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**FATE OF *SALMONELLA ENTERICA* SEROVAR
ENTERITIDIS DURING MANUFACTURE AND
STORAGE OF HOME MADE MAYONNAISE**
(With 6 Tables)

By

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**مصير ميكروب السالمونيلا انتريتيديس اثناء تصنيع
وتخزين المايونيز داخل المنزل**

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يهدف البحث الى تصنيع المايونيز داخل المنزل وذلك بتحضير ستة وصفات مختلفة من المايونيز باستخدام عصير الليمون المركز من ٢٠ الى ٤٠ ميللي لتصل حموضة المنتج pH الى ٤،٥٥ ؛ ٣،٣١ ؛ ٤،٣٠ ؛ ٣،٤١ ؛ ٤،٠٠ و ٣،٣٠ على التوالي وكذلك اضافة كلا من ٢٥٠ ميللي من الزيت و ٢٠ جرام من صفار البيض؛ من صفر الى ٣ جرام من الملح ؛ واحد جرام من المستردة ؛ نصف جرام من السكر و ربع جرام من الفلفل الابيض بنسب مختلفة في جميع الوصفات وفحصها حسيًا عقب تصنيعها مباشرة وقد اتضح ان الوصفة رقم واحد وثلاثة وخمسة هي احسن الوصفات في الطعم والقبول عن باقي الوصفات الاخرى. وبدراسة مصير ميكروب السالمونيلا انتريتيديس في كل الوصفات اثناء تخزينها عند ٥ و ٢٢ درجة سيليزيوس على التوالي وجد أنه يزداد تواجدها مع قلة كمية الليمون المركز وقلة حرارة التخزين حيث أن التخزين عند درجة حرارة منخفضة وهي ٥ درجة سيليزيوس يعمل على حماية الميكروب من تأثير حمض الستريك. ولهذا ومن اجل انتاج مايونيز آمن داخل المنزل ينصح باستخدام كمية من الليمون لا تقل عن ٢٥ ميللي او أكثر وتخزينه في درجة حرارة دافئة عند ٢٢ درجة سيليزيوس لمدة ٧٢ ساعة قبل الأكل او التخزين.

SUMMARY

In this study, six different recipes of home made mayonnaise were prepared using lemon juice in order to bring down pH to 4.55 ± 0.01 ; 3.31 ± 0.10 ; 4.30 ± 0.06 ; 3.41 ± 0.04 ; 4.0 ± 0.11 and 3.30 ± 0.03 , respectively. The content of oil (250 ml), egg yolk (20 g), citric acid (> 5 % w/v) (20-40 ml), salt (0-3 g), mustard (1 g), sugar (0.5g) and white pepper (0.25g) varied among the different recipes. All recipes were scored for their sensory evaluation. The results showed that Recipe 1; 3 and 5 gained higher grades and higher acceptability for most sensory attributes than the other recipes. The behavior of *Salmonella enterica*

serovar Enteritidis in home made mayonnaise was studied at two holding temperatures (5 and 22°C). The presence value increased whereas the amount of citric acid and storage temperature decreased. Storage of mayonnaise at low temperature (5°C) protected the *Salmonella enteritidis* against the effect of citric acid. The fate of *Salmonella enteritidis* appeared to be largely dependent on the temperature of the storage. Therefore, for production of safe home made mayonnaise, the use of citric acid at 25 ml or more and storage in warm place at 22°C for 72 hours before consumption or refrigeration was recommended.

Key words: *Home made mayonnaise, Salmonella enteritidis, sensory analysis, holding temperature.*

INTRODUCTION

Mayonnaise is a widely consumed product, forming the foundation of one half of all salad dressings and the basis of many other products such as tartar sauce and coleslaw (Hoffmann, 1989; Radford and Board, 1993). It is a kind of semi solid oil in water emulsion, traditionally prepared by carefully mixing a mixture of egg yolk, and oil to maintain closely packed foam of oil droplets. It may also include salts, mustard, sweeteners and other optional ingredients. The emulsion is formed by slowly blending oil with a premix consists of egg yolk, citric acid and or vinegar as well as mustard because mixing the oil and aqueous phase at once result in formation of water in oil emulsion (Anon, 1989; McClements, 1999; Smittle, 2000; Liu *et al.*, 2007 and Kim *et al.*, 2009).

Mayonnaise was first produced commercially in the early 1900's, and became popular in America from 1917 to 1927 (Anon, 2000 and Grodzka *et al.*, 2005) and recently in Japan, where sales increased by 21% a year from 1987 to 1990 (Brabant, 1992). A wide range of commercial mayonnaise is available, but many individuals and caterers still prefer to use home made mayonnaise due to its lowered tartness and better texture (Depree and Savage, 2001 and Rodrigue *et al.*, 2008). However, the home made mayonnaise has been identified as one of the causes of *Salmonella* food poisoning. Most of the outbreaks of food poisoning are transmitted through the home made mayonnaise which made from fresh eggs with *Salmonella enteritidis* (Murchie *et al.*, 2008). This situation might be due to several factors such as the habit of preparing mayonnaise frequently in home or in restaurants and a relatively high percentages of eggs being contaminated with *Salmonella*

enteritidis (Pearales and Audicana, 1989; Whiting and Buchanan, 1997; Stadelman, 1999; Davis and Reeves, 2002; Morgan *et al.*, 2007 and Irwin *et al.*, 2008).

Salmonella enteritidis is a food borne pathogen that causes food borne illness. Symptoms of this illness mainly consist of nausea, vomiting, abdominal pain, headache, chills and diarrhea (Knabel, 1995 and Holtby *et al.*, 2006). The fate of *Salmonella enteritidis* in home made mayonnaise is determined in part by the type and concentration of the acidulant, as well as, the time and temperature of storage (Pearales and Garcia, 1990; Radford, 1994 and Ordonez *et al.*, 2009). Considering the possible contamination and proliferation of pathogenic *Salmonella enterica serovar Enteritidis* during processing and storage of home made mayonnaise which may lead to public health hazard, this study was conducted to determine the conditions in which *Salmonella enteritidis* could be destroyed depending on the concentration of the acid used, as well as, the time and temperature of storage.

MATERIALS and METHODS

Eggs:

Specific pathogen free (S.P.F.) eggs were obtained from Nile S.P.F. project, Agriculture research center (Kom- Oshim- Fayoum governerate).

Culture:

Salmonella enterica serovar enteritidis strain was obtained from the culture collection of Health ministry central laboratories on a slope of nutrient agar and then was maintained on XLD agar (Oxoid) at 37°C for 48 hours.

Mayonnaise preparation:

The experiment is based on input materials provided by six types of mayonnaise recipes according to Xiong *et al.* (1999).

Table 1: The experimental design table.

Ingredients	Recipe 1	Recipe 2	Recipe3	Recipe 4	Recipe 5	Recipe 6
Sunflower oil/ml	250	250	250	250	250	250
Egg yolk/g	20	20	20	20	20	20
Citric acid/ml (>5% w/v)	20	40	25	40	30	40
Salt/g	3	0	1.5	1.5	0	3
Mustard/g	1	1	1	1	1	1
White pepper/g	0.25	0.25	0.25	0.25	0.25	0.25
Sugar/g	0.5	0.5	0.5	0.5	0.5	0.5

The mayonnaise was prepared using the following recipe: 20 g egg yolk, 250 ml pure sunflower oil, 1 g mustard, 0.5 g sugar, 0.25 g white pepper and different amounts of the citric acid solution (20 to 40 ml), and salt (0-3 g). Lower content of citric acid and egg yolk could not produce mayonnaise. Mayonnaise was prepared as follows: (I) all ingredients except the oil were mixed with an electronic mixer and (ii) oil was poured steadily while blending at high speed. After preparation, pH values were measured at room temperature for all mayonnaise samples, using a pH meter (Orion, digital, pH). Fresh mayonnaise samples were analyzed for sensory qualities. The experiment was conducted in three repetitions.

Sensory analysis:

For the purpose of evaluating sensory qualities, a commission composed of three qualified and experienced tasters trained in the field of emulsions was appointed, while sensory attributes of coded mayonnaise samples were tasted in a standard sensory laboratory (in order to guarantee objectivity as far as the sample brand names are concerned). The samples were evaluated by the same sensory commission. Strict confidentiality rules were observed during testing. All testing posts in the sensory laboratory had identical conditions. The room temperature was approximately 20 °C. Lighting of the room was also the same throughout the experiment. The commission was given representative mayonnaise samples of 5 grams placed on white china plates. The sensory commission assessed the samples separately one by one in groups composed of 6 samples. The break between the groups to be tasted was one hour. The test had three rounds, meaning that each mayonnaise sample had been prepared by following the same recipe in three batches. The analysis was performed by scoring sensory attributes by assigning a non-structured scale from 1 to 10 points, where higher score means more expressed attribute according to Abu-Salem and Abou-Arab (2008).

Inoculation and incubation of Mayonnaise:

A loopful of *cultutre strain of Salmonella enterica serovar enteritidis* from a XLD agar at 4°C was inoculated into 10 ml 0.1% peptone water (Oxoid) containing 10% egg yolk emulsion (Oxoid). The culture was incubated at 37°C for 24 hours (h). The culture was then added to the mayonnaise at the rate of 1ml per 100 g to give approximately 10^6 cfu/g and blended with stomacher for 2 min. No separation of mayonnaise was observed due to blending. The inoculated mayonnaise samples were immediately transferred into sterile containers

and stored at 5°C and 22°C for 9 days according to Marshall (1992) and Roberts *et al.* (1995).

Microbiological analysis:

The samples were taken at time intervals after contamination for viable counts. At each time interval, 10 g contaminated mayonnaise were weighed into a stomacher bag, 90 ml Buffered Peptone Water (BPW) (Oxoid) added and the mixture homogenized for 1 min with stomacher. Serial ten fold dilutions in 0.1% peptone diluents were plated onto XLD agar (Oxoid) using the surface spread method. The XLD agar plates were incubated at 37°C for at least 24 hours and the viable colonies then enumerated. For presence/absence tests, a 25 g mayonnaise sample was added to 225 ml 1% Buffer peptone water in a sterile container at each time interval. The mixture was swirled well and incubated at 37°C for 24 hours. After incubation, 0.1 ml of the mixture or enrichment was added to 4ml Rappaport-Vassiliadis broth (Difco) and incubated at 42°C for 18 h. Loopfull from the secondary enrichment was streaked on XLD agar media and incubated at 37°C for at least 24 hours according to Jay (1992) and Donaghy and Madden (1993). All experiments in this study were performed in triplicate.

RESULTS

Table 2: Sensory evaluation and pH values of home made mayonnaise recipes during processing at zero time.

Sensory evaluation and pH values	Recipes					
	1	2	3	4	5	6
Taste	9±0.01	5±0.01	9±0.06	6±0.01	9±0.03	8±0.06
Flavor	9±0.01	6±0.03	9±0.03	7.5±0.06	9±0.03	8±0.01
Color	9.5±0.03	9±0.06	9±0.01	9.5±0.01	9.5±0.01	9±0.03
Appearance	9±0.01	9.5±0.03	9.5±0.06	9±0.03	9.5±0.05	9.5±0.03
Overall acceptability	9.1±0.01	7.3±0.03	9.1±0.05	8±0.01	9.2±0.03	8.6±0.01
pH	4.55±0.01	3.31±0.10	4.30±0.06	3.41±0.04	4.0±0.11	3.30±0.03

*± Standard Error, S.E.

Table 3: Growth of *Salmonella enteritidis* in home made mayonnaise recipes during storage at 5°C.

Time	Recipes (cfu/g)					
	1	2	3	4	5	6
0 time	6x10 ⁵ ±0.04	6x10 ⁵ ±0.06	6x10 ⁵ ±0.05	6x10 ⁵ ±0.04	6x10 ⁵ ±0.03	6x10 ⁵ ±0.07
24h	58x10 ⁴ ±0.03	24x10 ⁴ ±0.03	54x10 ⁴ ±0.02	26x10 ⁴ ±0.04	58x10 ⁴ ±0.12	19x10 ⁴ ±0.03
48h	48x10 ⁴ ±0.12	21x10 ⁴ ±0.02	42x10 ⁴ ±0.11	18x10 ⁴ ±0.02	56x10 ⁴ ±0.06	13x10 ⁴ ±0.03
72h	69x10 ³ ±0.06	44x10 ³ ±0.05	57x10 ³ ±0.10	36x10 ³ ±0.01	64x10 ³ ±0.38	31x10 ³ ±0.06
96h	55x10 ³ ±0.03	16x10 ³ ±0.04	32x10 ³ ±0.22	23x10 ³ ±0.22	61x10 ³ ±0.05	17x10 ³ ±0.23
120h	45x10 ² ±0.01	6x10 ² ±0.01	36x10 ² ±0.31	18x10 ² ±0.04	55x10 ² ±0.01	10x10 ² ±0.06
144h	85x10±0.01	72x10±0.02	59x10±0.16	44x10±0.11	35x10±0.50	24x10±0.11
168h	48x10±0.02	42x10±0.11	44x10±0.01	37x10±0.05	41x10±0.03	34x10±0.05
192h	99±0.03	35±0.01	55±0.02	34±0.01	43±0.05	30±0.01
216h	ND	ND	ND	ND	ND	ND

*± Standard Errors, S.E. ** ND, not detected.

Table 4: Presence (+) /absence (-) of *Salmonella enteritidis* in home made mayonnaise recipes during storage at 5°C.

Time Recipes	24h	48h	72h	96h	120h	144h	168h	192h
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
6	D	D	d	d	D	d	d	D

d* = doubtful, one positive result from three experiments.

Table 5: Growth of *Salmonella enteritidis* in home made mayonnaise recipes during storage at 22°C

Time	Recipes (cfu/g)					
	1	2	3	4	5	6
0 time	6x10 ⁵ ±0.09	6x10 ⁵ ±0.06	6x10 ⁵ ±0.11	6x10 ⁵ ±0.04	6x10 ⁵ ±0.08	6x10 ⁵ ±0.06
24h	11x10 ³ ±0.11	84±0.06	30x10 ³ ±0.08	12x10 ² ±0.11	32x10 ³ ±0.07	13x10 ² ±0.11
48h	55x10 ² ±0.06	ND	75±0.05	ND	89±0.01	ND
72h	300±0.01	ND	ND	ND	ND	ND
96h	84±0.03	ND	ND	ND	ND	ND
120h	62±0.02	ND	ND	ND	ND	ND
144h	ND	ND	ND	ND	ND	ND
168h	ND	ND	ND	ND	ND	ND
192h	ND	ND	ND	ND	ND	ND
216h	ND	ND	ND	ND	ND	ND

*± Standard Errors, S.E. ** ND, not detected.

Table 6: Presence (+)/ absence (-) of *Salmonella enteritidis* in home made mayonnaise recipes during storage at 22°C.

Time Recipes	24h	48h	72h	96h	120h	144h	168h	192h
1	+	+	+	+	+	-	-	-
2	+	d	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-
4	+	d	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-
6	+	d	-	-	-	-	-	-

*d, doubtful, one positive result from three experiments.

DISCUSSION

Sensory analysis

The sensory analyses were carried out on all recipes of mayonnaise samples include: color, taste, flavor, appearance, and overall acceptability were recorded in Table 2. The taste, flavor, color, appearance and over all acceptability showed higher scores in recipes 1, 3 and 5 compared with the other recipes. These mayonnaise recipes samples contained balanced proportion of salt, citric acid and mustard that contributed to their taste. In recipes of relatively high content of citric acid, mayonnaise samples were characterized by a sour taste with pH value between 3.30-3.41 while recipes of low concentration of citric acid had pH values between 4.00-4.55. All recipes containing no salt were characterized by a flat and poor flavor. However, the color and appearance were not significantly affected in all recipes of mayonnaise, being in the range of 9-9.5. These results were in accordance with Meyer *et al.* (1995); Kishk (1997); Stefanow (1998); Xiong *et al.* (2000); Stern *et al.* (2001); Yang *et al.* (2001); Karas and Skvaraa (2002). Color is one of the most important quality attributes of mayonnaise which the consumer uses to select the mayonnaise brand from the grocer's shelf (Peressini *et al.*, 1998; Depree and Savage, 2001 and Rujirat *et al.*, 2007). The yellowish color is primarily provided by egg yolk carotenoids, while oil and mustard do not contribute a color simulating that provided by egg yolk (Abu-Jdayil, 2003 and Batista *et al.*, 2006).

The fate of *Salmonella enterica* serovar *enteritidis* in mayonnaise recipes prepared with citric acid solution was recorded in Tables 3&4.

The initial pH of home made mayonnaise of six recipes samples were 4.55 ± 0.01 ; 3.31 ± 0.10 ; 4.30 ± 0.06 ; 3.41 ± 0.04 ; 4.0 ± 0.11 and 3.30 ± 0.03 , respectively (Table 2). The initial level of *Salmonella enteritidis* in all recipes was around 6×10^5 cfu/g. *Salmonella enteritidis* was able to grow in all mayonnaise recipes during storage at 5°C regardless the initial pH in the samples. Comparing Tables 5&6 it could be seen that inactivation of *salmonella enteritidis* at 22°C was more rapid than at 5°C. The time for reduction of *Salmonella enteritidis* to the undetectable level at 5°C was after 192 hours, while at 22°C no microorganisms were detected after 48 -72 hours in all the recipes except recipe 1 which had no viable cells after 120 hours. The presence value increased whereas the amount of citric acid and storage temperature increased. Twenty five ml or more citric acid at 22°C were the boundary conditions (maximum or minimum requirements) for the absence of *Salmonella enteritidis* in citric acid home made mayonnaise after 72 hours storage. This confirmed that storage of mayonnaise at a lower storage temperature protects the *Salmonella enteritidis* to some extent against antimicrobial effects of organic acid used. Nearly similar findings were recorded by Xiong *et al.* (2000) and Ordonez *et al.* (2009). Our results disagree with Erickson and Jenkins (1991) and Saeed and Koons (1993) who reported that *Salmonella enteritidis* was unable to grow in home made mayonnaise at 22°C, where as Davis and Wray (1996) and Leuschner and Boughtflower (2001) reported that *Salmonella enteritidis* was inactivated in mayonnaise adjusted to pH 4 and stored at 4°C. This difference may be attributed to the different mayonnaise formulations used in the studies, the interaction of pH, temperature, antimicrobial ingredients, the physical and chemical properties of mayonnaise recipes used, the physical positioning of ingredients and the character of microorganisms throughout the mayonnaise recipes (Radford, 1994 and Yang *et al.*, 2001). The shelf life of mayonnaise recipes samples stored at 22°C was much shorter than at 5°C. All mayonnaise samples stored at 22°C were spoiled by molds after 7 days, while no visible molds were observed after 9 days of storage at 5°C. Acidic pH in mayonnaise prevents the growth of most microorganisms commonly associated with food spoilage. However, spoilage of these products does occur as a result of the growth of *lactobacilli*, *bacilli*, and *yeasts* (Xiong *et al.*, 2002 and Fialova *et al.*, 2008). It was found that in recipe 1, addition of 20 ml citric acid, did not affect *Salmonella enteritidis* up to 120 h at 22°C, while in other recipes *Salmonella enteritidis* was inactivated by addition of at least 25 ml citric acid at 22°C within 72 hours. This suggests that

mayonnaise recipes using less than 25 ml citric acid solution considered unsafe unless pasteurized eggs are used. Therefore, raw eggs would be a very high risk in food poisoning. If safe mayonnaise recipes could be eliminated, outbreaks of food poisoning attributed to home made mayonnaise may be reduced significantly or even eliminated (Clay and Board 1991; Humphrey, 1994; Knabel, 1995; Murphy, 1999; Smittle, 2000; Chouard, 2005; Guilmineau and Kulozik, 2007 and Cherington *et al.*, 2008). To maintain *Salmonella enterica* serovar *enteritidis* free mayonnaise prepared with pure lemon juice (citric acid concentration >5% (w/v). So, it is recommended that pH should be in the range of 3.3 - 4.3 using 25-40 ml pure lemon juice, and the product should be held at 22°C for at least 72 h before consumption or refrigeration. This recommendation is made with regard to the advice of the Chief Medical Officer that eating raw eggs should be avoided for vulnerable people such as elderly, sick, babies and pregnant women, while pasteurized egg should be used (Department of Health, 1998). Pepper, sugar, and mustard have no effect on *Salmonella enteritidis*. Nearly similar findings were reported by Membere *et al.* (1997); Baron *et al.* (2000); Guerzoni *et al.* (2002) and Leuschner and Juliette (2002). In contrast, Xiong *et al.* (1999 and 2002) recorded that high salt content affect the viability of *Salmonella enteritidis* in mayonnaise.

The results of the present study concluded that, to produce safe mayonnaise free from *Salmonella enterica* serovar *enteritidis*, lemon juice (concentration \geq 5% w/v), at least 25 ml citric acid should be used as an acidulant to achieve pH between 3.3 - 4.3 and stored in a warm place at 22°C for 72 hours before being consumed or refrigerated.

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