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# MICROBIOLOGICAL QUALITY OF COOKING BUTTER IN BENI-SUEF GOVERNORATE

(With 2 Tables)

By

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## الجودة الميكروبيولوجية لزبد الطهى فى محافظة بنى سويف

***عرفه مشرف سليمان مشرف***

أجريت هذه الدراسة على عدد 60 عينة من زبد الطهى، جمعت عشوائيا من محافظة بنى سويف وذلك لتعيين حالتها الميكروبيولوجية ومعرفة مدى سلامتها وأثرها على صحة المستهلك. وقد وجد أن 100 ، 100 ، 36.7 ، 31.7 ، 31.7 و 23.3 % من العينات المفحوصة كانت ملوثة بالبكتريا المحبة للبرودة، الفطريات والخمائر، الميكروب القولونى، الميكروب القولونى البرازى، الميكروب العصوى القولونى (الايشيريشيا كولاى) والميكروب العنقودى الذهبى المكور على التوالى. بينما لم يتم عزل ميكروب السيدوموناس ايروجينوزا من اى من عينات الزبد المفحوصة. وقد تبين من الفحص الكيميائى أن متوسط الملح كان 0.57 ± 0.05 % بينما كان متوسط نسبه الحموضة فى العينات 0.20 ± 0.013 %. هذا وقد دلت النتائج ان زبد الطهى المصنع بواسطة الفلاحين منتج تحت ظروف صحية غيرسليمة. كما تم مناقشة الأهمية الصحية لزبد الطهى والشروط الصحية الواجب توافرها لتحسين جودة هذا المنتج.

## SUMMARY

A total of 60 random samples of cooking butter, were collected from different farmer's houses in different villages, Beni-Suef Governorate, Egypt. The microbiological examination revealed that 100, 100, 36.7, 31.7, 31.7 and 23.3 % of the examined samples were contaminated by psychrotrophic bacteria, Molds and Yeasts, coliforms, faecal coliforms, *E. coli* and *S. aureus*, respectively. None of the examined cooking butter samples contained Ps.aeruginosa. The means values of sodium chloride and titratable acidity were 0.57 ± 0.05 % and 0.20 ± 0.013%, respectively. The present study concluded that the cooking butter is produced under unhygienic condition and without good manufacturing practice. The Public health significance and suggestive control measures were discussed.

***Key words:*** *Butter, microorganisms, acidity, sod. chloride*

# INTRODUCTION

Cooking butter is one of the most popular types of fat consumed in Egyptian houses. It is produced in villages by rural women that are usually using their traditional knowledge during manufacturing. It is consumed as butter and used as oil for food preparation or for cooking.

Cooking butter is obtained by churning of mechanically separated cream, after storing in skin bag called "Kerba" made from a goat skin. After churning, the butter is manually worked and stored in refrigerators till sold or consumed.

Although the butter is not a highly perishable food, it does undergo spoilage by bacteria and molds. The main source of microorganisms of butter is cream, whether sweet or sour, raw or pasteurized (Jay, 1996). Yeast and molds are important spoilage microorganisms of butter and can result in surface discoloration and off-flavor. Psychrotrophic Gram negative bacteria may develop and result proteolytic and lipolytic changes (ICMSF, 2005).

Microbiological analysis of butter for specific pathogens is not considered justified and testing is restricted to potential spoilage microorganisms; together with Escherichia coli and coliform bacteria. (Varnam and Sutherland, 1994).

Likewise, many studies have been carried out in Egypt to evaluate the microbiological quality of cooking butter (Ghoneim, 1963; Khalafalla *et al.,* 1974; Bakheet, 1979; El-Essawy, 1980; Ahmed *et al.,* 1987; Henin and Kaldes, 1992 and El-Kholy, 1994). However, recent information concerning the microbiological quality of cooking butter in Beni-Suef governorate are sketchy or totally absent. Therefore, this work was planned to evaluate the rate of contamination and hygienic quality of cooking butter in Beni-Suef governorate.

# MATERIALS and METHODS

**1- Collection of samples:**

A total of 60 random samples of refrigerated cooking butter were aseptically collected from different farmer's houses in different villages, Beni-Suef Governorate, Egypt during winter period (2009). All samples were taken in sterilized bottles and transported under refrigerated condition to the laboratory. Analyses were started without any delay.

**2- Microbiological analyses:**

Samples preparation and serial dilutions were made according to IDF (1992), and then subjected for the following examinations:

a. Psychrotophic colony count was carried out using plate count agar (Difco) after incubation at 7°C ± 1°C for 10 days (Frank *et al.*, 1992).

b. Total coliforms, faecal coliforms and E.coli were estimated by a three tube most probable number (MPN) technique as described by Christen *et al.* (1992).

c. Enumeration and isolation of S.aureus, was carried out by surface plating technique onto Baird Parker agar (Oxoid) according to technique described by Flowers *et al.* (1992).

d. Enumeration and isolation of Ps.aeruginosa, was carried out on Cetrimide agar (Biolife) according to technique described by King *et al.* (1954).

e. Molds and yeasts were enumerated on Sabouraud dextrose agar after incubation at 30°C for 3 days (Tantaoui-Elaraki *et al.*, 1983).

**3- Sanitary and chemical analyses:**

a- The Titratable acidity (TA) (as lactic acid %) was measured as method described by Atherton and Newlander (1977).

b- NaCl concentration was determined using the method described by O'Connor (1995).

**4- Statistical analysis:**

SPSS pocket program for windows was used for the statistical analysis. Values of different parameters were expressed as the mean ± standard error (±S.E).

## RESULTS and DISCUSSION

**Table 1:** Statistical analytical results of different microbial groups/g of examined cooking butter samples.

| Organisms | Min. | Max. | Mean | ± S.E. |
|---|---|---|---|---|
| Psychrotrophic count | $1 \times 10^1$ | $6 \times 10^5$ | $3.5 \times 10^4$ | $1.3 \times 10^4$ |
| Total coliforms | <3 | $1.5 \times 10^4$ | $8.9 \times 10^2$ | $4.05 \times 10^2$ |
| Faecal coliforms | <3 | $1.5 \times 10^4$ | $6.33 \times 10^2$ | $3.28 \times 10^2$ |
| E.coli | <3 | $1.5 \times 10^4$ | $3.16 \times 10^2$ | $2.52 \times 10^2$ |
| S.aureus | <100* | $3 \times 10^3$ | $1.55 \times 10^2$ | $6.6 \times 10^1$ |
| Ps.aeruginosa | <100* | <100* | 0 | 0 |
| Molds & Yeasts | $4 \times 10^1$ | $3 \times 10^4$ | $6.3 \times 10^3$ | $1.07 \times 10^3$ |

* Not detectable colonies on the plates

**Table 2:** Frequency distribution of different microbial groups/g of examined cooking butter samples.

| Intervals | Psychrot-rophic | | Total coliforms | | Faecal coliforms | | E.coli | | S.aureus | | Molds &Yeasts | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % | No | % |
| <3 | - | - | 38 | 63.33 | 41 | 68.3 | 41 | 68.3 | - | - | - | - |
| 3- $<10^1$ | - | - | 5 | 8.33 | 6 | 10 | 7 | 11.6 | - | - | - | - |
| $10^1$- $<10^2$ | 6 | 10 | 7 | 11.67 | 4 | 6.7 | 6 | 10 | 46 | 76.7 | 2 | 3.3 |
| $10^2$- $<10^3$ | 18 | 30 | 5 | 8.33 | 5 | 8.3 | 4 | 6.7 | 11 | 18.3 | 12 | 20 |
| $10^3$- $<10^4$ | 16 | 26.7 | 3 | 5 | 3 | 5 | 1 | 1.7 | 3 | 5 | 34 | 56.7 |
| $10^4$- $<10^5$ | 13 | 21.7 | 2 | 3.33 | 1 | 1.7 | 1 | 1.7 | - | - | 12 | 20 |
| $10^5$- $<10^6$ | 7 | 11.6 | - | - | - | - | - | | - | - | - | - |
| Total | 60 | 100 | 60 | 100 | 60 | 100 | 60 | 100 | 60 | 100 | 60 | 100 |

**Table 3:** Statistical analytical results of Titratable acidity and NaCl% of examined cooking butter samples.

| Test | Min. | Max. | Mean | ± S.E. |
|---|---|---|---|---|
| Acidity % | 0.04 | 0.55 | 0.20 | 0.013 |
| NaCl % | 0.10 | 1.8 | 0.57 | 0.05 |

The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used to manufacture the butter and the environmental and sanitary conditions during packaging and handling of such product (Richter *et al.*, 1992).

Results given in Tables 1&2 show that the psychrotrophic bacteria could be detected in 100 % of the examined cooking butter samples with a mean count of $3.5x\ 10^4 \pm 1.3 \times 10^4$ cfu/g. In previous studies, nearly similar level of psychotropic counts were reported by Ahmed *et al.* (1987) and Henin and Kaldes (1992) with mean values of $3.06 \times 10^4$ and $3.01x\ 10^4$ cfu/g, respectively. On the contrary, none of the examined cooking butter samples contained detectable level of psychrotrophic bacteria (<10/g) was reported by El-Sherief (2007).

Psychrotrophic Gram negative bacteria such as *Pseudomonas* spp. and *Flavobacterium* spp. may develop and cause off-odour formation and rancidity. Growth of Alteromonas putrefaciens or Flavobacterium malodoris may lead to surface taints very quickly affecting the mass of the product and accompanied by development of a putrid, decomposed or cheesy flavour that render the product unmarketable, leading to economic losses (ICMSF, 2005).

Spoilage of butter may result from the presence of heat resistant proteases and lipases produced by psychrotrophic bacteria during storage of raw milk or cream even after death of spoilage organisms (Kornacki *et al.*, 2001).

Total coliforms and faecal coliforms were detected in 36.7and 31.7 % of the examined butter samples with mean values of $8.9 \times 10^2 \pm 4.05 \times 10^2$ and $6.33 \times 10^2 \pm 3.28 \times 10^2$ cells/g (MPN) respectively (Tables 1&2).

Many reports dealing with occurrence of coliforms in butter have been accumulated. In those studies, various rates of coliforms were obtained as 35, 67, 82, 77.5, 72, 100 and 66.7 % of examined cooking butter samples by Ghoneim (1963); Bakheet (1979); El-Essawy (1980); Ahmed *et al.* (1987); Henin and Kaldes (1992); El-Kholy (1994) and Karagozlu and Ergonul (2008), respectively.

According to Egyptian standards (1992), total coliforms and faecal coliforms counts of butter should not exceed the limit 10/g and standards suggested by Robinson (2002) should be < 10 /g, 17 (28.3%) out of 60 samples were found to be highly contaminated with coliforms, whereas 13 (21.7%) out of 60 samples were found to be highly contaminated with faecal coliforms over this limit. The presence of coliforms in butter is an indicative of poor hygiene and potential risk of food poisoning (Wilbey, 2002).

*E. coli* is an indicator of faecal contamination and the possibility of the presence of enteric pathogens. *E.coli* was found in 19 (31.7%) out of 60 examined cooking butter samples. Moreover, the mean count of E.coli was $3.16 \times 10^2 \pm 2.52 \times 10^2$ organisms/g (MPN) (Tables 1&2). On the contrary, higher incidence rates were reported by Ahmed *et al.* (1987); Henin and Kaldes (1992) and Karagozlu and Ergonul (2008) as they found 55, 56 and 64.4 % of the samples were contaminated with *E. coli*, respectively. All positive samples do not comply with both Egyptian standards and standards suggested by Robinson (2002), since both recommended the freedom of 1g of butter from *E.coli.*

In the present study, 14 samples yielded *S. aureus* ranging from $10^2$ to $3 \times 10^3$ cfu/g and 23.3. % of the samples do not comply with the Egyptian standards and standard suggested by Robinson (2002) of freedom of 1 g of butter from S.aureus (Tables 1&2). The detection rate of S.aureus was not in agreement with the result of Karagozlu and Ergonul (2008) who reported no S.aureus in their butter samples. The presence of *S. aureus* in cooking butter may be from an endogenous source, i.e. using raw cream for manufacture of butter or from an exogenous source, i.e. a result of handling and inadequate personnel hygiene of farmer's wife.

Neither the absence of S.aureus nor the presence of small numbers of organism can provide complete assurance that the butter is

safe, since conditions inimical to the survival of *S. aureus* may result in a diminished population or death of viable microbial cells, while sufficient toxins remains to elicit symptoms of staphylococcal food poisoning (Bennett and Monday, 2003).

None of the examined cooking butter samples contained Ps.aeruginosa (Table 1). This finding is completely in agreement with those of Nasr *et al.* (1992), who stated that this may be due to the effect of high acidity and salt content.

It could be seen from Tables 1&2 that 100 % of the examined cooking butter samples contained Molds and Yeasts with a mean count of $6.3x\ 10^3 \pm 1.07x10^3$ cfu/g. The examined butter is considered having unacceptable hygienic quality, since all of the examined samples were above the limit of < 10 cfu/g. suggested by Robinson (2002).

Many reports are dealing with the occurrence of molds and yeasts in butter. In those studies, various rates of molds and yeasts were reported as 9.4, 50 and 92.7 % of the examined butter samples by Hankin and Hanna (1984); Paula *et al.* (1989) and Con (1990), respectively. Recent studies found that 56 & 84 % and 75 & 85 % of examined cooking butter samples were contaminated with molds and yeasts (Bahout, 2001 and El-Diasty and Salem, 2009), respectively. Several previous studies recorded higher levels of molds and yeasts contamination in butter as $5.3x10^4$, $2.7x\ 10^4$, $7.11\ x\ 10^4$ and $6.99x10^4$ by Ozalp *et al.* (1978); Ozdemir (1986); Ahmed *et al.* (1987) and Henin and Kaldes (1992), respectively.

The presence of molds and yeasts in butter are objectionable as they grow at a wide range of temperature and pH values resulting surface discolouration and off-flavour (ICMSF, 2005). Molds contamination not only cause deterioration of the foods but also adversely affect the health of humans and animals, since they produce toxic metabolites called mycotoxins (Ismail and Sabreen, 2001).

Generally, the microbial contamination of butter could be attributed to the fact that it is usually made from raw cream, in addition to the primitive way of processing, handling, storage and marketing.

On the other hand, Table 3 showed that the mean values of Titratable acidity (TA) and NaCl % were 0.20 ± 0.013 and 0.57 ± 0.05%, respectively. Similar TA was reported in earlier study on cooking butter by El-Kholy (1994). On the contrary, higher mean values of TA were showed by Sagdic *et al.* (2002) and Mourad and Nour-Eddine (2006).

The mean NaCl% in the present study was nearly similar to those reported by Sagdic *et al.* (2002) and lower than those recorded by El-Sadek *et al.* (1975); Hayaloglu (1999) and Mourad and Nour-Eddine (2006). While, El-Kholy (1994) and Samet-Bali *et al.* (2009) noted lower mean values than in this study.

According to the Egyptian Standards, all of the samples were in comply with the limit not exceed 2 % salt. Butter usually contains 1.5-2% NaCl will contain 9- 12.5 % in the aqueous phase, a concentration strongly inhibitory to most microorganisms. On the other hand, lactic acid level produced as a result of natural souring during storage of cream in Kerba may be sufficiently high to exert an inhibitory effect (Varnam and Sutherland, 1994 and Kornacki *et al.,* 2001). Likewise, Minor and Marth (1972) reported that *S. aureus* was able to grow in butter with 0- 1% salt. This can conclude that the role of NaCl% (0.57%) in this study on controlling the microbial growth in cooking butter may be questionable and the probability of growth of pathogenic microorganisms like *S. aureus* is high.

In conclusion, the current investigation has indicated that butter is produced under unhygienic condition at Beni-Suef governorate. The counts of microorganisms above the recommended criteria and the presence of pathogenic bacteria may pose a risk for public health. Therefore, there is a necessity for developing the hygienic status of locally produced butter through provision of information to rural women on good process hygiene and to consumers on how to handle their foods including correct storage to protect them from infection and to save a lot of products from being deteriorated. Also, 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.

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