely consumed allover the world . It is obtained by lactic acid fermentation of pasteurized milk by the starter culture containing *streptococcus thermophilus* and *lactobacillus delbrueckii* spp *bulgaricus* . The role of these two genera in yoghurt manufacture can be summarized as milk acidified and synthesis of aromatic compound ***(Sahan , et al . 2008*** and ***Serra , et al . 2009).*** Then the cultured pasteurized milk packed in plastic

container covered by aluminum foil , then warmed in an incubator for several hours, during this time the yoghurt thickens and develops its distinctive flavor. Non fat milk solids and stabilizers often added to thicken the yoghurt. After incubation to the desired acidity the yoghurt is kept in refrigerator quickly to stop the action of starter culture and then stored till sell.

Yoghurt is a very popular flavored and healthful dairy product in Egypt. Its production and consumption is growing continuously due to its therapeutic properties besides its high nutritive value ***(Karagol et al. 2004)***. The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose by maldigesters , lower blood cholesterol, improved immune response, help the body assimilate protein, calcium and iron and decreased risk of cancer ***(Perdigeon et al . 1998, Marona and Pedrigon , 2004 and Susanna et ai. 2008)***.

The quality and safety of yoghurt can be defined against a wide range of criteria such as chemical and microbiological characteristics.

Egyptian organization for standardization ***(ES, 1000 / 2005)*** stipulated that, yoghurt should be free from pathogenic microorganisms and their toxins *listeria monocytogenes* and *Escherichia coli*. Moreover, *coliform* and mould counts should not exceed 10 cell / gm from each . In addition the fat % and solid not fat % should not less than 3% and 8.25% respectively for such manufacturing from cow milk, also the acidity % should not more than 1.5 % calculated as lactic acid. Therefore, this study was done in order to give an information about chemical and microbiological evaluation during these processing in addition to sensory evaluation of the final product.

MATERIAL AND METHODS

A total of 90 random samples were taken during yoghurt manufacturing in a dairy plant in Kafr El-Sheikh governorate resembling 30 batches , of raw cow milk , standardized pasteurized milk and yoghurt (30 samples of each). The samples were directly transferred to the laboratory under aseptic condition and subjected to the following sensory, chemical and microbiological examination .

I - Sensory evaluation:

Final product (yoghurt) was evaluated for sensory characteristics according to ***American Dairy Science Association (ADSA, 1987)*** using a 20 points system with 10 points for flavor, 5 points for body and texture and 5 points for appearance.

II – Chemical examination:

All the samples from the manufacturing stages were subjected to quantities analysis of fat %, protein%, solid not fat%, moisture %, titratable acidity % and P^H value, in addition to the activity test for detection of antibiotic residues in raw milk samples only which recommended by ***Marshal, (1992)*** and ***AOAC, (2005)***.

III – Microbiological examination:

Preparation of samples:

The samples were prepared according to the method recommended by ***ICMSF, (1996)*** for the following tests:

A) Determination of total bacterial count: for raw and standardized pasteurized milk samples only according to ***IDF, (1991).***

B) ***Staphylococcus aureus count*****:** was performed according to ***ISO, (1995- 6888).***

C) Mould count: was carried out according to ***APHA, (1992).***

D) ***Coliform count:*** was performed according to ***ICMSF, (1996).***

E) ***Bacillus cereus*** **count:** was carried out according to ***Lancette and Harmon, (1980).***

F) Determination of ***Listeria monocytogenes*****:** were performed according to ***ISO, (1995 - 11290)***

G) Determination of ***Clostridia perfringens*** **:** was carried out according to ***ICMSF, (1996).***

I) Detection of ***Salmonella*****:** was carried out according to ***ICMSF,(1996).***

H) Determination of ***Escherichia coli*** **:** were performed according to ***Feng and Hartman, (1982).***

RESULTS AND DISCUSSION

I- **Raw milk:**

Raw milk is milk in its natural (unpasteurized state) and according to the ***Egyptian standard 154 – 1 (2005)*** the fat % and solid not fat % not less than 3% and 8.25% respectively, also it should be free from pathogenic microorganisms and their toxins, *Listeria monocytogenes* and *Salmonella*. Moreover, *Clostridia perfringens, Bacillus cereus* and *Staphylococcus aureus* counts should not exceed 1 cfu / ml , 1 cfu / ml , and 100 cfu / ml , respectively.

Test for antibiotic residues were carried out to prevent problems with fermentation whereas stated that a level as low as 0.005 I.U. penicillin had been known to inhibit strain of *streptococcus thermophilus* used in yoghurt manufacture ***(Packham et al. 2001)***.

Titratable acidity plays a fundamental role and represents average high important parameter for technical evaluation of the quality of milk and valuable as a guide manufacturing operation and for measuring the quality of dairy products ***(Smit, 2005)***. Determination of P^H value is an effective predictor of instability in the end product and is the most useful made on the milk after bulk storage as it indicate its real acidity.

Data stipulated in table (1) revealed that all the examinee samples were negative for the activity test for detection of antibiotic residues in milk . The obtained results were in agreement to those reported by ***Abd El-Hafeiz and Mervat, (2004)***. Also showed that the mean values of fat% , protein%, S.N.F%, moisture%, P^H value and acidity% were 3.68 ± 0.07 ., 3.36 ± 0.02 ., 8.86 ± 0.05 ., 86.85 ± 0.10 ., 6.51 ± 0.03 and 0.16 ± 0.003 respectively.

According to the ***Egyptian standard 154 – 1 (2005)*** these results lied within the permissible limits and agreed to a certain extent with ***Bille et al. (2009)***.

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with microorganisms. Therefore, the microbial content of milk is a major feature in determining its quality. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed soil, faeces and grass ***(Coorevits et al. 2008)***.

Concerning the microbiological analysis, table (4) cleared that incidence and the mean values of total bacterial count, *Staphylococcus aureus* , Mould , and *Coliforms* counts (cfu /ml) were 30 (100%) and $2.9x10^5 \pm 8.1x10^4$., 30 (100%) and $1.7x10^2 \pm 3.5x10$., 18 (60%) and $1.3x10^2 \pm 1.1$ x10., 30 (100%) and$5.4x10^2 \pm 1.9$ $x10^2$ respectively .

Total bacterial count is usually used to assess the over all sanitation and storage conditions of raw milk and all dairy regulation included to grade raw milk for the producer quality payment scheme.

The obtained results indicated 3 (10%) of the examined samples exceeded the permissible limits according to ***Mehari and Gashe, (1990)*** who reported that the total bacterial count should not exceed 10^6 cfu / ml. Nearly similar findings were recorded by ***Eman and Nahed, (2008)***.

Also the study revealed that 9 (30%) of examined samples disagreed with the ***Egyptian standard 154 – 1 (2005)*** due to hading *Staphylococcus aureus* count more than 100 cfu / ml. ***Abd El-Shaheed (2004)*** reported relatively higher count.

Furthermore, the study clarified that mould was present in all samples examined and the obtained results were nearly similar to those recorded by ***Fawzy et al. (2003)***.

Presence of mould in raw milk is indicative for the neglected sanitation during the milking transportation and cleaning equipments and is of great concern not only to human health but also it can cause lipolytic and proteolytic spoilage which influence the biochemical characters and flavor of the product and commercially be undesirable and unsuitable for yoghurt production ***(Beuver et al. 1997)***.

Coliforms were detected in relatively high count in examined samples . Nearly similar findings were reported by ***Bille et al. (2009)***. The presence of high numbers of *Coliforms* in milk provides an index of hygienic standard used in the production of milk which can contaminated the udder from different sources as manure, soil, feed, personnel and water ***(CDC, 1998)***.

Bacillus cereus, Listeria monocytogenes, Clostridia perfringens, Salmonella and *E. coli* could not be detected in all examined samples. Which comply the ***Egyptian standard 154 – 1 (2005)***.

II-**Standardized pasteurized milk:**

The chemical composition of the milk affects the nature of the final product (yoghurt). So its general practice to standardize milk composition to ensure consistency and maximum yield standardization were by skimming raw milk or addition of reconstitute milk powder.

The data presented in table (2) revealed that the mean values of fat%, protein%, S.N.F%, moisture%, P^H value and acidity% of examined pasteurized milk samples were 3.48± 0.04 ., 3.91± 0.01 ., 10.80±0.08 ., 86.20±0.11 ., 6.57±0.01 and 0.18±0.002 respectively.

The study showed that , only minor differences could be observed in the chemical composition of the raw and standardized pasteurized milk samples except solid not fat which is to some extent higher in standardized pasteurized milk samples which may be attributed to adding some material such as non fat dry milk , whey protein concentrate and some other dairy ingredients . Nearly similar results were achieved by ***Abd El-Hafeiz and Mervat, (2004)***.

Almost all potential microbiological hazards can be eliminated with a heat treatment milk which has proved to be successful to control classical zoonoses as well as new foodborne pathogens such as *salmonella* ***(IDF, 1994)***.

The data presented in table (5) showed that the incidence, and the mean values of total bacterial count (cfu /ml) were 24 (80%) and $1.7x10^3 \pm 1.5x10^2$ respectively . The presented count may be due to the presence of thermoduric bacteria which can resist the pasteurization temperature. Also the study clarified that *Staphylococcus aureus*, Mould, *Coliforms, Bacillus cereus, Listeria monocytogenes, Clostridia perfringens, Salmonella* and *E . coli* could not be detected in all of the examined samples.

The obtained results were nearly similar to those recorded by ***Ammara et al. (2009)***.

III- **Final product**

The chemical , microbiological and sensory tests were carried out for verification that the final product comply with the quality and safety specifications stated by the Egyptian legislation ***(ES, 1000 / 2005)***.

The data recorded in table (3) revealed that the mean values of fat % , protein% , S.N.F% , moisture%, P^H value and acidity% were 3.52± 0.03 ., 3.90± 0.01 ., 10.57±0.05 ., 85.35±0.08 ., 4.56±0.02 and 1.21±0.03 respectively . These findings of all samples were within the required limits according to ***ES, 1000 (2005)*** and agree with those obtained by ***Shahid, et al. (2002)***.

Regarding the microbiological analysis, the data stipulated in table (6) showed that, incidence and the mean values of Mould, and *Coliforms* counts (cfu /ml) were 15(50%), 1.46x10±0.17x10 and 6 (20 %) and 2.17x10 ± 0.32 x10 respectively. Also the study declared that,

Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Clostridia perfringens, Salmonella and *E. coli* could not be detected in all of the examined samples. The observed results confirmed the findings of ***Eman* and *El-Kaseh, (2008)*** and disagreed from those reported by ***Kasthurie, (2005)***.

It is noticed from the aforementioned results that all the examined samples were lied within the permissible limits reported by ***ES, 1000 (2005)*** except 3 (10 %) of these samples due to hading mould and *Coliforms* counts more than 10 cfu /ml which may be attributed to post pasteurization contamination or using unsatisfactory sterilized cups in packing whereas, despite all the possible sites in the processing chain at which bacteria can be introduced. The step that has the greatest influence on the keeping quality of heat treated dairy products is the filling operation. Real contamination may also occur at the filling stage from condensation formed on the machines ***(Kasthurie, 2005)***.

Concerning sensory evaluation, the data given in table (7) showed that all the examined samples were in good condition and comply with the requirement of ***ES, 1000 (2005)*** except 1 (3.3 %) of these samples due to hading appearance defect (slight wheyed off). Yogurt manufacturers use stabilizers, such as, pectin and gelatin to try to prevent wheying-off. Another approach is to increase the total solids content of yoghurt milk, especially the protein content, to reduce wheying-off. ***(Lee and Lucey, 2010)***.

In conclusion if the yoghurt is manufactured under strict measures including use of high quality milk and starter , in addition application of GMP (good manufacturing practices) through the production line from receiving area till storage the final product will be safe and with high quality .

RESULTS

Table (1): Statistical analytical results of chemical analysis of raw milk used in yoghurt manufacturing (number of examined samples 30).

Chemical aspects	Fat%	Protein%	S.N.F%	Moisture%	P^H value	Acidity%	Activity test
Min	3	3	8.25	86.25	6.31	0.120	negative
Max	4.4	3.48	9.17	87.90	6.75	0.175	negative
Mean ±SE	3.68±0.07	3.36±0.02	8.86±0.05	86.85±0.10	6.51±0.03	0.16±0.003	negative
Accepted limits	Not less than 3	Not less than 3	Not less than 8.25	Not more than 88	Not more than 6.8	Not more than 0.18	negative

Min = minimum S.E = standard error

Max = maximum S.N.F= solid not fat

Table (2): Statistical analytical results of chemical analysis of standardized pasteurized milk used in yoghurt manufacturing (number of examined samples 30).

Chemical aspects	Fat%	Protein%	S.N.F%	Moisture%	P^H value	Acidity%
Min	3.2	3.81	10.21	85.20	6.46	0.17
Max	3.8	3.99	11.80	87.00	6.69	0.20
Mean ±SE	3.48±0.04	3.91±0.01	10.80±0.08	86.20±0.11	6.57 ±0.01	0.18±0.002
Accepted limits	Not less than 3	Not less than 3	Not less than 8.25	Not more than 88	Not more than 6.8	Not more than 0.18

Table (3): Statistical analytical results of chemical analysis of final product (yoghurt) (number of examined samples 30).

Chemical aspects	Fat%	Protein%	S.N.F%	Moisture%	P^H value	Acidity%
Min	3.3	3.78	10.20	84.80	4.4	0.94
Max	3.8	4.00	11.40	85.90	4.7	1.43
Mean ±SE	3.52±0.03	3.90±0.01	10.57±0.05	85.35 ±0.8	4.56±0.02	1.21±0.03
Accepted limits	Not less than 3	Not less than 3	Not less than 8.25	Not more than 86	Not more than 4.6	Not more than 1.5

Table (4): Statistical analytical results of microbiological examination of raw milk used in yoghurt manufacturing (number of examined samples = 30).

Microbiological aspects cfu / ml	Accepted limits	Positive samples		Exceeded samples		Min	Max	Mean± S. E
		No	%	No	%			
Total bacterial count	Not more than 10^6 cfu / ml	30	100	3	10	$8x10^5$	$2x10^6$	$2.9x10^5 \pm 8.1x10^4$
Staph aureus count	Not more than 10^2 cfu / ml	30	100	9	30	2x10	$7x10^2$	$1.7x10^2 \pm 3.5x10$
Mould count	not defined	18	60	0	0	4x10	$3x10^2$	$1.3x10^2 \pm 1.1$ x10
Coliforms count	not defined	30	100	0	0	1.5x10	$4.8x10^4$	$5.4x10^2 \pm 1.9$ x10^2
Bacillus cereus count	not more than 1 cfu / ml	0	0	0	0	0	0	0
Listeria monocytogenes	absent	0	0	0	0	0	0	0
Clostridia perfringens count	not more than 1 cfu / ml	0	0	0	0	0	0	0
Salmonella	absent	0	0	0	0	0	0	0
E . coli count	absent	0	0	0	0	0	0	0

No = number % = percentage

Table (5): Statistical analytical results of microbiological examination of standardized pasteurized milk used in yoghurt manufacturing (number of examined samples = 30).

Microbiological aspects cfu / ml	Accepted limits	Positive samples		Exceeded samples		Min	Max	Mean± S. E
		No	%	No	%			
Total bacterial count	not defined	24	80	0	0	$5x10^2$	$4x10^3$	$1.7x10^3 \pm 1.5x10^2$
Staph aureus count	absent	0	0	0	0	0	0	0
Mould count	absent	0	0	0	0	0	0	0
Coliforms count	absent	0	0	0	0	0	0	0
Bacillus cereus count	absent	0	0	0	0	0	0	0
Listeria monocytogenes	absent	0	0	0	0	0	0	0
Clostridia perfringens count	absent	0	0	0	0	0	0	0
Salmonella	absent	0	0	0	0	0	0	0
E. coli count	absent	0	0	0	0	0	0	0

Table (6): Statistical analytical results of microbiological examination of yoghurt (final product) (number of examined samples = 30).

Microbiological aspects cfu / ml	Accepted limits	Positive samples		Exceeded samples		Min	Max	Mean± S. E
		No	%	No	%			
Total bacterial count	not defined	NE	NE	NE	NE	NE	NE	NE
Staph aureus count	absent	0	0	0	0	0	0	0
Mould count	not more than 10 cfu / ml	15	50	3	10	1x10	4x10	1.46x10± 0.17 x10
Coliforms count	not more than 10 cfu / ml	6	20	3	10	0.4x10	4.3x10	2.17x10± 0.32 x10
Bacillus cereus count	absent	0	0	0	0	0	0	0
Listeria monocytogenes	absent	0	0	0	0	0	0	0
Clostridia perfringens count	absent	0	0	0	0	0	0	0
Salmonella	absent	0	0	0	0	0	0	0
E . coli count	absent	0	0	0	0	0	0	0

NE = not examined

Table (7): Statistical analytical results of sensory examination of final product (yoghurt) (number of examined samples = 30).

Sensory trait	Score	No.of sample	%
1- Flavor			
A-Normal	10	30	100
B-Slight acetaldehyde	9	0	0
C-Definite acetaldehyde	7	0	0
2- Body and texture			
A-Normal	5	30	100
B-Slight too thin	4	0	0
C-Definite too thin	3	0	0
D-Slight too firm	4	0	0
E-Definite too firm	3	0	0
3- Appearance			
A-Normal	5	27	90
B-Slight wheyed off	4	1	3.3
C-Definite wheyed off	3	0	0
D-Slight gel like	4	0	0
E-Definite gel like	3	0	0

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التقييم الحسى والكيميائى والبكتيريولوجى للزبادى اثناء مراحل تصنيعه

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معهد بحوث صحة الحيوان – المعمل الفرعى بالإسكندرية **

تم تجميع 90 عينة من احد مصانع الالبان بمحافظة كفر الشيخ اثناء خطوات التصنيع الخاصة بمنتج الزبادى تمثل 30 عينة لكل من اللبن الخام ، اللبن المبستر المعدل والمنتج النهائى وذلك لتقييم المنتج خلال مراحل تصنيعه من الناحية الحسية ، الكيميائية والبكتيريولوجية وأسفرت النتائج عن الأتي:-

- بالنسبة لعينات اللبن الخام كانت جميع الخصائص الكيميائية (الدهن، البروتين، المادة الصلبة غير الدهنية ، محتوى الرطوبة ، درجة الاس الهيدروجينى، نسبة الحموضة واختبار الكشف عن المضادات الحيوية) داخل الحدود المسموح بها طبقا للمواصفة القياسية المصرية رقم (154 – 1 / 2005) الخاصة باللبن الخام وكذلك الفحوصات الميكروبيولوجية (العد البكتيرى الكلى ، العنقودى الذهبى ، القطريات ، الكوليفورم ، الباسيلس سيرس ، الليستريا مونوسيتوجينز ، الكلوستريديا بيرفرينجينز ، السالمونيللا والايشيريشيا كولاى) داخل الحدود ماعدا 3 (10 %) و 9 (30 %) من العينات لاحتوائها على الميكروبات الكلية والعنقودى الذهبى على التوالى اكثر من الحدود المسموحة .

- بالنسبة لعينات اللبن المعدل المبستر كانت هناك اختلافات غير جوهرية للخصائص الكيميائية عن تلك الخاصة لعينات اللبن الخام . اما بالنسبة للفحوصات الميكروبيولوجية فقد اتضح ازالة لكل الميكروبات نتيجة لحرارة البسترة ماعدا تلك المقاومة لحرارة البسترة .

- اما بالنسبة للمنتج النهائى كانت الخصائص الكيميائية (الدهن ، البروتين ، المادة الصلبة غير الدهنية ، محتوى الرطوبة ، درجة الاس الهيدروجينى، نسبة الحموضة) داخل الحدود المسموح بها طبقا للمواصفة القياسية المصرية رقم (1000 / 2005) الخاصة بالزبادى وكذلك الفحوصات الميكروبيولوجية (العد العنقودى الذهبى ، الفطريات ، الكوليفورم ، الباسيلس سيرس ، الليستريا مونوسيتوجينز ، الكلوستريديا بيرفرينجينز ، السالمونيللا والايشيريشيا كولاى) داخل الحدود ماعدا 3 (10 %) من العينات لاحتوائها على فطريات وكوليفورم اكثر من الحدود المسموحة ، كذلك كانت جميع العينات مطابقة لتلك المواصفة ماعدا عينة واحدة نتيجة لعيوب فى القوام نتيجة لفصل مصل اللبن .

- وقد تم مناقشة النقاط اللازمة لتحسين جودة وسلامة المنتج .