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MICROBIOLOGICAL QUALITY OF COMMERCIAL MAYONNAISE SOLD IN ASSIUT CITY (With 2 Tables)

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الجودة الميكروبيولوجية للمايونيز التجاري المباع بمدينة أسيوط

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يستخدم المايونيز كثيراً هذه الأيام بمفرده أو يضاف إلى الأطعمة كأحد المشهيات بجانب الوجبات الرئيسية وذلك على نطاق واسع. لذلك أجريت هذه الدراسة وفيها تم جمع عدد ٣٠ عينة عشوائية من المايونيز التجاري المعروض للبيع والمتداول بالأسواق من العديد من المحال التجارية في مدينة أسيوط لفحصها ميكروبيولوجياً وذلك لتحديد مدى حالتها الصحية وسلامتها للمستهلك. وقد تم تقدير كلاً من aerobic plate count و psychrotrophs و *S.aureus* و anaerobes و yeasts & molds. وقد أظهرت النتائج أن متوسط العدد الكلي لكل من aerobic plate count و psychrotrophs و thermoduric enterococci و coliforms و fecal coliforms و *E.coli* و *B.cereus* 3x10⁴، 1.7x10³، 2.7x10⁴، 2.9x10³ لكل جرام من عينات المايونيز، على التوالي. ولقد تم عزل *B.cereus* من 20% بمتوسط أعداد 1x10³ لكل جرام من عينات المايونيز. وقد لوثت yeasts & molds 26.67% من العينات وذلك بمتوسط أعداد 2.2x10² لكل جرام. وبالبحث عن coliforms تبين من الفحص أن كل عينات المايونيز قد وجدت خالية من هذا الميكروب (أقل من 3 للجرام) وبالتالي لم يتم عزل fecal coliforms ومن ثم *E.coli*. كذلك *S.aureus* و anaerobes لم يتم عزلها من كل العينات. وقد ظهر من هذه النتائج أن عينات المايونيز التجاري المباع بمدينة أسيوط تعتبر جيدة من الحالة الميكروبيولوجية والجودة الصحية. ورغم إن الحمل الميكروبي تحت معدل الخطر إلا أن خطورة هذه الميكروبات مازالت موجودة ولذلك فقد تم إتاحة الفرصة لبعض الميكروبات الممرضة لكي تنمو وتتكاثر وذلك من خلال الجزء الثاني من هذه الدراسة حيث تم إجراء تجربة معملية بهدف معرفة التأثير المباشر للمايونيز على ٥ عترات لميكروبات ضارة وهي: *S.typhimurium*, *E.coli* O157:H7, *L.monocytogenes*, *S.aureus* and *B.cereus*، حيث تم حقن كل عينة مايونيز بميكروب كل على حده ثم التحضين عند درجة حرارة الغرفة حيث أنها الدرجة التي يتم توزيع وعرض وتخزين المايونيز فيها. وتم أخذ عينات بعد الحقن بمعدل عدد ساعات: ٦، ٢٤، ٤٨، ٧٢ ساعة. وقد أسفرت النتائج عن التأثير الفعال والمميت للمايونيز على الميكروبات المحقونة رغم بقاء كلا

من *L.monocytogenes* and *B.cereus* بصورة أطول نسبياً عن باقي العزلات. هذا وقد تمت مناقشة الإجراءات الصحية والشروط الواجب إتباعها لتحسين جودة المايونيز وكذلك تم مناقشة مدى خطورة هذه الميكروبات على صحة وسلامة المستهلك.

SUMMARY

A total of 30 random samples of commercial mayonnaise were collected from different retailers in Assiut city. To assess their quality, the samples were examined microbiologically for the incidence and counts of aerobic plate count (APC), psychrotrophs, thermotolerant, enterococci, coliforms, fecal coliforms, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, anaerobes and yeasts & molds. The obtained results verify that the total bacterial, psychrotrophs, thermotolerant and enterococci counts averaged 3×10^4 , 1.7×10^3 , 2.7×10^4 and 2.9×10^3 /g of the examined mayonnaise samples, respectively. *B. cereus* could be isolated from 20%, in numbers averaged 1×10^3 /g, of the examined mayonnaise samples. Yeasts & molds contaminated 26.67% of the examined mayonnaise samples and existed in numbers averaged 2.2×10^2 /g of the samples. All the examined mayonnaise samples failed to yield coliforms (less than 3/g), and therefore fecal coliforms and *E. coli* could not be recovered from all of the examined samples. Also, *S. aureus* and anaerobes could not be detected in any of mayonnaise samples examined. The results prove that the examined commercial mayonnaise samples sold in Assiut city are of quite good quality and considered as microbiologically safe products. Although, the microbial loads are below the hazard point, the health hazard of such microorganisms still exists if they are allowed to grow and multiply, and that what was studied through the second part of the present study, in which the survival and viability of *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* in commercial mayonnaise kept at room temperature were studied. Each mayonnaise sample was inoculated separately with one of the mentioned pathogenic microorganisms and then incubated at room temperature (about 25°C) as commercial mayonnaise is usually distributed, shelved and stored at this temperature. After that, the inoculated mayonnaise was sampled after 6, 24, 48 and 72 hours. The results revealed the lethal effect of mayonnaise on the inoculated microorganisms although the survival and viability of *L. monocytogenes* and *B. cereus* was relatively longer than others. Suggestive hygienic measures for improving the quality of mayonnaise and also to safeguard the consumer were discussed.

Key words: *Microbiological quality, Commercial mayonnaise, Salmonella typhimurium, Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus.*

INTRODUCTION

Mayonnaise is a remarkable stable food product because of its resistance to most microbial spoilage and its unfavorable media for growth and survival of most bacteria especially pathogens. Consequently mayonnaise is of little public health concern as its overall microbiological content is low with a very low incidence of spoilage.

The microbiological content of mayonnaise is dictated primarily by the high acetic acid concentration found in their aqueous phase. The major preservative effect is from the acetic acid content with a minor influence from salt or sugar concentration (Smittle, 1977). Erickson and Jenkins (1991) reported that commercial mayonnaise represents negligible consumer's safety risks. However, mayonnaise has been found to be associated with outbreaks of food poisoning due to salmonella and staphylococci (Radford and Board, 1993).

The nutrient content of mayonnaise is suitable for supporting growth of many spoilage microorganisms such as yeasts, few bacteria and molds (Jay, 1978), therefore, bacilli and yeasts are the organisms commonly found (Smittle, 1977). The growth of microorganisms takes place in aqueous phase of mayonnaise and causes alterations of sensory quality and changes with potentially toxic effects (Traveria, 1992).

This study was designed as an attempt to throw spotlight on the microbial aspects of commercial mayonnaise sold in Assiut city, in addition to, studying the survival of 5 strains of pathogenic microorganisms (*S. typhimurium*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *B. cereus*) that were inoculated separately in commercial mayonnaise samples and kept at room temperature.

MATERIALS and METHODS

Collection of samples:

A total of 30 random samples of commercial mayonnaise were collected from different retailers in Assiut city. All samples were dispatched directly to the laboratory under strict hygienic measures with a minimum of delay.

Preparation of samples:

The samples were opened aseptically and 10 g of each sample were aseptically weighed and added to 90 ml of 0.1% sterile peptone water and homogenized for 2 minutes to obtain a dilution of 10^{-1} (APHA, 1992), then decimal dilutions were made and followed by microbiological examination.

Microbiological examination:

- 1- APC, (APHA, 1992).
- 2- Psychrotrophic count, (APHA, 1985).
- 3- Thermotrophic count, (APHA, 1985).
- 4- Enterococci count, (Deibel and Hartman, 1982).
- 5- Coliforms count (MPN/g), (AOAC, 1980).
- 6- Enumeration of fecal coliforms (MPN/g), (AOAC, 1980).
- 7- Enumeration of *E. coli* (MPN/g), (AOAC, 1980).
- 8- Enumeration and isolation of *B. cereus*, (Kim and Goepfert, 1971) followed by:
 - a) Nitrate reduction test, (Speck, 1976).
 - b) Gelatin liquefaction test, (Cowan and Steel, 1974).
 - c) Voges-Proskauer test, (Speck, 1976).
- 9- Isolation of *S. aureus*, (Finegold and Martin, 1982) followed by:
 - a) Gram staining, (Speck, 1976).
 - b) Anaerobic mannitol fermentation, (Baired-Parker, 1962).
 - c) Coagulase test, (Cruickshank *et al.*, 1969).
- 10- Detection of anaerobes by stormy fermentation test, (Cruickshank *et al.*, 1969).
- 11- Yeasts & molds count, (Harrigan and McCance, 1976).

Strains used and inoculation technique:

Five pathogenic strains were used individually as an inoculum involving *S. typhimurium* NC000074 that was obtained from National Collection of Type Cultures, London; *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *B. cereus* that were obtained from Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt. Each strain was grown individually in 100 ml sterile nutrient broth, at 37°C for 24 hours. Serial dilutions were done for counting cfu/ml. One ml of the inoculum of known cfu/ml was added aseptically to mayonnaise sample under a static hood to obtain a concentration of 7×10^3 , 1×10^4 , 3×10^4 , 1×10^4 and 4×10^5 cfu/g of mayonnaise for each microorganism, respectively. The inoculated sample was thoroughly mixed for 2-3 minutes with sterile spoon for

uniform distribution and then corked effectively with metal-screw caps and incubated at room temperature (about 25°C) and sampled periodically after 6, 24, 48 and 72 hours.

pH measurement:

pH was measured using a digital laboratory pH meter (model 701A / digital ionalyzer, Orion).

RESULTS

Table 1: Microbial counts of the examined commercial mayonnaise samples.

Microbiological examination	Positive samples		Counts/g		
	No./30	%	Minimum	Maximum	Average
APC	22	73.33	$<10^{2*}$	3.2×10^5	3×10^4
Psychrotrophic count	6	20	$<10^{2*}$	3×10^4	1.7×10^5
Thermotrophic count	26	86.67	$<10^{2*}$	2.3×10^5	2.7×10^4
Enterococci count	2	6.67	$<10^{2*}$	8×10^4	2.9×10^3
<i>B. cereus</i> count	6	20	$<10^{2*}$	1×10^4	1×10^3
Yeasts & molds count	8	26.67	$<10^{1*}$	3.5×10^3	2.2×10^2

*No colonies could be counted on the plates.

Table 2: Behavior of some pathogenic microorganisms in commercial mayonnaise samples (cfu/ml or g).

Time (hour)	<i>S.typhimurium</i>	<i>E.coli</i> O157:H7	<i>L.monocytogenes</i>	<i>S.aureus</i>	<i>B.cereus</i>
Inoculum	7×10^3	1×10^1	3×10^4	1×10^4	4×10^5
6 hours	-	-	3×10^3	-	6×10^2
24 hours	-	-	1×10^3	-	5×10^2
48 hours	-	-	-	-	-

DISCUSSION

The summarized results presented in Table 1 pinpoint that 73.33% of the examined commercial mayonnaise samples had countable numbers of total bacteria, ranged from $<10^2$ to 3.2×10^5 with an average of 3×10^4 /g. Comparatively, lower counts were obtained by Fabian and Wethington (1950), who mentioned that the microbial count of mayonnaise was very low (<10 /g). It is worth to mention that higher total bacterial counts in the examined mayonnaise samples did not prove the presence of pathogens but they reflect the unhygienic

status of such product during processing, post manufacturing or during handling. It may also occur through using of raw and cracked eggs (Palmu, 1992 and Radford and Board, 1993).

The results obtained from the examined mayonnaise samples in Table 1 revealed that 20% had numbers of psychrotrophs averaged $1.7 \times 10^3/\text{g}$. It is precisely evident from these results that the high incidence and counts of psychrotrophic bacteria could be attributed to ineffective processing and sanitizing methods, as well as use of unpasteurized eggs. Most of psychrotrophic bacteria are destroyed by a mild heat treatment, however, their presence implies post processing contamination and existence of heat resistant types such as *Bacillus* and *Clostridium* (Bhadsavle *et al.*, 1972). Generally, in foods that are kept refrigerated till reach consumers or served, large numbers of psychrotrophic bacteria may indicate a history of unsanitary handling. Furthermore, some species of psychrotrophic bacteria are heat resistant or spore forming which cause problems in heat-treated products, as they produce heat resistant proteolytic and lipolytic enzymes that may cause spoilage of refrigerated products (Stevenson and Rowe, 1994 and Celestino *et al.*, 1996).

From the aforementioned results in Table 1, it was noticed that high percentage of mayonnaise samples (86.67%) contained thermotolerant with an average of $2.7 \times 10^4/\text{g}$. Also, enterococci as recorded in Table 1 existed in 6.67% of the examined mayonnaise samples in numbers varied from $<10^2$ as a minimum to 8×10^4 as a maximum, with an average count of $2.9 \times 10^3/\text{g}$. No acceptable level of these bacteria can be stated because enterococci counts vary with the holding condition, time of storage and other factors. Nevertheless, the public health significance cannot be denied, especially when the organism is allowed to multiply and exist in tremendous numbers in the product, as they have been implicated in several food poisoning outbreaks (ICMSF, 1978).

It is obvious from the achieved results (Table 1) that 20% of the examined mayonnaise samples were contaminated by *B. cereus* in numbers ranged from less than 10^2 as a minimum to 1×10^4 as a maximum with an average count of $1 \times 10^3/\text{g}$. Although, *B. cereus* existed in low percentage (20%) with non-significant numbers, its public health hazard should not be neglected. It has been well documented that high numbers of *B. cereus* is needed to elicit symptoms of food poisoning. Therefore, the few numbers of *B. cereus* contaminating the samples should not be overlooked, as they can resist

heat processing (thermoduric) and can grow and multiply during refrigeration storage to reach numbers able to produce sufficient toxins to induce symptoms of food poisoning or even induce spoilage of the product (Richter *et al.*, 1992 and Meer *et al.*, 1993).

According to the results obtained, 26.67% of the examined mayonnaise samples were contaminated by yeasts and molds in numbers averaged 2.2×10^2 /g (Table 1). Higher incidence was obtained by Bahout *et al.* (1996), who found 100% of samples contained yeasts & molds. From the viewpoint of public health, certain strains of yeasts and molds when grow and multiply could be implicated in food poisoning outbreaks (Mossel, 1982).

None of the examined mayonnaise samples had coliforms (less than 3/g). These data prove that the examined mayonnaise samples had insignificant numbers of coliforms and all of the examined samples failed to recover fecal coliforms. Fortunately, no *E. coli* could be detected. However, coliforms and fecal coliforms still continue to be considered as indicator organisms of choice in examining foods, their absence indicate the good microbiological quality of the product. In recent years much attention has been paid towards *E. coli*, because of its importance as an organism of true fecal origin with possible existence of associated enteric pathogens.

As staphylococci count declines in high acid foods (Minor and Marth, 1972), *S. aureus* could not be detected in all of the examined mayonnaise samples. Also, anaerobes could not be detected at all. The obtained results are in accordance with Doyle *et al.* (1982) viewing mayonnaise of quite good microbial quality with levels of contamination beyond the hazard point.

It is known that the commercially prepared dressings such as mayonnaise have never been directly identified as a cause of any food borne illness. This performance is largely due to the intrinsic bactericidal nature of these products. Organic acids and acid ingredients contribute a desirable flavor for these products and are toxic to food borne pathogens (Brudzinski and Harrison, 1998). Acetic acid is the predominate acid which is added as various types of vinegars (Smittle, 2000).

The use of unpasteurized eggs in commercial mayonnaise was discontinued in the early 1970's, with most manufactures using exclusively USDA-certified pasteurized eggs. However, on rare occasions, sporadic low-levels contamination with *Salmonella*, *Listeria* and *E. coli* may occur in pasteurized eggs. Hence, the ability of the

acidity in mayonnaise to destroy food borne pathogens is of important consideration. However, 2 recent outbreaks in the Pacific Northwest epidemiologically implicated mayonnaise as a vehicle of *E. coli* O157:H7 transmission.

Therefore, the purpose of the experimental work in this study was to determine the fate of some pathogenic microorganisms in commercial mayonnaise samples to evaluate the antimicrobial properties of such product as it is considered one of the microbiological safe food products.

The summarized results in Table 2 revealed that *S. typhimurium* could not recover after 6 hours of its inoculation in mayonnaise and that could be attributed to its high acid content of acetic acid, which has greater antimicrobial effects than most other acids. Several studies have demonstrated that types of the acidulants would have different effects on microorganisms with acetic acid giving the greatest killing effect (Faber *et al.*, 1989 and Sorrels *et al.*, 1989). An acetic acid level of 0.7% or more in the aqueous phase which is commonly used in commercial mayonnaise is likely sufficient to kill Salmonella (Jeanne-Marie *et al.*, 1997). Salmonella was observed to die off in homemade mayonnaise acidified with acetic acid (vinegar) more rapid than with citric acid (lemon juice) at the same pH (Perales and Garcia, 1990). Commercial pasteurized egg white is important in the manufacturing of cholesterol-free mayonnaise due to the presence of a synergistic effect of acetic acid and lysozyme or other antimicrobial substances in egg white on inactivating Salmonella in mayonnaise. Because microorganisms need water to grow and the oil is anhydrous, the water phase milieu or the water-oil interface is the portion of these products that is of primary concern. However, Radford *et al.* (1991) found that mayonnaise prepared from oil high in phenolic compounds, such as olive oil, can accelerate the death of Salmonella, the acid effects on growth and survival are primarily concentrated in the water phase components.

Concerning *E. coli* O157:H7, no viability was noticed (Table 2) as sudden death was occurred after 6 hours of inoculation and that could be attributed to the low inoculum used (1×10^1 cfu/ml). However, Hathcox *et al.* (1995) commented as regardless of the inoculum level, death of *E. coli* O157:H7 is most rapid at room temperature (20°C to 30°C) at which mayonnaise is stored, distributed and offered for sale in the marketplace. Weagant *et al.* (1994), Zhao and Doyle (1994), Raghubeer *et al.* (1995) and Hathcox *et al.* (1995)

demonstrated that *E. coli* O157:H7 could not survive in real mayonnaise or reduced-calorie mayonnaise dressing commercially prepared with good manufacturing practices.

Although *L. monocytogenes* had not been associated with mayonnaise, considering the wide spread distribution of this organism and the possibility of *Listeria* contamination of eggs, it would be beneficial to have an understanding of inactivation kinetics of *L. monocytogenes* in mayonnaise. This is particularly important considering the relatively high heat tolerance of *L. monocytogenes* in liquid whole egg as reported by Foegeding and Leason (1990) who revealed that the minimal heat processing of 60°C for 3.5 minutes for pasteurization of liquid whole eggs would kill only 2 to 3 log₁₀ *L. monocytogenes*/ml, thus the heavily contaminated whole eggs may be unsafe even after pasteurization for this pathogen. Therefore, the survival behavior of *L. monocytogenes* in the present study was required and recommended.

As recorded in Table 2 that *L. monocytogenes* could not able to persist viable after 24 hours after inoculation and that could be explained by the lethal effect of acetic acid accompanied by higher incubation temperature as *L. monocytogenes* has psychrotrophic nature (Glass and Doyle, 1991). Also, Erickson and Jenkins (1992) speculated that the anti-listerial activity was due to the egg white lysozyme. Our findings was established before by FDA, as >10⁴/g *L. monocytogenes* were inactivated within 72 hours holding period and this level of kill provides sufficient margin of safety considering that low levels of *Listeriae* (<10 cfu/g) normally are present when *L. monocytogenes* is detected in ingredients. As an additional margin of safety, under normal shipping and distribution practices, commercial mayonnaise must be 2 to 4 weeks old before it reaches the store shelves. Even excessive high levels (>10⁷ cfu/g) of *L. monocytogenes* contamination, which is extremely unlikely situation, would be killed under these conditions.

Regarding *S. aureus*, it could not be detected after 6 hours (Table 2). Dolye *et al.* (1982) reported that commercial mayonnaise prevents growth of *S. aureus*. However, *S. aureus* has a potential hazard in mayonnaise and related products as the water activity of the product is not sufficiently low to preclude the growth of *S. aureus*. The organism is killed in mayonnaise with a pH <4.1 (Smittle, 1977). Therefore, Radford and Board (1993) concluded that in the preparation of homemade mayonnaise, vinegar should be used as the acidulant,

with pH less than or equal 4.1 and initial incubation at room temperature for 24 hours before refrigeration.

With focusing on the survival and behavior of *B. cereus*, it persisted alive till 24 hours and reached a count of 5×10^2 cfu/g (Table 2).

It is apparent from the obtained results that none of the pathogenic microorganisms inoculated into mayonnaise could be detected after 48 hours and that could be attributed to the antimicrobial properties of mayonnaise, in which, the acidity is the most important intrinsic characteristic in controlling the growth and survival of pathogenic bacteria. Secondly, salt and sugar play minor roles, but they have an interactive effect with acetic acid in vinegar on inhibiting the growth of food borne pathogens (Entani *et al.*, 1998). Therefore, it could be concluded from the aforementioned results (Table 2) that 48 to 72 hours holding at room temperature was sufficient to kill the pathogenic microorganisms inoculated into commercial mayonnaise samples having a pH of 4.95 to ensure safeness of the products. This conclusion was in agree with the requirement of federal regulations that commercially manufactured dressings such as mayonnaise made with unpasteurized eggs must have pH of less than or equal 4.1, an acetic acid level of the aqueous phase greater than or equal to 1.4%, and holding period of 72 hours before the product is shipped. These conditions were established to assure destruction of Salmonella, Listeria (Kathleen and Michael, 1991) and enterohaemorrhagic *E. coli* (Errol *et al.*, 1994). Unfortunately, these inhibitory effects of mayonnaise could be compromised if this product is contaminated in a food service establishment and then diluted in preparation of other foods. Therefore, it is important that food service establishment and households be aware that when preparing food with mayonnaise, the acidity and pH derived from the mayonnaise can not ensure the inhibition of growth of pathogenic bacteria by cross-contamination of such prepared food.

Finally, good manufacturing practices and the implementation of Hazard Analysis Critical Control Point (HACCP) program in food manufacturing and food preparation should be done to improve quality and control pathogenic microorganisms of commercial mayonnaise.

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