Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(2):412-418

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Comparative analysis of antimicrobial characteristics of mustard paste and powder in mayonnaise

Morteza Adeli Milani[1*], Maryam Mizani [2], Mehrdad Ghavami[2] and Parvin Eshratabadi[3]

[1]*Department of Food Science and Technology, Hidaj Branch, Islamic Azad University, Hidaj, Iran*
[2]*College of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[3]*Institute of Standard and Industrial Research, Karaj, Iran*

ABSTRACT

The present research was undertaken to make a comparison between the effects of different concentrations of yellow mustard powder and its paste on pH and microbial population. This research was an attempt to replace potassium sorbate and sodium benzoate with higher concentrations of yellow mustard to make free-preservatives mayonnaise. First, 0.00%, 0.10%, 0.20%, 0.30%, 0.40%, and 0.50% concentrations of yellow mustard powder were applied to the mayonnaise formula. According to the results, the higher the concentration of yellow mustard, the less the microbial population and the longer the shelf life. However, undesirable changes in taste and color as a result of using mustard powder was observed. Applying heat treatment eliminates the enzyme in higher concentrations of mustard past (0.00 to 1.50) %, responsible for undesirable sensory changes(p<0/05).These results concluded that the mayonnaise sample formulated with 1.00% mustard paste, free from chemical preservatives, was the most suitable sample to reduce microbial population and to prolong shelf life.

Keywords: Yellow mustard paste and powder; Antimicrobial characteristics; Sinalbin; 4-hydroxybenzyl isothiocyanate; Preservative-free Mayonnaise

---

INTRODUCTION

Mustard oil seed contains high levels of protein. Various genera of this plant, including more than 200 wild and 40 cultivated species, are in the U.S. and Canada. From an economical and practical point of view, three main species are known worldwide, namely yellow mustard, brown mustard and oriental mustard [1]. Beginning in the 1980s, production of low-acid/low-calorie salad dressings and condiments were widely promoted. Using natural additives, such as mustard, in condiments and salad dressings has been highly valued. This increasing adherence was a result of their ability in decreasing the acetic acid, oil content, concentrations of organic acid and salt and increasing pH and water phase of the product. Thus, in the new formulae of various food products, such as condiments and meat products, mustard has been used as a flavoring that improves the physicochemical properties and prolongs the shelf life. Nowadays, appreciating the functional properties of mustard and its variable applications in food industry seems crucial. In the yellow mustard seed, a kind of aromatic glucosinolate compound is found, which has the chemical formula of 4- hydroxybenzyl isothiocyanate known as Sinalbin [2, 3]. This compound lies in Aleuronic layer and vacuoles, which are surrounded by a membrane called Tonoplast. There exist about 200 µmol/g combinations of glucosinolate in mustard. As a result of the enzyme activity, these combinations are hydrolyzed and changed into a 4-hydroxybenzyl isothiocyanate compound [4].

Previous research shows that the resulting compound, known as IsoGard™, at relatively low-level usage, is an effective antimicrobial agent. When 25 mg/L of this compound is used in acidified fruit drinks, it will present

preservative characteristics against gram-negative, acid-tolerant bacteria, such as Glocunobactar and preservative-resistant yeast such as Zygosaccharomyces bailii. In addition, it improves the stability of the product up to 28 days against microbial growth at ambient temperature [5, 6]. Previous studies in neutral pH peptone broth under refrigerated conditions (6.5ºC), demonstrated that IsoGard™ acts against various pathogenic and spoilage bacteria, including Escherichia coli, staphylococcus aureus, Campylobacter jejuni, pseudomonas aeruginosa, Salmonella enteritidis and Shigella boydii. Using 360 mg/L of 4HBITC has lead into 6-log decrease in the microbial population. When injected into beef steaks as part of brine, an initial 4HBITC concentration of 46 mg L-1 yielded a one log reduction in aerobic plate count (28 days, 5-10ºC).

Antimicrobial effects of mustard flour and acetic acid were proved against three bacteria species, including Escherichia coli, Salmonella typhimurium and Listeria monocytogenes. In this study, 10-20% concentrations of mustard powder with 1% acetic acid exhibited synergistic antimicrobial effect. At 22ºC, this antimicrobial effect is more than 5ºC [7].

In active and modified atmosphere packaging of bread, the fungicidal effects of the isothiocyanate, existing in mustard, were tested against a range of fungi, such as penicillium roqueforti, penicillium commune, Asprgillus flavus and Endomyces fibuligra. Nielson and Rios (2002) have investigated the application of mustard essential oil in active packaging. In comparison with other spices, mustard essential oil in a concentration of 1 µl, proved to have the strongest effect to completely inhibit the growth of all the microorganism at 25ºC within 7 days. Moreover, active packaging was particularly useful to increase the shelf life of bread [8].

Studying mutagenicity effect of isothiocyanate compounds on Salmonella typhimurium exhibited the occurrence of an epoxide intermediate in allyl isothiocyanate metabolism. Another metabolic pathway, namely hydrolysis to allyl alcohol and oxidation to acrolein, a known mutagen, inhibitor of aldehyde dehydrogenase, can slightly increase the mutagenic potential [9].

Han et al. (2005) have studied the antimicrobial effect of mustard flour on Escherichia coli O157:H7 in ground beef under nitrogen-flushed packaging. In this research, along with an increase in the concentration of mustard powder, an increase in the antimicrobial effect of isothiocyanate on E-coli O157:H7 was observed. The results showed that using mustard flour at levels of >5–10% limits the growth of E. coli O157:H7 in fresh ground beef [10].In their MS thesis, Adeli Milani et al. (2005) have investigated the functional properties and application of yellow mustard in food industries to demonstrate its emulsifying, stabilizing, antioxidant, flavoring, and antimicrobial properties[11].

The purpose of this study is to investigate the effects of different concentrations of yellow mustard powder and paste on pH, microbial population, shelf life, and organoleptic properties of mayonnaise.

MATERIALS AND METHODS

Materials

Yellow mustard powder was obtained from G.S.DUN, a Canadian company. Other materials such as salt, vinegar, eggs, sugar, water, acetic acid, potassium sorbate and sodium benzoate were prepared, following the National Standards of Iran.

Samples Preparation

In this experiment, yellow mustard as a natural condiment was selected to investigate its antimicrobial and flavouring properties. Attempt was made to develop a useful formula of mayonnaise free from chemical preservatives and finally producing a product with desirable sensory properties. First, different samples of mustard powder concentrations were prepared to reduce microbial population of mayonnaise, and thus prolonging its shelf life. Then, sodium benzoate and potassium sorbate were eliminated from the formula of mayonnaise, while higher concentrations of mustard paste were used to produce chemical-preservative-free mayonnaise (Table 1 and 2).

Table 1: The treated mayonnaise samples with mustard powder and without sodium benzoate and Potassium sorbate

0.00%	0.10%	0.20%	0.30%	0.40%	0.50%	Mustard (%)
B	D11	D12	D13	D14	D15	Sample name

Table 2: The treated mayonnaise samples with mustard paste and without sodium benzoate and Potassium sorbate

0.00%	0.75%	1.00%	1.25%	1.50%	Mustard (%)
B	W1	W2	W3	W4	Sample name

The method for combining the materials of mayonnaise were taken from James Peterson and Mary et al. in

1998[12]. The whole production process was conducted under full hygienic condition, i.e. cleansing and disinfecting the equipment. At first, using a laboratory mixer, the beaten eggs were mixed with 15 grams of soybean oil. Then, the total amount of powder materials and half of the whole water were added. Next, soybean oil was gradually added within 6 minutes. After 4 minutes passed, the rest of the water was added; and after one minute, the vinegar was added. Finally, it took a total of eight minutes to create a homogeneous mixture. For different types of mayonnaise, weight of each batch was determined to be 3Kg, following the functional formula and in a way to meet the National Standards of Iran. The percentage recipes of free-preservatives mayonnaise are listed in Table 3[13, 14].

Table 3: Percentage recipes of the mayonnaise without potassium sorbate and sodium benzoate (wt, %)

Ingredients	Weight (%)
Egg	9.00
Sugar	3.85
Soybean oil	63.26
Vinegar	5.20
Xanthan gum	0.40
Salt	1.30
Water	17.37
Guar gum	0.10
Citric acid	0.14
Potassium sorbate	0.00
Sodium benzoate	0.00
Mustard	0.00-1.50

Experiments

In the present study, a number of experiments on determining antimicrobial properties, blanching and preparing mustard paste were carried out. To this end, mustard powder in samples of (0.0%, 0.10%, 0.20%, 0.30%, 0.40% and 0.50%), and paste in samples of (0.0%, 0.75%, 1.00%, 1.25% and 1.50%) were used. Subsequently, a number of tests were conducted, including microbial cultivation test, pH test, color test, and analysis of sensory properties of mayonnaise, i.e. flavor, smell and color.

Yellow Mustard Composition Analysis

The analysis of mustard was in accordance with the national standard of the Institute of Standards and Industrial Research of Iran; Mustard- Specifications and test methods. ISIRI no 246. 3rd revision, Karaj: ISIRI; 1999. [In Persian]

Yellow Mustard Paste Preparation

To inactivate the myrosinase enzyme of yellow mustard, 120g of mustard powder was mixed with 720g of vinegar according to the National Standards of Iran (NO; 355, 2008). This mixture was respectively in proportion of 1-6 in order to make a mustard-vinegar emulsion and facilitate heat-transfer. The resulting emulsion was heated in (800 cc) bottles by a thermostatic water bath (Grant Instruments, Cambridge, UK) for 10 min at 75°C. With reference to the aforementioned studies, the researchers had claimed that heating to the temperature of 75°C for 10 min would cause a noticeable decrease in the activities of Myrosinase enzyme [15]. It should be noted that, after the blanching process, the compound was cooled in a refrigerator (5° C) to prevent the side effects of heating. Followed by the thermal process, the resulting mustard solutions were filtered by Buchner funnel. The residue of this process on the upper Decanter chamber of the filter paper was mustard paste.

Microbial analysis

Counting tests for the total number of microorganisms were based on the national standards of Iran with the following numbers using the method of mixed cultivation and two time repetitions. The plates and greenhouse stocking were put in an Incubator with the temperature of 30° C for a period of 72±3 hours.

-Institute of Standards and Industrial Research of Iran, Microbiology of mayonnaise sauce and salad sauce – Specifications and test methods. ISIRI no 2965. 2nd revision, Karaj: ISIRI; 2002. [In Persian]

-Institute of Standards and Industrial Research of Iran, Mustard Powder- specifications. ISIRI no 3404. 3rd revision, Karaj: ISIRI; 1999. [In Persian]

-Institute of Standards and Industrial Research of Iran, Mayonnaise and salad souses -Specifications. ISIRI no 2454. 3rd revision, Karaj: ISIRI; 2003. [In Persian]

Microbial tests of produced treatments were carried out in intervals of 0 hours (immediately after production), 24 hours, one month, two months, three months after production, and in three concentrations of 0.1, 0.01, 0.001 and

two times of microbial cultivations for each concentration.

pH Measurement
The pH values of mayonnaise samples were measured at a temperature of 20 ± 0.5°C, using a Metrohm pH meter (Model 622, Switzerland), which was calibrated at a temperature of 20°C before use according to ISIRI 2454. 3rd revision, 2003.

Sensory Evaluation
A sensory evaluation of mayonnaise samples was conducted after preparation and during storage at 5°C for 3 month. Sensory characteristics: taste, flavor, smell, color, texture and overall acceptability were evaluated by a 30-member panel on 5-point hedonic scale, with 1 being the lowest and 5 the highest according to Barath et al. (2003). The panelists were mostly the experts from the Institute of Standard and Industrial Research of Iran. The mean of the results was used for the statistical analysis [16].

Statistical Analysis
The results were collected through duplicate measurements of tree samples by using the Statistical Analysis System program (version 8.1; SAS). Microbiological data were analyzed by the General Linear Models (GLM) procedure and Duncan s multiple range test with significant difference ($p< 0.5$) at storage interval for individual treatment. Sensory data were analyzed by ANOVA and t-test for separation of mean differences.

RESULTS AND DISCUSSION

Specification of Consumed Yellow Mustard (CYM)
Moisture Content
The moisture content of Consumed Yellow Mustard (CYM) was 3/9 %. This amount of moisture is in line with the National Standard of Iran (No. 3404, 1998) where the maximum acceptable ash percent in mustard is 7% (ISIRI 3404, 1998).

Total Ash
According to the results, the total amount of ash in CYM was 4%. This amount of ash is in line with the National Standard of Iran (No. 3404, 1998) where the maximum acceptable ash percent in mustard is 6% (ISIRI 3404, 1998).

Sensory Evaluation
The results of evaluating smell and taste in consumed mustard was indicative of a mild desirable bitter taste in CYM and its homogeneity with the national Standard of Iran (ISIRI 3404, 1998).

Antimicrobial Effect of Yellow Mustard Powder and Yellow Mustard Paste in Treated Samples
Table 4 shows the antimicrobial effects of different concentrations of yellow mustard powder on samples (D11-D15) in concentrations of 0.10%, 0.20%, 0.30%, 0.40%, 0.50% and the effect of different concentrations of mustard paste on samples (W1-W4) in amounts of 0.75%, 1.00%, 1.25%, 1.05% and also 0.00% in the resulting mayonnaise.

As shown in table 4, the microbial population, at the beginning of the test, decreased in all mustard-containing samples (D_{11}-D_{15}), in comparison with the control sample (B). Regarding the existence of Isothiocyanate in yellow mustard, the increase in amount of mustard powder leads into decrease in microbial population. In other words, the antimicrobial properties had a direct relation with an increase in the concentration of consumed mustard powder in mayonnaise samples. The decreased concentration of microbial population was concomitant with an increase in Isothiocyanate. As shown in table 4, counting the total number of microorganism three months after production, in samples (D_{11}-D_{15}) showed a 37.3% decrease in microbial population in sample D_{11}, 47.9% in sample D_{13} and 58.5% in D_{15}. Counting the total number of microorganism at intervals of 24 hours indicated the continuation of decreasing process in microbial population in samples W_1, W_2, W_3, and W_4, respectively containing 0.75%, 1.00%, 1.25% and 1.50% mustard paste other than the control sample (B).Microbial population increased to 670 CFU/g in control sample (B) and decreased to 290 CFU/g in sample W_1, and 190 CFU/g in sample W_4. This shows a direct relationship between the decrease in microbial population and the increase in the consumed mustard paste in mayonnaise.

The results obtained from counting the total number of microorganism at the intervals of 2 and 3 months after production was indicative of an increase in microbial population in all treated products. The microbial population, however, decreased by an increase in consumed mustard paste in mayonnaise.

Table 4: Results of the antimicrobial effects of different concentrations of yellow mustard powder on treated samples[a]

Time(h)										Sample Name
2160		1440		720		24		0		
B	590±10/00	b	480±10/00	b	430±20/00	b	410±10/00	B	535±20/00	D11
C	520±20/00	b	450±10/00	bc	400±10/00	b	390±10/00	B	510±20/00	D12
cd	490±20/00	c	405±20/00	c	370±10/00	c	330±10/00	C	460±10/00	D13
ecd	470±00/00	c	390±10/00	cd	350±20/00	cd	310±10/00	D	405±10/00	D14
gf	390±20/00	cd	380±10/00	d	310±10/00	cde	300±10/00	Ed	380±10/00	D15
gf	390±10/00	cd	360±10/00	d	310±10/00	cde	290±10/00	Ed	370±20/00	W1
gf	370±20/00	ed	330±10/00	d	290±20/00	def	260±10/00	Efg	340±20/00	W2
gh	340±10/00	ef	310±10/00	ed	270±10/00	fgh	230±10/00	Fg	320±20/00	W3
H	310±20/00	f	290±10/00	e	230±20/00	h	190±10/00	G	300±20/00	W4
A	940±10/00	a	880±10/00	a	740±10/00	a	670±10/00	A	580±20/00	B

[a] *Mean value of tree measurements.*

Table 5: Changes rate of microbial population in treated samples (W_1-W_4) to the control sample (B)

Time(h)					Sample Name
2160	1440	720	24	0	
59/79%	59.55%	58.66%	57.35%	37.28%	W1
61.85%	62.92%	61.33%	61.76%	42.37%	W2
64.94%	65.16%	64.00%	66.17%	45.76%	W3
68.04%	67.41%	69.33%	72.05%	49.15%	W4

Microbial population changes in samples (W1-W4), compared with the control sample, were analyzed on the interval of 3 months after production (table 5). It showed a 59.8% decrease in microbial population in sample W1 (containing 0.75% mustard powder), 61.8% in sample W2 (containing 1.00% mustard powder), 64.9% in sample W3 (containing 1.25% mustard powder) and 68% in sample W4 (containing 1.50 mustard powder). Accordingly, the heating treatment in the production process of mustard paste and the decrease in IsoGard™, stemmed from the decline in Myrosinase enzyme activity, necessitate an increase in the concentrations of mustard paste. This proliferation compensates the reduction of released Isothiocyanate and the increase in antimicrobial properties of mustard [17].

pH of Mayonnaise Samples

In table 6, the pH of treated samples is shown, affected by the different concentrations of yellow mustard powder (0.00%, 0.10%, 0.20%, 0.30%, 0.40%, and 0.50%) in samples (D11-D15) and yellow mustard paste (0.00%, 0.75%, 0.10%, 1.25% and 1.50%) in samples (W1-W4).

Table 6: Means of pH in treated samples[a]

*pH**	Sample Name
Kj 3.86±0.005	D11
Hj 3.87±0.003	D12
Gf 3.88±0.003	D13
Fe 3.86±0.005	D14
Fed 3.89±0.010	D15
Ed 3.90±0.003	W1
Cd 3.91±0.003	W2
B 3.92±0.006	W3
A 3.96±0.008	W4
Gf 3.88±0.003	B

**Mean value of tree measurements.*

Statistical analyses of GLM variance by SAS software showed the existence of a statistically significant difference in the means of pH test in the produced treatments ($p \leq 0.05$). Duncan test showed a statistically significant difference between the control sample and the treated samples. As shown in table 6, an increase in consumed mustard causes an increase in pH, but gradually, this proliferation decreased considerably. This reduction is a result of an increase in H+ which emanates from the effect of Myrosinase enzyme on Sinalbin of yellow mustard. As a consequence of a decrease in the activity of Myrosinase enzyme caused by heating treatment and the decrease of H+, the pH of samples containing 1.25% and 1.50% concentrations of mustard paste respectively increased to 3.92% and 3.96%. Accordingly, the mean of pH in mayonnaise sample W4 (containing 1.50% mustard paste) increased to 1.02%, compared with control sample. Regarding the results of pH measurement, all the treated samples were determined to be 3.6 – 4.1, which is consistent with the National Standard of Iran (ISIRI 2454, 2003). Regarding the pH limitation determined in National Standard of Iran (ISIRI 2454, 2003), the increase of pH in sample W4 (containing 1.50%

mustard paste) shows a proximity to the critical point. Hence, an increase in the concentrations of yellow mustard in the treated samples requires some changes in the formula of mayonnaise or a modern production process.

hese results of this study were in line with the findings of Xing et al. (1999) about the effects of mayonnaise components, such as mustard, on pH of the mayonnaise. This research also confirmed the findings of Buskov et al. (2000) about the effect of enzyme myrosinase on Sinalbin of yellow mustard [4, 18].

Sensory Evaluation of Treated Samples

In table 7, the color hue, taste, odor, stability and overall acceptability of mayonnaise samples are shown as effected by the different concentrations of yellow mustard powder (0.00%, 0.10%, 0.20%, 0.30%, 0.40%, and 0.50%) in samples (D11-D15) and mustard paste (0.00%, 0.75%, 1.00%, 1.25% and 1.50%) in samples (W1-W4) and control sample (CS). ANOVA GLM at the 95% level of significance showed a meaningful difference in the data analyzing texture, color, flavor, and general acceptances of different samples. At the same time, in treated samples, there is a lack of statistically significant difference in the data considering texture. Furthermore, analyzing the results of Duncan test showed a statistically significant difference in the data, obtained from the aforementioned tests and those from control group, excluding the data from the analysis of texture properties. Therefore, according to table 10, the aforementioned sensory evaluation group did not differentiate the texture properties of mayonnaise samples. Regarding the flavor, the lowest score (3.93) was allocated to the control sample (B). Along with the increase in mustard content from 0% to 0.3%, flavor score improved considerably to 4.80.

Table 7: Score Means of the sensory evaluation in treated samples[a]

Sample name	Consistency*	Color*	Odour*	Flavor*	Overall acceptability*	Texture
D11	4.63±1.02	4.80±1.06	4.73±0.98	4.33±1.22	4.70±1.20	4.93±1.01
D12	4.70±1.03	4.50±1.13	4.10±0.92	4.63±1.12	4.93±0.98	5.10±1.02
D13	4.73±1.01	4.46±1.16	4.13±0.89	4.80±1.06	4.40±0.93	5.00±1.08
D14	4.83±0.98	4.33±0.88	4.30±0.98	4.20±1.06	4.33±0.88	4.93±1.04
D15	5.00±0.90	4.20±0.96	4.53±1.10	4.13±1.00	4.13±1.07	5.23±1.04
W1	5.20±0.96	4.93±1.04	5.13±0.89	4.60±1.19	5.03±0.46	5.20±1.03
W2	5.23±0.81	4.80±0.96	4.83±1.01	4.93±0.98	5.33±0.80	5.10±1.06
W3	5.40±0.77	4.63±1.03	4.70±1.02	4.60±1.27	4.80±1.06	5.13±1.17
W4	540±1.06	4.40±0.96	5.10±0.95	4.43±0.88	4.40±0.96	5.10±1.06
B	4.50±1.10	5.40±0.96	4.30±0.93	3.93±1.15	4.10±0.88	5.03±0.99

[a] *Means value of 30 measurements. Statistically significant ($p \leq 0.05$)*.*

This proves the advantage of yellow mustard as the main flavouring component of mayonnaise. According to the results, the flavour score of mayonnaise decreased as a result of the increase in the concentration of mustard powder to 0.40% and 0.50%. It was a consequence of an increase in the content of Isothiocyanate, following the activity of Myrosinase enzyme in mustard powder. In higher concentrations of the yellow mustard powder, this proliferation causes a pungent flavour in mayonnaise. With the increase of mustard paste by 1.00%, the mean of flavour score increased to 4.76. The application of heating treatment in mustard paste led into Myrosinase enzyme deactivation, Isothiocyanate reduction and thus reduction of pungent flavour in mustard paste.

Therefore, thanks to the use of heating treatment and the production of mustard paste, the use of high concentrations of mustard paste (o.75%-1.50%) effectuated [15,19]. The results were in line with the findings of Barath et al. (2000) and Balint et al. (2006). The sensory evaluating group gave higher scores to the smell samples of mayonnaise, containing mustard paste (0.75%-1.50%), in comparison with the samples, containing mustard powder (0.10%-o.50%), and control sample [16, 20].

CONCLUSION

Applying heating treatment at the second stage of the experiment deactivated the myrosinase enzyme, which is responsible for creating the pungent flavour in yellow mustard. Hence, along with an increase in the amount of Isothiocyanate, the bitterness decreased. The heating treatment also destroyed the pigments of Anthocyanin in yellow mustard, which brought about a lighter colour of mustard paste, and improved its colour score. The results of the experiments on mayonnaise samples (W1-W4) using mustard paste and in high concentrations (0.75%-1.50%) was obtained without 2 chemical preservatives, namely potassium sorbate and sodium benzoate. This condition, in comparison with mustard powder in concentrations (0.10%-0.50%), intensively decreased the microbial population and prolonged the shelf life of mayonnaise. In addition, undesirable changes in colour and taste were reduced. This study specifies that 1.00% yellow mustard paste was the best concentration to decrease the microbial population and to prolong the shelf life of mayonnaise.

Acknowledgments
This research has been supported by Institute of Standards and Industrial Research Organization of Iran, Food and Agricultural Research Organization and Mahnam Food Company. The author would like to appreciate their financial support and their attempt to kindly provide laboratory facilities.

REFERENCES

[1] Clifford S, *Springdale Crop Synergies Ltd*, Rudston, East Yorkshire, **1999**, pp7.
[2] Cluad B, *Spices and mustard in today's dressings*, the R.T.French Company, **1965**, pp 62.
[3] Cui SW, Eskin NM, Liu U, *J Food Chem*, **2006**, 95, 83-93.
[4] Buskov S, Olsen CE, *Journal of Biochemical and Biophysical Methods*, **2000**, 43, 157-174.
[5] Douglas R, *Grain Research Laboratory Concentration of Canadian Grain Commission*, Canada, **2005**, 2-12.
[6] Liu M, Oleson, *Situation and outlook. Bi- weekly Bulletin*, Canada, **2005** ,pp12.
[7] Rhee M, Dougherty RH, kang D H, Lee y , *Department of Food Science and Human Nutrition*, Washington, **2003**, 164,2959-296**3**
[8] Nielsen V, Rios R, *J Food Microbiology*, **2000**, 60, 219-229.
[9] Henschler D, Neudecker T, *J mutation Research and Genetic Toxicology*, **1985**, 156(1-2): 269-278.
[10] Han JH, Holley R A, Nadarjah D. *J Food Microbiology*, **2005,** 99(3): 257-267.
[11] Adeli Milani M, Mizani M, Ghavami M, Eshratabadi P , *J Food Techno Nut*, **2007**, 4, 67-83.
[12] Peterson J, *First edition chapter 14, Mayonnaise Based sauces*, **1992**, 363-379.
[13] Astrom A, Langton M, Marie A. The Swedish institute for food and biotechnology". Got ebon Sweden, **1998**, pp 152.
[14] Codex Stan. 168-1989-Volume 11-**1994.**
[15] Hendrickx M, VanEylen O, Van Loey, A, J *Food chemistry*, **2006**, 97, 263-271.
[16] Barath A, Czukor B, Markus Z, *J Food Sci Emerge Techno*, **2000, 1**, 251-254.
[17] Bones A, Rossiter , *a Department of Biology, Norwegian University of Science and Technology London*, UK, **2006**,1053-1065.
[18] Xing R, Xie G ,*food research group,leeds characteriza university*, **1999**,49-56
[19] Edmonson AS, Meullenet JF, xie G, *J Food control*, **2002**,13(8):525-533.
[20] Balint c, IldikoSG, Klara KA, *J Food Sci Emerge Techno*, **2006**, 7, 74-79.