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Psychrotrophic count influence on oxidative stability and aflatoxins in milk and cooking butter

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Abstract

Milk and butter are among the precious foods susceptible to spoilage and rancidity due to psychrotrophic microorganisms' activities, which grow in abundance due to the richness of milk and butter in the nutrients and their ability to resist the cold environment milk and butter are stored. In this study, the total psychrotrophic bacterial and fungal counts were recorded. The rancidity represented by the Thiobarbituric acid reactive substances value and aflatoxins B1 and M1 levels were also measured by high-performance liquid chromatography. The results reflected a strong correlation between the total number of psychrotrophic bacteria, the rate of rancidity and the total number of molds, and the levels of the aflatoxins in the milk and butter. In conclusion, the psychrotrophic bacterial and mold counts in the milk and butter must be monitored carefully and be added as a routine examination to the list of the butter examinations.

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Introduction

Milk is of particular significance for human nutrition as the most nearly complete single food, but milk is also essential as the raw material from which a variety of nutritious products is established to satisfy all tastes. These products may contain all or only some of the nutrients present in the milk, but each product can make a nutritionally significant contribution to our diets (1). The butter is obtained from the fresh cream or milk using churning. The churning action causes the fat-in-water emulsion of cream to break down and change into the water-in fat emulsion of butter. Butter's nutritional value depends almost entirely on the total content of fat and fat-soluble vitamins, particularly carotene and retinol. Butter is an energy-producing food and yields about 730 Kcal per 100 gm, and it is also an excellent source of vitamin A; 14gm of butter supplies a child with about one-third of his daily requirements of this vitamin (2). Butter is responsible for forming the excellent aroma of the food during cooking (2). Comparable to all precious foods, butter is subject to be spoiled easily by chemical rancidity and microbial activity (3,4).

In order to slow the chemical rancidity, the butter is stored in cold temperatures. However, Psychrotrophes; can flourish and grow well during the extended period of storage in cold temperature, producing a variety of off-flavors and rancidity, and physical defects (2). These microorganisms played a role in deteriorating the manufactured products of milk through the production of proteolytic or lipolytic enzymes during their growth. Some enzymes are heat-stable and can withstand milk processing temperature, decreasing quality and keeping the quality of milk and milk products (1).

Furthermore, molds and yeasts also are growing over an extensive range of temperatures (1-3). Therefore, these organisms can be present on all food at almost any temperature under which food is held. The storage of food products for an extended period at refrigeration temperature has resulted in new quality problems for the food industry. These problems are related to the growth and metabolic

activities of psychrotrophic yeasts and molds at low temperatures and the secretion of mycotoxins which are considered of significant human health hazard (4). Several studies have been reported the production of volatile organic compounds (VOCs) leading to spoilage of dairy products due to the activity of psychrotrophic (4). These organisms are more or less abundant in nature and are the most common cause of spoilage of refrigerated fatty dairy products. Hydrolytic oxidation in butter occurs due to the lipolytic enzymes of psychrotrophes during the cold storage of butter, leading to rancidity and reduction in the butter quality; undesirable flavors, rancid odors, unpleasant taste resulting in shortening of shelf life and decreasing their nutritional quality (2). Aging, inflammatory bowel disease, cell membrane damage, heart diseases, and various malignancies are all linked to lipid oxidation, too (2,4). Heat, oxygen, light, and some metals, particularly iron and copper, all contribute to the production of butter oxidation (3).

Aflatoxins (AF) are mold byproducts that contaminate food and milk. Aflatoxin M1 (AFM1) is metabolized from aflatoxin B1 (AFB1) by the cytochrome P450 enzyme in the liver. AFB1 and AFM1 are both hepatotoxic and carcinogenic. Since pasteurization or processing of dairy derivatives cannot destroy or remove AFs from milk, it has been proven that they can originate and progress liver, lung, and colon cancer (1,2).

This work was planned to achieve enumeration of total psychrotrophic bacteria and yeast and mold count in the market milk and cooking butter, estimation of the oxidative stability, and aflatoxins B1 and M1 in the cooked butter.

Materials and methods

Samples collection

A total of 140 random samples of market milk and cooking butter (n=105 for milk and n= 35 for butter) were collected from the local markets in Assiut City (27°11′N 31°10′E), Egypt.

Total psychrotrophic bacterial count

The determination of total psychrotrophic bacterial count was carried out according to Al-Rudha (5). Briefly, for each sample, one mL of the prepared peptone serial dilutions was transferred into petri dishes in duplicate and was carefully mixed with 15 mL of melted and tempered 45.0°C standard plate count agar (Oxoid, UK). After solidification, the inoculated plates were incubated at seven °C for ten days. After that, the plates showing 30-300 colonies were counted, and the colony-forming units (CFU) of the psychrotrophic bacterial count were calculated in mL or g of an examined sample.

Total psychotropic yeasts and molds count

To detect the psychotropic yeasts and molds, count Yassein and Zghair (6) instruction was followed. One mL of each sample's previously prepared serial dilutions was transferred into petri dishes in duplicate and then carefully mixed with10-15 mL of melted and tempered at 45°C malt extract agar (Oxoid, UK) (containing 500 mg each of chlortetracycline HCL and chloramphenicol). After media solidification, the inoculated plates were incubated at 7 °C for ten days. Psychrotrophic yeasts and molds CFU/mL or g of examined samples were calculated and recorded.

Oxidative stability estimation

The butter samples of the higher psychrotrophic bacterial counts were further examined with the oxidative stability test after seven days of cold storage. The oxidative reaction in the butter was determined based on the level of thiobarbituric acid reactive substances (TBARS), according to Zhang *et al.* (7). The extraction of butter serum was performed according to AOAC (8). The butter samples of 100 g were melted in a water bath at 40-50°C until water and oil separated, the clear supernatant was filtered and used in the measuring. TBARS determination was carried out as follows: 5 mL TBA Reagent (0.02 M2-thiobarbituric acid in 90% glacial acetic acid was added to the prepared, then 1 g of the extracted butter oil sample was added to 5 mL of TBA reagent. Then, the mixture was immersed in a boiling water bath (A1102, USA) for 35 min. The TBAR blank reagent blank was also prepared. A portion was transferred to a cuvette, and the optical density was recorded and determined against the blank at a wavelength of 538 nm using a spectrophotometer (YCW-04M, USA).

Aflatoxins detection

The Aflatoxins B1 (AFB1) and M1 (AFM1) were detected in the butter samples revealed the high mold counts by HPLC technique according to Saud and AL-Zuhariy (9).

Results

Total psychrotrophic bacterial count

The total psychrotrophic bacterial count in milk samples from markets, street vendors, dairy farms, and cooking butter samples were recorded in table 1. This revealed a 100% contamination of all samples by psychrotrophic bacteria. The TPBC ranged from $1.25x10^2$ to $6.20×10^5$ CFU/mL in different milk samples. While butter samples were shown, a count ranged between $3.30×10^2$ and $9.80×10^5$ CFU/g.

Total psychrotrophic yeasts count

The yeast was contaminated (83: 88.6%), while 100% of butter samples were contaminated (Table 2.).

Total psychrotrophic mold count

The milk samples from the street vendors showed the highest percent and counts of mold contamination (100% and $1.00×10^4$ CFU/mL), respectively (Table 3).

Table 1: Total psychrotrophic bacterial count (CFU/mL or g) in examined samples

Examined samples	No. examined	No. +ve (%)	Total psychrotrophic bacterial count (CFU/mL or g)		
			minimal	maximum	mean
Market milk	35	35(100)	1.25×10^2	3.04×10^6	5.20×10^5
Street vendor milk	35	35(100)	2.00×10^3	4.20×10^6	7.50×10^5
Dairy farm milk	35	35(100)	2.80×10^3	6.20×10^5	1.17×10^5
Cooking butter	35	35(100)	3.30×10^2	9.80×10^5	9.30×10^4

Table 2: Total psychrotrophic yeasts count (CFU/mL or g) in examined samples

Examined samples	No. examined	No. +ve (%)	Total psychrotrophic yeasts count (CFU/mL or g)		
			minimal	maximum	mean
Market milk	35	29(83)	10	1.00×10^3	0.75×10^2
Street vendor milk	35	31(88.6)	10	2.00×0^3	2.06×10^2
Dairy farm milk	35	29(83)	2	2.00×10^2	0.28×10^2
Cooking butter	35	35(100)	10	5.30×10^3	5.74×10^2

Table 3: Total psychrotrophic mold count (CFU/mL or g) in examined samples

Examined samples	No. examined	No. +ve (%)	Total psychrotrophic mold count (CFU/mL or g)		
			minimal	maximum	mean
Market milk	35	32(91.4)	10	2.00×10^2	0.35×10^2
Street vendor milk	35	35(100)	10	1.00×10^4	1.50×10^3
Dairy farm milk	35	33(94.3)	2	3.00×10^2	0.36×10^2
Cooking butter	35	35(100)	10	6.00×10^2	1.33×10^2

Oxidative stability

Figure 1 reflects the MDA values as an oxidation indicator in the butter samples. The MDA and oxidation are higher in butter samples of high TPBC than those of lower counts.

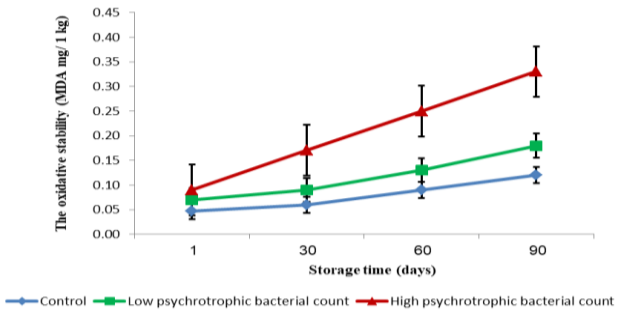

Figure 1: The oxidative stability of MDA mg/1 kg in examined butter samples.

Aflatoxins levels

Both AFB1 and AFM1 were measured in the butter samples. The results in Tables 4 and 5 revealed that the higher the TMC, the higher the AFB1 0.37 ppb and AFM1 0.091ppb.

Table 4: Aflatoxin B1 concentration (ppb) in examined butter samples

Examined samples	No. examined	No. +ve (%)	No. exceeded ECR(%)	Aflatoxin B1 concentration (ppb)		
				minimal	maximum	mean
Market milk	5	1 (20)	0 (00)	0.003	0.04	0.0112± 0.016
Street vendor milk	10	5 (50)	1 (10)	0.070	0.15	0.0920± 0.025
Cooking butter	10	7 (70)	5 (50)	0.180	0.37	0.2240± 0.059

Table 5: Aflatoxin M1 concentration (ppb) in examined butter samples

Examined samples	No. examined	No. +ve (%)	No. exceeded ECR(%)	Aflatoxin B1 concentration (ppb)		
				minimal	maximum	mean
Market milk	5	0 (00)	0 (00)	0.000	0.000	0.000± 0.000
Street vendor milk	10	3 (30)	0 (00)	0.021	0.024	0.0218± 0.001
Cooking butter	10	5 (50)	4 (40)	0.043	0.091	0.0618± 0.019

Discussion

The mean value of total psychrotrophic bacterial count (TPBC) in the examined milk was 5.20×10^5 CFU/mL with a minimum of 1.25×10^2 CFU/mL and a maximum of 3.04×10^6 CFU/mL. On the other hand, lower values were reported by Elshaghabee *et al.* (10); Júnior *et al.* (11). These variations may be due to differences in climatic, hygienic conditions, and examination.methods Spoilage of the milk by psychrotrophic microorganisms is usually proteolytic and lipolytic and involves various off-tastes, clot-formation, and, in some cases, virtually completes digestion of the protein. The average value of TPBC in the examined milk was 7.5×10^5 CFU/mL with a minimum of 2.0×10^3 CFU/mL and a maximum of 4.2×10^6 CFU/mL. Lower values were reported by Cebeci (12). The average value of TPBC in the examined milk samples was 1.17×10^5 CFU/mL with a minimum of $2.8x10^3$ CFU/mL and a maximum of 6.20×105 CFU/mL. Higher values were reported by Xin *et al.* (13), while nearly similar results were obtained by Mcphee and Griffiths (14), Cempírková, and Mikulová (15). The TPBC per g of butter samples varied from 3.3×10^2 CFU/g to 9.8×10^5 CFU/g with a mean value of 9.3×10^4 CFU/g nearly similar levels of psychrotrophic counts was reported by Ahmed *et al*. (16), Meshref (17). The variation observed in the TPBC in the milk and butter samples may be due to several factors such as the raw milk quality, the hygienic conditions of the animal, farm, dairy plant, and equipment involved in the production and manufacturing of the milk and butter .

The average count of psychrotrophic yeast in the positive samples of the examined milk was 0.75×10^2 CFU/mL with a minimum of 1.00×10^1 CFU/mL and a maximum of 1.00×10^3 CFU/mL. Psychrotrophic yeasts constituted 39.06% of the total mesophilic yeasts examined in positive samples. Lower values were obtained by El-Shinawy (18). Psychrotrophic fungi (yeasts and molds) can present problems, have been found in milk and dairy products, such degradation of milk components leads to off-flavor and odors that may cause the raw milk to be unfit for processing or consumption as fluid milk, also may not be suitable for other dairy products (19).

The average count of psychrotrophic yeast in the positive samples of the examined milk was 2.06×10^2 CFU/ml with a minimum of 1.00×10^1 CFU/mL and a maximum of 2.00×10^3 CFU/mL. Psychrotrophic yeasts constituted 5.07% of the total mesophilic yeasts examined in positive samples. Higher values were obtained by Korashy and Wahbba (19). The average count of psychrotrophic yeasts in the positive samples of the examined farm's milk was 0.28×10^2 CFU/mL with a minimum of 2.00 CFU/mL a maximum of 2.00×10^2 CFU/mL. Psychrotrophic yeasts constituted 39.09% of the total mesophilic yeasts examined in positive samples. Higher values were obtained by Torkar and Teger (20). The average counts of psychrotrophic yeasts in the positive samples of the examined cooking butter samples were 5.74×10^2 CFU/g with a minimum of 1.00×10^1 CFU/g and a maximum of 5.30×10^3 CFU/g. Compared with other studies, higher counts of psychrotrophic yeasts in cooking butter were detected by Sagdic *et al.* (21). The mean value of total psychrotrophic molds count (TMC) in the positive samples of the examined milk was 0.35×10^2/mL with a minimum of 0.10×10^2 CFU/mL and a maximum of 2.00×10^2 CFU/mL higher values were obtained by El-Shinawy *et al.* (18).

The average count of psychrotrophic mold in the positive samples of the examined farm's milk was 1.5×10^3 CFU/mL with a minimum of 0.1×10^2 CFU/mL and a maximum of 1.0×10^4 CFU/mL. Psychrotrophic molds produce proteolytic and lipolytic enzymes even at low temperatures that change the composition, leading to releasing of mycotoxins which are difficult to destroy during milk processing (22). The average count of psychrotrophic mold in the positive samples of the examined farm's milk was 0.36×10^2 CFU/mL with a minimum of 0.02×10^2 CFU/mL and a maximum of 3.00×10^2 CFU/mL higher values were obtained by Júnior *et al.* (11). The average count of psychrotrophic molds in the positive samples of the examined cooking butter samples was 1.33×10^2 CFU/g with a minimum of 1.00×10^2 CFU/g and a maximum of 6.00×10^2 CFU/g.

It was noticed that the examined cooking butter samples had high mold counts, either mesophilic (results not involved) or psychrotrophic, that may be attributed to the neglected hygienic measures during manufacture. The mycological contamination of the cooking butter could be attributed to the fact that it is usually made from raw cream and the primitive way of processing, handling, storage, and marketing. Therefore, butter should not be manufactured from raw cream or, if it is, it should be used only for cooking where it will receive adequate heat treatment as recommended by Meshref (17). Generally, the microflora of butter reflects the quality of the cream, the sanitary conditions of the equipment used to manufacture the butter, and the environmental and sanitary conditions during the packaging and handling of such product (23).

The rancidity or oxidation is the most significant problem in the fatty foods industry. Rancidity leads to a decrease in the quality and shelf-life of butter, resulting in several chemical components of health hazards. The peroxides produced from the oxidation of unsaturated fatty acids lead to rancid flavor. In this study, the rancidity or oxidation in the cooking butter samples spectrophotometrically, by TBARS kits. TBARS is a marker for lipid oxidation. TBARS value exposes the content of malondialdehyde (MDA) mg present in 1 kg of sample. MDA is a secondary product that develops from the lipid oxidation of polyunsaturated fatty acids with more than two double bonds (24).

Increase in MDA content in the butter samples contaminated by the highest levels of psychrotrophes than the control 0.09 and 0.05 mg/kg, respectively. During the cold storage, more MDA contents were detected in days 30, 60, and 90 in a significant routine. According to the lipase

enzyme secreted by such microorganisms and the water contents of butter 16%. This accelerates the hydrolysis of fat. Similar results were recorded by Wheatley (24) during an experimental work comparing the effect of packaging and light on the oxidation of butter. Abbas *et al.* (25) evaluated the oxidation of cow and buffalo butter during the cold storage and recorded TBARs values ranging from 0.08 to 0.46 mg/kg according to cold storage and animal species.

The formation of the aflatoxins in the food is mainly the responsibility of molds. Afaltoxin B1 (AFB1) is the most common type of aflatoxins in food (1). Aflatoxin M1 is the 4-hydroxylated metabolite of AFB1 in dairy foods principally due to liver detoxification towards AFB1, which reaches the animal liver through feeding on contaminated feeds with mold and AFB1. According to Ali *et al.* (26), 1-3% of totally ingested AFB1 may convert into AFM1 .

The results showed that the high mold counts in the samples led to high levels of AFB1. Among ten butter samples showing TMC from 10 to 99 CFU/mL tested for AFB1, 50% (n=5) of samples were contaminated with AFB1 ranged from 0.07 to 0.15 ppb. While the butter samples showed the highest mold count up to $6x10^2$ CFU/ml revealed higher AFB1 levels 0.18 to 0.37 ppb, exceeding the European Committee Regulations (ECR) 2 ppb in 50% of samples. These results are in parallel with Hassan *et al.* (27). These findings ensure that; the higher the mold contamination of food, the higher the chance for aflatoxins production.

Table 5 exposed the control butter samples prepared hygienically and were free from AFM1. At the same time, the samples (n=10) of high mold count were contaminated by AFM1 50% and 40% of samples exceeding the ECR 0.05 ppb. Similar percentages of 45% were detected in butter by Iqbal *et al.* (28). Similarly, Abbas *et al.* (25) detected AFM1 in 87% of the butter samples, 51% exceeding EU maximum limits. Tekinçen and Uçar (29) also recorded 28% of the butter in Turkey exceed the legal limits of the AFM1. Moreover, very high AFM1 levels were recorded>250 ng/kg in 16% of the butter samples. Lower percentages were detected by Hassan *et al.* (27) in Qatar and by Khalifa and Shatta (1) in Egypt. They detected AFM1 in 67% of butter samples. All were below the EUTL 0.05 ppb. The variations on the findings may be in relation to several factors, including; feed quality of dairy animals, feed storage and climate condition appropriate mold growth and mycotoxins secretion, milk quality, dairy processing technologies-moreover, the differences in the analytical methods and techniques Tekinçen and Uçar (29).

Conclusion

The contamination of the milk and butter by psychrotrophic bacteria and molds is significant in reducing the milk and butter quality and shelf-life through the rapid development of the rancidity and formation of the mycotoxins.

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Conflict of interest

The authors declare that there is no conflict of interest.

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مدى التأثر العددى لأليفات البرودة في الحليب والزبد على معدل التزنخ والسموم الفطرية

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الخلاصة

يعد الحليب أكمل الأغذية وأصلحها لجميع الفئات العمرية. والحليب ومشتقاته وخاصة الزبد من الأطعمة الغنية المعرضة للتلف والتزنخ السريع بسبب نشاط الكائنات الحية الدقيقة كالبكتريا والفطريات وخاصة أليفات البرودة منها، والتى تنمو بسبب احتواء الحليب والزبد على جميع العناصر الغذائية تقريبا اللازمة لنموها وتكاثرها، وكذلك قدرتها على مقاومة البيئة الباردة وانخفاض درجات الحرارة التى يتم حفظ الحليب والزبد فيها (الثلاجة). في هذه الدراسة تم تسجيل عدد البكتريا والفطريات المحبة للبرودة بدقة. كما تم أيضا قياس معدل التزنخ فى الزبد، ممثلة بقيمة المواد التفاعلية لحمض الثيوباربيتوريك وتم قياس مستويات الأفلاتوكسينات بى وام. وقد عكست النتائج النهائية وجود علاقة ارتباط قوية بين العدد الإجمالي للبكتيريا محبة البرودة ومعدل التزنخ، وكذلك بين العدد الإجمالي للفطريات والأعفان محبات البرودة وبين مستويات الأفلاتوكسين فى اللبن والزبد. وفي الختام، نوصي بوجوب مراقبة اعداد البكتيريا والأعفان أليفات البرودة فى الحليب والزبد بعناية وإضافتها كفحص أساسي إلى قائمة فحوصات الزبد الروتينية. منعا لتدهور قيمتها الغذائية أثناء التخزين والحفظ.