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RISK ASSESSMENT OF *ESCHERICHIA COLI, SALMONELLA TYPHIMURIUM, AND LISTERIA MONOCYTOGENES* CONTAMINATION IN COMMERCIAL REDUCED CALORIE MAYONNAISE

(With 3 Tables and 3 Figures)

By

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تقدير مدى خطورة تلوث المايونيز منخفض السعرات الحرارية بميكروبات الايشيريشيا كولاي والسالمونيلا تايفيميوريم والليستيريا مونوسيتوجينز

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تـم إجراء تجربة معملية لتصنيع نوع تجارى من صلصة المايونيز ذات السعرات الحرارية الخالـية مـن الكوليستيرول بنسب مختلفة من حمض الخليك (٠,٣ ، ٠,٥ وبدرجة حموضة للمنـتج الـنهائى تتراوح بين ٣,٩ : ٤,١ و٠,٧%)٠ وقد تم تقييم هذه التركيزات من حيث تأثـيرها المباشر على ثلاثة عترات تم حقنها فى المنتج وهى ميكروب الايشيريشيا كولاي O157:H7 ومـيكروب السـالمونيلا تايفيمـيوريم ومـيكروب الليسـتيريا مونوسيتوجينز F5069(46) بعـد مـبدئى ١٠ ^٦ /جـرام تقريباً وتم تحضينها عند ٢٣,٩ درجة مئوية لمدة أسـبوعين٠ وقد أسفرت النتائج عن الآتـى: عترة الليستيريا مونوسيتوجينز ظلت حية لمدة أطـول (١٤ يومـاً) عـن ميكروب الايشيريشيا كولاي (٥ أيام) ثم ميكروب السالمونيلا تايفيمـيوريم (يومـين)٠ وأثبتت التجربة أن تركيز ٠,٧% من حمض الخليك كان له التأثير الفعـال والمميـت للميكروبات المحقونة٠ وقد أثبتت هذه الدراسة ضرورة حفظ هذا المنتج المصنع من خامات غير معالجة حرارياً بعد تصنيعة لمدة لا تقل عن أسبوعين وبتركيز من حمـض الخلـيك لا يقـل عن النسبة الموصى بها (٠,٧%) وذلك ليكون المنتج آمن لصحة المستهلك٠

SUMMARY

One type of commercial cholesterol-free, reduced-calorie mayonnaise (CFM) made with different levels of acetic acid, was evaluated to determine the survival characteristics of *Salmonella typhimurium, Listeria monocytogenes* F5069 serotype (4b) and *E. coli* O157:H7. Three formulation of CFM made with 0.3, 0.5 or 0.7% acetic acid in the aqueous phase were evaluated. The initial pH of the products after

equilibration ranged from 3.9 to 4.1 which were adjusted by addition of HCL. Products were inoculated separately with 3 strains of *Salmonella typhimurium, Listeria monocytogenes and E. coli* O157:H7 respectively; at Ca 10^6 cfu/g and held at 23.9°C for up to 2 weeks. *Listeria monocytogenes* survived longer than *E. coli* followed by Salmonella in equivalent preparation of mayonnaise.

No Salmonella was detected at 48 h in mayonnaise made with 0.7% acetic acid in the aqueous phase. No *Listeria monocytogenes* was detected at 14 days post-inoculation in CFM made with 0.7% acetic acid, also no *E. coli* O157: H7 was detected at 5 days after CFM inoculation at the same level f acetic acid. Results indicated that these new varieties of mayonnaise when formulated with 0.7% acetic acid in the aqueous phase will inactivate $>10^6$ Salmonella and $>10^3$, $>10^4$ cfu/g of *Listeria monocytogenes* and *E. coli* O157: H7 respectively within 48 h. holding time required for regular mayonnaise made with un-pasteurized eggs. Hence, properly acidfied (pH<4.1) reduced-calorie mayonnaise containing 0.7% acetic acid in the aqueous phase is a microbiologically safe product.

Key words: *Mayonnaise, E. coli, S. typhimurium, L. monocytogenes.*

INTRODUCTION

Consumer demand for low calorie and low cholesterol foods has prompted the development of a new generation of products that include both reduced-calorie and cholesterol-free mayonnaise.

Commercially prepared dressing such as mayonnaise has never been directly identified as a cause of any foodborne illness. This performance is largely due to the intrinsic bactericidal nature of these products.

Organic acids and acid ingredients contribute to the desirable flavor of these products and are toxic to foodborne pathogens at or below their Pk_a (Brudzinski and Harrison, 1998). Acetic acid is the predominate acid, which is added as a various types of vinegars, (Smittle, 2000).

In general this product is composed of a vegetable oil or fat-free oil substitute base, salt-containing water (aqueous phase), acetic acid in the water phase and to a lesser extend lactic or citric acids or both depending on the formulation. In addition, it is flavored by acid-containing ingredients such as butter milk, sour cream, blue cheese and other fermented dairy products (lactic acid), lemon juice (citric acid), (Entani *et al.*, 1998).

Federal regulations required that commercially manufactured dressing such as mayonnaise made with un-pasteurized eggs must have a pH of less than or equal 4.1, an acetic acid level of the aqueous phase of greater than or equal to 1.4%, and holding period of 72 h. before the product is shipped (CFR part 101.100). These conditions were established to assure destruction of Salmonella, Listeria (Kathleen and Micheal, 1991) and Enterohaemorrhagic *E. coli* (Errol *et al.*, 1994). The authors were determined that acetic acid was the ingredient in mayonnaise but using 1.4% acetic acid in the aqueous phase of cholesterol-free calorie mayonnaise would result in an organoleptically unacceptable product. Hence, manufactures must use lower levels of acetic acid for such products.

The use of un-pasteurized eggs in commercial mayonnaise was discontinued in the early 1970's, with most manufactures using exclusively USDA-certified, pasteurized eggs. However, on rare occasion sporadic low level contamination with Salmonella, Listeria and *E. coli* may occur in pasteurized eggs. Hence, the ability of the acidity of reduced calorie mayonnaise to destroy foodborne pathogens is an important consideration.

An acetic acid level of 0.7% or more in the aqueous phase which is commonly used in commercial, reduced calorie mayonnaise is likely sufficient to kill Salmonella, (Jeanne-Marie *et al.*, 1997). However, this has not been verified. In addition although *Listeria monocytogenes* 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 *Listeria monocytogenes* in reduced calorie mayonnaise. This is particularly important considering the relatively high heat tolerance of *Listeria monocytogenes* in liquid whole egg, reported by (Foegeding & Leasor, 1990 and Foegeding & Stanley, 1990) which revealed that the minimal heat processing of 60 °C for 3.5 min for pasteurization of liquid whole eggs would kill only 2 to 3 $\log_{10}$ *L. monocytogenes*/ml. That means heavily contaminated whole eggs may be unsafe even after pasteurization for this pathogens.

Two recent outbreaks in the Pacific Northwest epidemiologically implicated mayonnaise as vehicle of *E. coli* O157: H7 transmission, possibly after cross-contamination with meat products. This suggested that strain of *E. coli* 157: H7 might be more resistant to acetic conditions than previously believed, (Errol *et al.*, 1994).

This study is initiated to determine the microbiological safety of new generation mayonnaise products by studying the effect of different levels of acetic acid on the fate of *Salmonella typhimurium, Listeria monocytogenes* and *E. coli* 157: H7 in cholesterol-free reduced calorie mayonnaise.

MATERIALS and METHODS

One type of (Cholesterol-Free reduced-Calorie Mayonnaise) "CFM" was made in the laboratory with pasteurized liquid egg whites at 60 °C for 3.5 minutes, (Foegeding and Stanley, 1990) and had the following composition: 32.7% olive oil, 54.8% moisture, 2.32% salt (aqueous phase), 3.7% sucrose (aqueous phase), 0.10% potassium sorbet and 0.0077% EDTA, (Kathleen and Michael, 1991).

Three formulation of CFM were evaluated which differed on the basis of acetic acid and HCL content. The formulation contained 0.3, 0.5 and 0.7 % acetic acid in the aqueous phase, which the pH adjusted at the time of manufacture to 4.0 ± 0.1 to confirm with CFR part 101.100 criteria with HCL for all the formulation except 0.7% acetic acid. The percent of HCL added to each batch was 0.012 and 0.039 for 0.3% and 0.5 acetic acid formulation respectively. HCL was used to adjust pH because it is among the least bactericidal of acids which was used strictly for experiment purpose; it is not normally used in formulation of commercial; mayonnaise or salad dressing.

Product with 0.7% acetic acid only in the aqueous phase is representative of commercial product, hence no HCL was added. In addition, a formulation with no acetic acid or HCL (pH 5.8) was included as positive control.

Salmonella in reduced-calorie mayonnaise:

A) Inoculum:

One strain of *Salmonella typhimurium* obtained from Animal Health Research Institute Dokki-Giza was used as inoculum which was associated to eat meal (sandwiches) illnesses.

The pure strain was grown individually in 10 ml of tryptic soy broth (Difco, Detrio, M1) at 37 °C for 10 h.

Cells were sediment by centrifugation (3500xg for 20 min.) and washed in 0.1 m phosphate buffered saline (PBS), cells were suspended in 1.0 M (PBS) and adjusted to Ca 10^8 cfu/ml, and one ml of inoculum aseptically was added to 99 gm of product under a static hood.

The inoculum was mixed with the product for about 2-3 min. with sterile longer depressor for uniform distribution and the inoculated

jars covered securely with metal-screw caps. The number of Salmonella for inoculated strain was confirmed by enumeration on tryptic soy agar (Difco).

B) Incubation and sampling of mayonnaise:

The product was incubated at 23.9 °C and sampled (3 jars for each formulation) at 0, 6, 24, 48, 72, 96, 120, 168 and 336 h. The unacidified control product was sampled at 0, 6, 24 and 48 h. the purpose of this control was to verify that un-acidified mayonnaise could support the growth of Salmonella, Listeria and *E. coli*. This product spoiled within a few days during incubation at 23.9 °C, hence it was not sampled for as long as the other formulations.

Zero-time sampling was completed within 7.5 min. after inoculation to minimize the effect of acidic environment on Salmonella.

Samples were assayed for Salmonella by enumeration according to USFDA BAM, (1984).

In addition, pH was determined for each sample using a digital laboratory pH meter (Model No. EA 940. Orion) at zero time and at the end of incubation.

All tests were done in triplicate, with an average of the results reports. Un-inoculated samples were assayed for aerobic plate count (plate count agar, 30 °C for 48 h.) and considered as control negative.

Listeria monocytogenes in reduced-calorie mayonnaise:

A) Inoculum:

Listeria monocytogenes F5069 serotype 4b was used as inoculum which was obtained from Dr. Robert Weaver, Center for Diseases Control, Atlanta, G. A. This strain represents serotype most frequently isolated from food products.

Pure inoculated strain was grown individually in 10 ml of tryptose phosphate broth at 37 °C for 16 h. and washed in (0.1 ml PBS) according to the procedure described above, and adjusted to Ca. 10^8 cfu/ml which was confirmed by enumeration on tryptose phosphate agar.

The mayonnaise was inoculated according to the procedure described above for the salmonella study.

B) Incubation and sampling of mayonnaise:

Products were incubated at 23.9 °C and sampled (3 jars for each formulation) at 0, 6, 24, 48, 72, 96, 120, 168 and 336 h., un-acidified control product was sampled at 0, 6, 24 and 48 h.

Samples were assayed for Listeria monocytogenes according to the procedure recommended by McClain and Lee, (1989). Plate counts and pH were determined according to the procedure described above.

Escherichia coli O157: H7 in reduced-calorie mayonnaise:

A) Inoculum:

Pure strain of Enterohaemorrhagic *E. coli* O157: H7 (EHEC) obtained from Animal Health Research Institute Dokki-Giza. (Fecal contamination of food stuffs) was used as inoculum which was maintained on trypticase soy agar slants, stored at 4 °C. The strain was individually inoculated into tryptic soy 0.6% yeast extract broth and incubated at 35 °C for 24 h., cells were sedimented by centrifugation (3500 xg for 15 min.). The EHEC strain was suspended in sterile physiological saline and adjusted to Ca. 10^8 cfu/ml population density (plate count method).

One ml of the inoculum aseptically was added to 99 gm of the product producing $<10^7$ cells/gm of target product.

Inoculated jars were thoroughly mixed for 2 to 3 min. as described above and tight by metal-screw caps.

B) Incubation and sampling of mayonnaise:

The product was incubated at 23.9 °C and sampled (3 jars for each formulation) at 0, 6, 24, 48, 72, 96, 120, 168 and 336 h. The unacidified control product was sampled at 0,6, 24 and 48 h.

Samples were assayed for (EHEC) by enumeration according to the procedure recommended by AOAC, (1990). Plate count and pH were determined according to the procedures described above.

RESULTS

Acidity is the most important intrinsic characteristic of mayonnaise in determining the growth and survival of pathogenic bacteria. Secondarily, salt and sugar play minor roles, but they have an interactive effect with acetic acid in vinegar on inhibiting the growth of foodborne pathogens, (Entani *et al.*, 1998).

Salmonella was inactivated to levels undetectable by enrichment (recovery of injured cells) to >6 $\log_{10}$ cfu/g decrease in CFM made with 0.7% acetic acid in the aqueous phase and held at 23.9 °C for 48 h. (Table 1 and Figure 1).

At 24 h., Salmonella in CFM with 0.3, 0.5, 0.7% acetic acid in aqueous phase had decreased by 2, 3, and 5 $\log_{10}$ cfu/g of the product respectively, whereas reached under detectable levels after 7, 4 days in CFM made with 0.3 and 0.5% acetic acid respectively. These results indicated that CFM which is formulated with egg white, had greater anti-salmonella properties than reduced calorie mayonnaise (RCM) which was made with egg yolk in previous studies by Kathleen and Michael, (1991) whereas Salmonella was inactivated to undetectable levels at 2

weeks and 1 week of incubation for the 0.5 and 0.3% formulation, but similar result was obtained in 0.7% formulation.

Salmonella typhimurium growth (Ca-2 $\log_{10}$ cfu/g increase in 48 h.) occurred in un-acidified CFM that served as positive control. (Figure, 1).

The pH values of 0.3, 0.5, and 0.7% acetic acid in aqueous phase of CFM after equilibration were 4.3, 4.1 and 3.9 respectively. There was little or no change (≤0.1 pH unit) of pH throughout the study.

Listeria monocytogenes was more resistant than *Salmonella typhimurium* at 0.7% acetic acid in the aqueous phase formulation (Table 2 and Figure 2), *Listeria monocytogenes* was inactivated to undetectable levels by enrichment within 14 days in CFM compared with 48 h. for *Salmonella typhimurium*, while the death rate was 0.5, 1, 2 $\log_{10}$ cfu/g decrease within 24 h. for 0.3, 0.5 and 0.7% acetic acid concentration. Similar results were obtained by Smittle, (2000). *Listeria monocytogenes* growth (Ca- 2.5 $\log_{10}$ cfu/g increases) occurred in un-acidified CFM after 48 h. (Figure 2).

Enterohaemorragic *E. coli* O 157:H7 ((EHEC) in CFM was inactivated to levels undetectable by enrichment (>6-$\log_{10}$ cfu/g. decrease) after 5 days of 0.7% acetic acid in aqueous phase (Table 3 and Figure 3). Similar results were obtained by John *et al.* (1995), while the death rate of *E. coli* within the first 24 h. of incubation was <4-$\log_{10}$, <3-$\log_{10}$, <2-$\log_{10}$, cfu/g decrease in 0.7, 0.5 and 0.3% acetic acid concentration in aqueous phase formulation respectively. *E. coli* O157: H7 growth (ca- 2 $\log_{10}$ cfu/g increase at 48 h.) occurred in un-acidified CFM (Figure 3). Previous investigation on the effect of pH on *E. coli* O157: H7 showed that *E. coli* O157: H7 was inactivated within 10 days and 17 days at pH 3.5 and 4.0 respectively in TSB acidified with HCL and kept at 27 °C, however when the medium was acidified with lactic acid, the organism was inactivated within 24 h. and 7 days at pH 3.5 and 4.0 respectively. (Abdul-Raouf, *et al.*, 1993).

In fact that we didn't recover the organism at 5 days may be due to the presence of acetic acid, which has been shown to have greater antimicrobial effects than most other acids. Several studies have demonstrated that types of acidulant would have different effects on microorganisms with acetic acid giving the greatest killing effects (Faber *et al.*, 1989 and Sorrels *et al.*, 1989).

All un-inoculated acidified mayonnaise products generally contained low levels of microorganisms at the time of inoculation with the average aerobic plate count $<10^2$ cfu/g.

Table 1: Behavior of *Salmonella typhimurium* in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 °C.

Time/hour	Control positive Log_{10} cfu/g	Acetic acid concentrations		
		Log_{10} cfu/g at 0.3%	Log_{10} cfu/g at 0.5%	Log_{10} cfu/g at 0.7%
0	7.30	6.82	6.38	6.21
6	8.44	5.81	4.42	4.23
24	9.00	4.36	3.65	1.30
48	9.25	3.91	2.60	0
72	-	3.23	1.83	0
96	-	2.63	0	-
120	-	2.1	0	-
168	-	0	-	-
336	-	0	-	-

Table 2: Behavior of *Listeria monocytogenes* F5069 serotype 4b in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 °C.

Time/hour	Control positive Log_{10} cfu/g	Acetic acid concentrations		
		Log_{10} cfu/g at 0.3%	Log_{10} cfu/g at 0.5%	Log_{10} cfu/g at 0.7%
0	6.80	6.53	6.40	6.31
6	7.21	6.09	6.00	5.80
24	8.50	5.99	5.47	4.22
48	9.20	4.60	4.28	3.85
72	-	3.23	2.63	1.33
96	-	2.85	2.50	1.26
120	-	2.28	2.00	1.20
168	-	1.82	1.50	1.00
336	-	0	0	0

Table 3: Behavior of *Escherichia coli* O 157: H7 in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 °C.

Time/hour	Control positive Log_{10} cfu/g	Acetic acid concentrations		
		Log_{10} cfu/g at 0.3%	Log_{10} cfu/g at 0.5%	Log_{10} cfu/g at 0.7%
0	7.00	6.90	6.35	6.22
6	7.26	6.32	5.56	5.02
24	8.43	5.03	4.04	3.10
48	9.11	4.60	3.80	2.23
72	-	3.25	3.24	1.84
96	-	2.90	2.82	0.5
120	-	2.43	1.30	0
168	-	1.00	0	0
336	-	0	0	-

Fig 1: Behavior of Salmonella typhimurium in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 ° C.

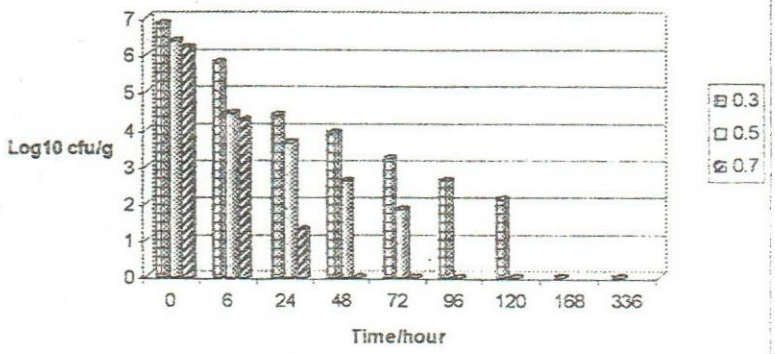


Fig. 2: Behavior of Listeria monocytogenes F5069 serotype 4b in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 ° C.

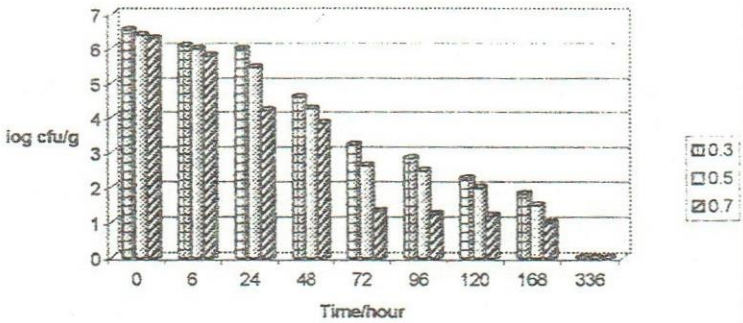
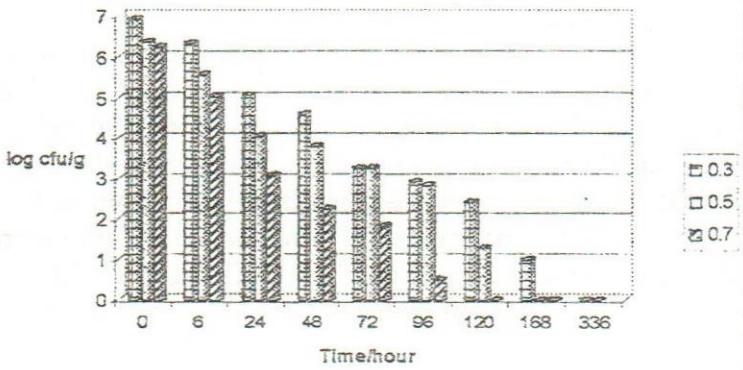


Fig.3: Behavior of Escherichia coli O 157: H7 in cholesterol Free Mayonnaise with 0.3, 0.5 and 0.7% acetic acid concentration at 23.9 ° C.



DISCUSSION

The purpose of this investigation was to determine the fate of *Salmonella typhimurium, Listeria monocytogenes* and *E. coli* O1547: H7 in the low pH CFM at normal storage conditions.

Our results indicated that several factors effect the survival of target bacteria either independently or synergistically with other components of the products.

Commercially 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, also Erickson and Jenkins, (1992) were speculated that the antilisterial activity was due to the egg white lysozome in CFM. Because microorganisms need water to grow and 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.

The storage temperature of the products in previously studies may explain differences in survivability of target microorganisms.

In acidified mayonnaise *Listeria monocytogenes* has been shown to die off more slowly at 4 °C than at 30 °C, (Parish and Higgins, (1989). Other studies (Glass and Doyle, 1991) reported that the lethal effect of acetic acid on *Listeria monocytogenes* was enhanced with higher incubation temperature due to the psychrotrophic nature of the organism. Abdul-Raouf *et al.,* (1993) have shown that population levels of *E. coli* O1547: H7 decreased on vegetable stored at 5 °C but increases at 12 and 21 °C with the most rapid increase observed at 21 °C.

Junkins and Doyle, (1992) discovered that many wild-type of EHEC isolates produced visible mucoid colonies with exopolysaccharide slime layers composed of colonic acid. This could partially explain EHEC acid tolerance in foods. The slime layer provides a physical protection barrier against hostile environmental conditions, there by blocking or delaying penetration of antimicrobial food ingredients into cells. Though EHEC is more acid tolerant than Salmonella in real mayonnaise but reproducible challenge study data

indicates that cellular protective factors are suppressed or destroyed in acetic acid-low pH (<3.9) cholesterol-free mayonnaise.

The importance of using acetic acid as acidulant of mayonnaise rather than other acids such as citric acid is substantiated by studies of Perales and Garcia, (1990). They observed that Salmonella was killed in home made mayonnaise acidified to pH 4.0 with acetic acid (vinegar; >5 $\log_{10}$ cfu/g reduction within 72 h) but not with citric acid (lemon juice; < 0.5 $\log_{10}$ cfu/g reduction within 120 days) at the same pH value.

New generation, reduced-calorie mayonnaise with 0.7% acetic acid in the aqueous phase eliminates detectable Salmonella within the time-temperature holding period regulated by the FDA, if un-pasteurized eggs are used by the manufacturer. Importantly, $>10^4$/g *Listeria monocytogenes* were inactivated within the 72 h. holding period established by the FDA, and this level of kill provides sufficient margin of safety considering that low levels of listeriae (<10 cfu/g) normally are present when Listeria monocytogenes is detected in ingredients.

As an additional margin of safety, under normal shipping and distribution practices, commercial mayonnaise is 2 to 4 weeks old before it reaches the store shelves. Even excessively high levels ($>10^7$ cfu/g) of *L. monocytogenes*, which is an extremely unlikely situation, would be killed under these conditions.

This study indicated that properly acidified (pH<4.1) reduced-calorie mayonnaise containing 0.7% acetic acid in the aqueous phase is a microbiologically safe product. Hence, acetic acid levels in the aqueous phase of greater than or equal t 1.4% as required by federal regulation can be reduced by 50% and still have microbiologically safe mayonnaise. However, it is incumbent on manufacturer to verify the microbiological safety of such formulations of the reduced-calorie mayonnaise and salad dressing that deviate substantially from the present acid and/or pH requirements of such products.

Good manufacturing practices and the implantation of Hazard Analysis Critical Control Point (HACCP) program in food manufacturing and food preparation can help to control pathogenic bacteria.

The prevention of cross-contamination of finished products with raw materials and unsanitary equipment, and proper personal hygiene are critical elements in controlling the contamination of food products with pathogens and spoilage microorganisms.

The inhibitory effects of mayonnaise could be compromised if this product is contaminated in a food service establishment and then

diluted in the preparation of other foods. More importantly, these products must be kept separated from possible sources of contamination, particularly meat and chicken products during storage and usage.

Therefore, it is important that food service establishment and house holds be aware that when preparing food with mayonnaise, the acidity and pH derived from the mayonnaise can't ensure the inhibition of growth of pathogenic bacteria by cross-contamination of such prepared food.

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