academicJournals

Vol. 7(20), pp. 2269-2273, 14 May, 2013
DOI: 10.5897/AJMR12.1291
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Microbial quality of retail mayonnaise-base salads

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Accepted 10 May, 2013

The purpose of this study was to determine the microbiological quality of retail mayonnaise-based salads samples. A total of 432 samples were collected between 1 February 2008 and 31 July 2009 in Ankara. In the present study, pH values of the samples were measured in the range of 4.05-7.10 (average pH=5.69). The samples with pH higher than 4.6 (n=236/432, 54.6 %) were carried out for microbiological analysis. In the present study, the samples were analysed for the presence of total aerobic bacteria (TAB), *Esherichia coli, Salmonella spp*. and *Staphylococcus aureus.* According to microbiological analysis, TAB was detected in 210 of 236 (89.4 %) retail mayonnaise based salads (range: $1.1x10^2$-2.9×10^6 cfu/g). *E.coli*, *Salmonella* and *S. aureus* were detected in 143 (60.6.%) of 236 samples each, with a range of 1.3×10^2-3.6×10^4 cfu/g, 2.4×10^2-7.1×10^4 cfu/g (62 samples, 26.3%) and 1.8×10^2-8.3×10^3 cfu/g (41 samples, 17.40%), respectively. The results indicate that the type of mayonnaise based salads analysed may contain pathogenic bacteria and thereby represent a risk to the consumers in regard to foodborne diseases. Thus, it is essential to include the effective hygiene practices as an important safety measure in the production of mayonnaise- based salads.

Key words: Mayonnaise-based salads, microbiological analysis, pathogens.

INTRODUCTION

Mayonnaise is an important ingredient for the manufacturing of various kinds of mayonnaise-based products. It can be used as a decorative element or raw material in production of salads, sauces and other delicaies. Mayonnaise is made by emulcification of vegetable oil and water phase of egg yolk. In general, water phase is composed of water, salt, sugar, vinegar, polysaccharides, additives and condiments, especailly mustard. Mayonnaise is a relatively microbiologically stable product due to its high fat content and addition of acidic ingredients. Organic acids and other acidic ingredients are toxic to foodborne pathogens, they also contribute to the desirable flavor and, decrease the final pH of product (Filova et al., 2008). Mayonnaise-based salads (MBS) are widely consumed to such an extent that they form the foundation of one-half of all salad dressings and the basis of many other products (Xiong et al., 2002). Ready-to-eat (RTE) foods by Codex Alimentarius is any food or beverage that is normally consumed in its raw state or otherwise prepared into a form in which it is normally consumed without further processing (Forsythe, 2010). MBS are ready for consumption without additional preparation and cooking by consumers.

Therefore presenting a potential microbiological risks to consumers (Hwang and Tamplin, 2005). MBS are simply a mixture of different types of foods. The ingredients may

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Abbreviations: TAB, Total aerobic bacteria; **MBS,** mayonnaise-based salads; **RTE,** ready-to-eat; **GMP,** good manufacturing practices.

be poultry, meat, eggs, fish, shellfish, grains, dry beans, pasta, potato, vegetables and fruits and, herbs or nuts. Apart from the mayonnaise and the main components, starch, sugar, spices, organic acids, preservatives and flavour/colour substances may be present in the product (ICMSF, 1998; ICMSF, 2005). MBS are made by mixing ingredients at an ambient or a specific range of cooling temperature. During this stage mayonnaise or dressing have to be in a special thickness. Some of the ingredients such as chicken and meat have to be cooked but others may not.

Typical ingredients that are used without a heat treatment are raw vegetables. They can introduce a very broad spectrum of microorganisms to the products (ICMSF, 2005). Due to the vinegar and other acids added, MBS usually have a pH of 3.5-5.5. The acetic acid level in the aqueous phase (0.2-0.5%) is much lower than that of mayonnaise itself (ICMSF, 1998; ICMSF, 2005). When the complete salad is mixed, it is packed in consumer tubs or in larger containers for sale by the retailer (counter salads). Most MBS are kept refrigerated to extend their shelf-life. The pH level of the salad depends on the initial amount of inhibitory acids and preservatives. Under chilled conditions, the shelf life varies between 2-8 weeks (ICMSF, 1998). It has been shown that some pathogens can survive in MBS for several weeks at 5°C (Brocklehurst and Lund, 1984; Zhao and Doyle, 1994). However commercial MBS, whether made for retail or foodservice, are normally held at an room temperature (23-25°C) till the opening moment (Zhao and Doyle, 1994). The aim of this study was to determine presence of *Esherichia coli*, *Salmonella* and *Staphylococcus aureus* in retail MBS during storage at refrigeration temperatures.

MATERIALS AND METHODS

Collection of samples

This study was carried out in Ankara. All kinds of MBS that are ready to eat were collected from the markets which are on sale on every Wednesday in a period of time for 18 months. A total 432 retail MBS samples collected during the study. All samples (200 g) were aseptically collected, placed in sterilized jars ice chests during transportation to the laboratory. pH analyses of the samples were conducted either immediately after arrival in the laboratory, following overnight storage in the refrigerator (4°C), and microbiologically examined on the next day.

pH measurements

pH values were measured at ambient temperature (20°±1°C) using a digital laboratory pH meter which was calibrated at a temperature of 20°C before used. pH readings were obtained on a digital laboratory pH meter (P Selecta). An aseptic portion of the MBS (10 g) and 100 mL deionized water were placed into a 400 mL stomacher bag. Then homogenized in a stomacher (IUL instruments Masticator) for 1 min before pH measurement. Samples of 10 mL homogenized solution was transferred and this procedure was performed in a 50 ml of beaker (Xiong et al., 2000; Hwang and Tamplin, 2005; Banwart, 1989; Ray, 2004; Evancho et al., 2001). The samples with higher pH than 4.6 (n=236/432, 54.6 %) were analyzed for microbiological load.

Microbiological procedures

Portion of the MBS samples (25 g) were weighed into sterile stomacher bags, homogenized with 225 mL sterile peptone water (0.1%) in a stomacher (IUL Instruments Masticator) for 2 min. Decimal dilutions up to 10^{-8} were prepared from the suspension with 0.1% peptone water and microbiological analyses were performed by using pour plate and spread plate methods. The samples were analysed for the presence of microorganisms by using standard methods. All samples were analyzed for aerobic plate count, *E. coli*, *Salmonella*, and *S. aureus*. All analyses were performed by using the standard procedures outlined in the American Public Health Association (APHA) Standard Guidelines (Fleming et al 2001; Smittle and Cirigliano, 2001). The media used, incubation periods and conditions for microbiological analyses and identification techniques are presented in Table 1 (Temeli et al., 2006; Legnani et al., 2004).

RESULTS AND DISCUSSION

In the present study, pH values of the MBS samples were measured in the range of 4.05-7.10 (average pH=5.69). The samples with pH higher than 4.6 (n=236/432, 54.6 %) were carried out for microbiological analysis. In the present study, samples were analysed for the presence of TAB, *E. coli, Salmonella spp*. and S.*aureus.* According to microbiological analysis, total aerobic bacteria (TAB) were detected in 210 of 236 (89.4%) retail MBS (range: $1.1x10^2$-2.9×10^6 cfu/g). *E.coli*, *Salmonella* and *S. aureus* were detected in 143 (60.6%) of 236 samples each, with a range of 1.3×10^2-3.6×10^4 cfu/g, 2.4×10^2-7.1×10^4 cfu/g (62 samples, 26.3%) and 1.8×10^2-8.3×10^3 cfu/g (41 samples, 17.4 0%), respectively. Results obtained from the microbiological analyses of retail MBS are presented in Table 2.

In this study, the samples were analysed for the presence of TAB, *E. coli, Salmonella spp*. and *S.aureus.* According to microbiological analysis TAB was detected in 210 of 236 (89.4 %) retail MBS (range: $1.1x10^2$-2.9×10^6 cfu/g). *E. coli*, *Salmonella* and *S. aureus* were detected in 143 (60.6%) of 236 samples each, with a range of 1.3×10^2-3.6×10^4 cfu/g, 2.4×10^2-7.1×10^4 cfu/g (62 samples, 26.3%) and 1.8×10^2-8.3×10^3 cfu/g (41 samples, 17.4 0%), respectively. The inital microflora of MBS is made up of the microbial load of the raw material used and much depends on the microbiological condition of the materials selected for the production of MBS. The mayonnasie typically has a low microbial count with no or a very limited contamination with microorganisms (Michles and Koning, 2000). Commercial MBS, microbiologically, have long shelf life and are extremely safe processed foods. The safety of these products is directly associated with synergistic formulation components of which aqueous

Table 1. Media and incubation conditions used in the microbiological analyses.

Microorganism	Media	Incubation conditions	
		Temperature (°C)	Time (h)
Total aerobic mesophilic bacteria	Oxoid CM 463	30	48
E.coli	Oxoid CM 451	35	24-48
	Brilliant green bile broth, Oxoid CM 31	35	24-48
	Lauryl tryptose broth, DIFCO 0314-01-0 Eosin methylene blue agar, Oxoid CM63 IMViC test	45.5	48
Salmonella	Rappaport-Vassiliadis broth, Oxoid CM866	42	24
	Selenite cystine broth,DIFCO 0687-05-5	37	24
	Hektoen Enteric Agar ,Oxoid CM 419	37	24-48
	Brilliant green agar, Oxoid CM 329	37	24-48
Staphylococcus aureus	Baird-Parker agar, Oxoid M275	37	24
	Egg yolk-tellurite emulsion, Oxoid SR 54 Staphytect Plus Test,Oxoid	37	24

Table 2. Results of microbiogical quality of mayonnaise based salads samples (n=236).

Bacteria	Positive sample		Range (cfu/g)
	n	%	
TAB	210	89.0.	1.1×10^2 - 2.9×10^6
E.coli	143	60.6	1.3×10^2 - 3.6×10^4
Salmonella	62	26.3	2.4×10^2 - 7.1×10^4
S. aureus	41	17..4	1.8×10^2 - 8.3×10^3

phase acetic acid and total formula pH levels are considered the most essential in inactivating foodborne pathogens such as *Salmonella* and *S. aureus* (Erickson and Jenkis, 1991). The bacteria would have been able to multiply in the MBS because it had not been allowed to cool properly. The mixture of potatoes and meat was still warm during the selling (Michles and Koning, 2000).

Some results confirm that the bactericidial activity of the acetic acid is greater than that of citric (lemon) acid. At the same time the low temperature may provide a protective effect to salmonella cells against the antimicrobial effects of the organic acids (Perales and Garcia, 1990). Storage at low temperatures appears to be protective the *Salmonella* against the effects of organic acid (Lock and Board, 1995).

It may be assumed that any pathogenic bacteria which may have been present in the raw meat were destroyed when the meat was cooked through on time. Either separately boiled meat and potatoes or the mixture of potatoes and meat must have been avoided from recontamination with bacteria (Beckers et al., 1985). During the Eurepean summit Conference in Maasstrich in 1981, two outbreaks of salmonellosis caused by *S. İndiana* were recorded. *S.indiana* was determined in the faecal specimens of the victims. On the other hand, the MBS which the victims had consumed, contained approximatly 10^7 *S.indiana/g* bacteria. That is more than enough to cause salmonellosis (Beckers et al., 1985). In various studies, it was found that any ingredient which is added to MBS the potential to increase in bacterial growth and number (Snyder et al, 1984; Smith and co-workers 1987; Erickson and co-workers 1993; Weagant et al., 1994; Zhao and Doyle, 1994).

Traditionally MBS, spoonable salad dressing have been classified as acid foods with a pH below 4.6. The reported highest manufacturing target pH for MBS, spoonable salad dressing and sauces is 4.4, which is below the reported inhibitory pH of 4.5 foodborne patho-gens in the presence of acetic acid (Michles and Koning, 2000). In the study of Swaminathan et al. (1981), sand-wiches prepared with turkey and mayonnaise were inoculated with 6-7x10^2 cfu/g nildixic-resistant *S. typhimurium* and incubated at 4, 21 or 30°C. Growth and/or survival was asses-

sed after 4, 7 and 24 h. Mayonnaise was found to have a significant inhibitory effect on *S. typhimurium* for these sandwiches. Mayonnaise did not prevent the growth of *S. typhimurium* when sandwiches were stored at 21 and 30°C for 8-24 h. In another study, Doyle et al. (1982) tested the ability of *S. aureus* and *S. typhimurium* to survive and/or multiply in meat salads prepared with different proportions of mayonnaise stored at 4, 20 or 32°C. The presence of mayonnaise in meat salads retar-ded the growth of these food-borne pathogens. It was recommended that the presence of mayonnaise should not be considered as a alternative for refrigeration. Vege-table tissue, such as carrot or cabbage, in mayonnaise has the effect of removing the toxicity of mayonnaise by absorbing acetic acid. The concentration of acetic acid in the mayonnaise component had diminished (Radford and Board, 1993).

The concentration of acetic acid and the pH in most present formulations of MBS is sufficient to inhibit growth of the majority of organisms and is lethal to many. The addition, however, of carrot or cabbage to mayonnaise is followed by the absorption of acetic acid by the vegetable tissue resulting in a decrease in acetic acid concentration and hence a rise in pH. The ability of pathogenic microorganisms to survive and grow at low temperatures may be important in food-borne infection, particularly when prepared foods are exposed for a long period of time in refrigeration cabinets (George and Levett, 1990). This was due to retention of acetic acid by the plant tissue. Deterioraiton in the physical properties of mayonnaise occurs before microbail spoilage and increases because of interaction of water and oil between the vegetable and mayonnaise phases. Manufacturers recommend 4-6°C for storage and 6-14 days for shelf-life (ICMSF, 1998; Erickson and Jenkis, 1991; Radford and Board, 1993).

In addition, the pH of commercial MBS increases when it is added to the other foods. *S. aureus* have been shown to able to grow at low pH values. Nevertheless, in commercial MBS, other factors besides pH should be taken into account when considering the potential risk of the product. Gomez-Lucia et al. (1990) demonstrated that *S. aureus* may grow at 22°C and sythesize enterotoxins in mayonnaise. Acidification is one of the methods commonly used in the food industry to control growth and survival of spoilage-causing and pathogenic microorganisms. However, it has been reported that microorganisms exposed to a moderately acidic environment may develop cells with increased resistance and longer survival time when transferred to a more acidic condition (Cheng et al., 2003). The presence of meat and fish is a potential source of food poisoning organisms in MBS (Zhao and Doyle, 1994; Raghubeer et al., 1995). These studies were performed in MBS consisting of different formulations and under a wide range of storage temperatures. It is therefore difficult to establish conditions clearly which would be expected to limit the survival of pathogens (McKeller et al., 2002). The processing of a MBS essentialy consists of a mixing of all ingredients with the mayonnaise base. Normally this does not include a heating step. The processing will not reduce the microbial counts and may even lead to increase when time and temperatures support microbial growth. Particularly when processing is not done hygienically, this may lead to build-up of an acid-adapted pathogenic and spoilage flora in the plant resulting in contamination of product (Michles and Koning, 2000).

Commercial MBS manufactured under good manufacturing practices (GMP) is not a public health concern. The principal basis of concern is abusive handling of MBS by foodhandlers. Commercial MBS which are pathogens-free is transferred from its original container to make in-store products. If unsatisfactory GMP and sanitation procedures are employed, MBS could be cross-contaminated by contact with dirty utansils, unclean table surface or raw ingredients, such as meats or vegetables. Hence, cross-contaminated of MBS with pathogens contaminated foods such as raw meat, or unsanitary foodhandling practices, infected foodhandlers are the major factors of concern (Zhao and Doyle, 1994). The pH was increased by 0.6 to 0.7 (5.0- to 5.7) unit when the salad ingredients were added to mayonnaise. No organoleptic changes could be observed when counts were above 10^8 CFU/g. Mishandling of contaminated MBS enhanced the possibility of the presence of pathogenic organisms in the product (Gomez-Lucia et al., 1987).

Infective pathogens which contaminate MBS, may be destroyed rapidly at low pH and high temperature. However, pathogens may survive for days or weeks under chilled conditions. Contamination with infective pathogens thus presents a distinct safety hazard for the consumers (Michles and Koning, 2000). Control of the safety and spoilage of MBS depends on these steps: There should be usage of cooked, blanched, or pasteurized animal products; vegetables should be washed and ensured that it is clean; usage of organic acids (acetic acid) to help keep pH low, strict hygiene in preparation, mixing, and storing MBS (ICMSF, 1998; ICMSF, 1980). MBS production under proper refrigeration and good hygienic practices represent negligible microbiological health hazards risks to consumers.

ACKNOWLEDGEMENTS

The authors would like to thank M.Cengiz Aktan and Dr. A Celebi Tayfur for support and assistance during this study.

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