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Effect of Electromagnetic Waves (Microwave) on Microbial Load and Shelf Life of Liquid Egg Whites

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ABSTRACT

Background: Microwave method of treating liquid egg whites to inactivate or destroy a sufficient amount of micro-organism contained therein without significantly reducing the egg protein functionality so as to provide extended shelf life of the egg white **Objective:** In this study, liquid white egg was pasteurized by microwave in 2 different frequencies (2950-4500 MHz) and both frequencies together for constant times of (3,5 ,6) sec and 2 pulse(1,2) in storage times (5, 15, 60, 180 and 360 days), were compared with thermal pasteurized samples. **Results:** Results showed that effect of 4 variations include applied frequencies, duration process, number of pulses and shelf life of pasteurized product on reduction of total the microorganism count were statistically significant and the number of bacteria in the 360th day in pasteurized samples stored were 1.8×104 cfu / ml and the blank samples 2.15×106cfu / ml the number of coliforms in pasteurized samples were less than 10 pcs/mL and in the blank sample 1.15×104cfu / ml and the number of molds & yeasts in samples of pasteurized were less than 30 pcs/mL and in blank samples were 7.5×103 cfu / ml it was. **Conclusion:** This method can be used for pasteurization of egg in industry scale because of good shelf life and low load of microbial contamination. Also in compare with traditional pasteurization in big amount of product using of this method can save money.

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INTRODUCTION

Eggs are one of the most commonly consumed food products. Many of the dishes like Caesar salad, hollandaise potential hosts and carriers for pathogenic microbes like *Salmonella Enteritidis*, due to their rich nutritive value. Heat pasteurization is a good solution for controlling these pathogens (Hank *et al*., 2001). Eggs are used as a vital ingredient in several foods, especially for their exceptional functional properties (Hank *et al*., 2001). These properties mainly depend on the protein quality of the eggs and are severely affected when heated, due to protein denaturation (Dev, 2007). Proteins are highly heat sensitive components of the egg. The functional properties like whip ability, foam ability, foam stability etc. which make the eggs an inevitable ingredient of various food products are severely affected by high temperatures (Hank *et al*., 2001). Microwaves can be used to raise the temperature of in shell eggs to the required pasteurization temperature in minutes. It is also a proven fact that microwave enhances the thermal destruction of microbes (Tajchakavit 1997). Microwaves are not ionizing radiations but the dielectric properties of the microorganism (i.e. heat generated within the microorganism) itself enhance its destruction in a microwave environment. The microwave power distribution inside the shell eggs also seems to be well suited for uniform pasteurization (Datta *et al*. 2005). There is only some works which done on making microwave pasteurization viable for industrial use and there is very limited literature available. Microwave pasteurization of the eggs can make the process faster and continuous and the total operation can be completed in a few minutes. The shell egg appears ideally suited for pasteurization in a microwave environment (Fleischman 2004; Rehkopf 2005). In this study after different storage times (5, 15, 60, 180 and 360 days) the effect cold pasteurization by electromagnetic waves (microwave) on liquid white egg on the population of aerobic mesophilic bacteria, coliforms, mold & yeast, were examined, then were compared with control samples.

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Methodology:
Materials:

Samples were selected from a fresh and healthy batch of eggs and it was broken by egg breaker equipment, and were separated (yolk and white egg), were filtered (to separate crust and small particles) and were homogenized. These were performed by selecting 190 samples (100 g) from 100kg liquid egg whites randomly and they were filled in special poly ethylene bags automatically after filtering, they were stored at 0 to 4ºC until experiments.

Instrumentals specifications:

Electromagnetic pasteurizer was used (model SCP150, Afra Sanat Kimia Mashhad co, Mashhad, Iran). This device was able to work in variety of temperature, times and frequencies.

Methods:

190 pcs of samples 100 g in accordance with those mentioned above were prepared and 60 samples randomly selected within the reservoir system was placed and the frequency of 2950 MHz as pasteurized was applied in 10 samples in a pulse of 3 seconds -10 sample a pulse of 5 seconds -10 samples in a pulse of 6 seconds -10 sample two pulses of 3 seconds (6 seconds) -10 samples two pulses of 5 seconds (10 seconds) -10 samples two pulses of 6 seconds (12 seconds) and also randomly selected 60 samples exactly the same way and at a frequency of 4500 MHz was pasteurized. 60 samples with random acts of two frequencies were pasteurized and 10 samples were selected as control samples (non-pasteurized) were selected. And then effect of storage times on microorganisms samples treated in the appropriate conditions (0-4 C °) to a period of 5, 15, 60, 180, 360 days were studied . At the designated time, 36 samples from 18 treatment of the (two repeats) and two control samples for microbial cultures transferred to the laboratory for cultivation with culture medium of plate count agar in 2 repeat, and cultivated in 30 °C for 72 hours according to national standard NO. 5272,13248, and Total Aerobic Mesophilic Count (TAMC) and with culture medium of violet red bile glucose agar in 2 repeat, and cultivated in 37 °C for 24 hours according to national standard NO. 11166,13248, and total coliforms and with culture medium of yeasts extract glucose chloramphenicol agar in 2 repeat, and cultivated in 25 °C for 120 hours according to national standard NO. 10899, 13248 and molds & yeasts were determined.

Statistical analysis:

Each treatment was analyzed as randomized complete block design with ten replications and the data were assessed by analysis of variance (ANOVA) and Duncan's multiple range tests using MSTAT-C software program. Differences among treatments were tested with minimum significant difference (LSD) test ($P<0.05$). Besides, correlation analyses were performed to clarify the relations among parameters considered in this study. Microsoft Excel 2007 was used to plot apparent.

RESULTS AND DISCUSSION

The effect of different frequency electromagnetic waves (microwave) and different times of contact on microorganisms:

Microwave energy inactivates micro-organisms through thermal kill. It also has the potential to cause biological damage as well as alteration of the cell membrane and metabolic functions. This injurious effect on the living organisms may very well induce additional lethality and impaired recovery from injuries by them during the subsequent time period of product storage (Huang, 1989). In this study, two frequencies 2950, 4500 MHz and both together (4500 +2950 MHz) with a pulse time of 3,5, 6 and 2 pulse time 3.5, 6 seconds (6,10,12 seconds total times) on liquid white egg was investigated. results showed that in pasteurization with electromagnetic waves, the microorganisms present in the liquid white egg, reduced so that in the first test in the fifth day the average total number of bacteria in mesophilic aerobic samples pasteurized 2.05×102 cfu / ml and the control sample 7.2×103 cfu / ml, and coliforms , molds & yeasts were found in pasteurized samples (table1). Results confirmed with kozempel *et al*. (1999) which reported microwave energy has a significant effect on reduction of microbial population in egg liquid (Kozempel *et al*., 1999). Results also agreed with Dev *et al*. (2008) which showed in this study, microwave heating has been considered for in-shell egg pasteurization. First of all, the effects of temperature (0-62°C) and frequency (200 MHz to 10 GHz) on the dielectric properties of egg components were investigated. Laboratory trials on microwave heating of in-shell eggs indicated that the heating rates of both albumen and yolk were similar. Therefore, microwave heating appeared perfectly suited for in-shell egg pasteurization (Dev *et al*., 2008). According to results, that obtained from statistical analyzes, effect of adding frequency variants ($P<0.05$) and pasteurized product durability ($P<0.0001$) on reduction of total count of aerobic mesophilic in whites egg in α level was 0.05 (95% probability) and it was statistically significant, and interactions were observed between studied variants in cold pasteurization process. Storage time which was studied up to 360 days has a higher effect on TAMC than various frequencies applied during so the value of

increasing TAMC was higher than reduction of it during liquid white egg pasteurization process by different electromagnetic waves. results showed (Figure 1) the total of treatments, the frequency 4500 MHz with a total of two frequencies (4500 and 2950) was almost similar and the average number of aerobic mesophilic bacteria was 3.6 cfu /ml log (4×103 cfu/ml), but the 2950 MHz frequency have poorer performance and aerobic mesophilic bacteria's in the samples treated with was cfu / ml 9.3 log (8.3×103 cfu/ml). That it showed 2450 MHz frequency is better than 915 MHz frequency in pasteurization and sterilization, and higher frequencies will increase killing effects (Tang *et al*., 2002). In similar study; Lotfian et.al (2012) yolk liquid was pasteurized by using electromagnetic method in 3 different frequencies (2950-3950-4500 MHz) for constant time of 12 sec (6 sec of second pulse) and then results were compared with samples which were pasteurized at 65 C for 120 sec and control samples, after different times of storage(1,3,5,8,10 and 15 days). Results showed that used electromagnetic waves had significant effect on reduction of logarithmic count of aerobic mesophilic bacteria.

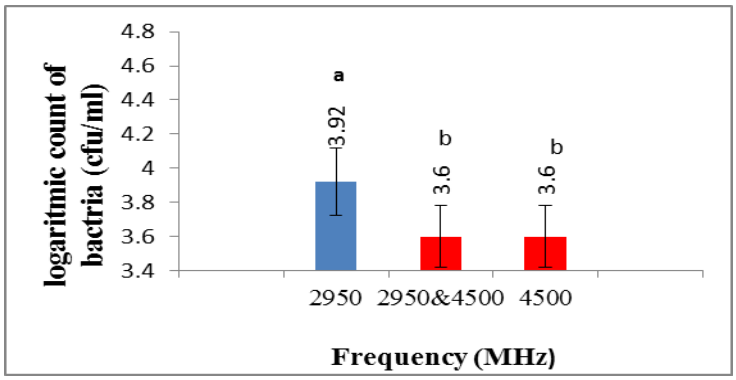

Fig. 1: Effects of different frequencies on TAMC in liquid white egg in cold pasteurization procedure.

Effect of process time of the fatality of microorganisms and the short time pasteurization process and produce a higher quality product:

Results also showed that the process time is effective on the fatality rate, and more time any time the higher frequency, higher case fatality rate. The number of aerobic mesophilic bacteria at 3, 5, 6 were 3.63, 3.72, 3.83 log cfu / ml respectively (Figure 2). In the higher pulse number, the fatality rate was higher and total count of bacterias was lower (Figure 3) .Microwave sterilization can deliver products that have good taste because microwaves are able to heat the product 3-5 times faster than conventional sterilization systems. The microwave -sterilized product is not temperature abused, so the food looks better, has better texture, and tastes better than products processed by any other available technologies (Harlfinger, 1992). There are significant reduction in process time which is important in results of other studies, also in this study confirmed total time of process is in most of the time (time off and relax in two 6-second pulse) only 15 sec that it is very short comparing with 120 sec time of thermal pasteurization, and why does not adversely impact on the functional properties of white egg. Tang and others (2010) showed that it is possible to reduction packaged food pasteurization time from 1.1 to 1.4 in compared with regular methods (Tang *et al*., 2010). In comparison with regular methods for heating foodstuffs, pasteurization by microwave potentially could improve organoleptic properties, appearance and nutritional value. Ohlson (1987) reported that very short time length in microwave process causes in producing a product with very higher grade and quality comparing with products obtained from regular heating methods (Ohlson, 1987). Also David Reznik (1995) showed that there are many obstacles in egg pasteurization because of its unique properties. Because of these contortions and according to trading practical respects, egg liquid thermal pasteurization is very difficult (Reznik, 1995). Results of this research showed that usage of both heat and waves could have stronger effect on lethal rate that it confirmed Kozmpel *et al.* which demonstrated that microwave energy may complete or strengthen thermal effects (Kozmpel *et al*., 1999). Such that Sanvo co. (2010) used microwave method combine with 65°C heating and claimed that this methods very quickly and total time of operations is only a part of second and product heated to only coagulation and coagulation point and consequently the product was completely like fresh egg. This process decreases total amount of bacterias 10 times more than regular pasteurization methods. Studies showed that the method will protect egg functional properties 20% more than regular methods (Colavitti *et al*., 2010). Different studies have severally referred to physical damages to egg and damages on its functional properties in temperatures above 60 °C, for instance .Wang et.al. (2009) showed that thermal denaturation was affected by dielectric properties and polar compounds in egg, and these changes (higher loss coefficient) occur in temperatures higher than 60°C which is effective on egg functional properties and results in losing some of this properties (Wang *et al*.,2009). In a similar study by Huang; egg white-containing mixture was inoculated with Escherichia coli (ATCC #25922) at a concentration of 10 cfu per ml, and subjected to microwave heating in various product temperatures. The resultant samples

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were tested to determine bacterial survival and water soluble protein content. An Arrhenius plot of the inactivation rate vs. temperature (1/T) was then constructed. The rate of bacterial fatality increased at a greater pace than that of the protein denaturation. Based upon the study results, it is believed that the advantage of having bacterial killed by increasing the processing temperature outweighs the drawback of losing some protein functionality (Huang, 1989).

Table 1: Treated samples by electromagnetic waves compared with blank samples in growth of microorganisms (cfu/ml) during storage of white egg.

Day/resuls	Total count(cfu/ml)		Coliforms(cfu/ml)		Molds/ yeasts (cfu/ml)	
Storage (day)	Blank sample	Treatment sample	Blank sample	Treatment sample	Blank sample	Treatment sample
5	7.2×10^{3}	2.05×10^{2}	9	0	40	0
15	2.6×10^{3}	6.2×10^{2}	85	0	1.2×10^{2}	1
60	6.05×10^{3}	1.1×10^{3}	3.5×10^{2}	1	6.0×10^{2}	2
180	3.9×10^{3}	1.8×10^{3}	1.0×10^{3}	2	1.9×10^{3}	3
360	2.15×10^{6}	1.8×10^{3}	1.15×10^{4}	6	7.5×10^{3}	22

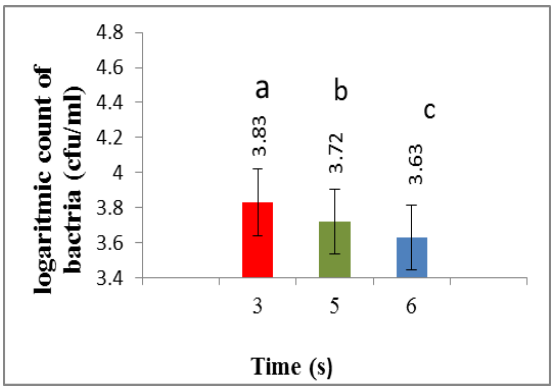

Fig. 2: Effects of different time process on TAMC in liquid white egg in cold pasteurization procedure.

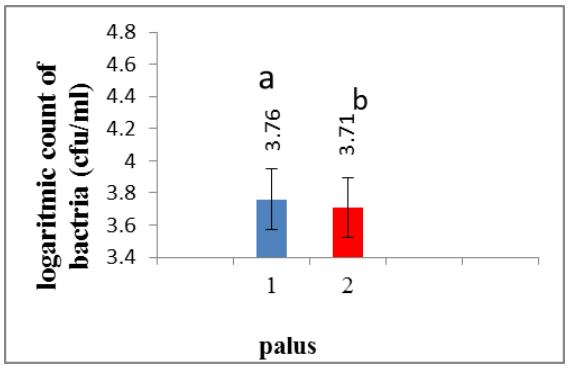

Fig. 3: Effect of different pulses on TAMC in liquid white egg in cold pasteurization procedure.

Determination pasteurized sample's storage time according to Iranian national standard limit:

The numbers of aerobic mesophilic bacteria in pasteurized samples were 2.31 log cfu / ml in the fifth day and 4.25 log cfu /ml in the 360 th day. This means that during the year, about 1.94 cycle logarithmic increase. Whereashe number of aerobic mesophilic bacteria in blank samples was 3.86 log cfu / ml in the fifth day and 7.33 log cfu / ml in the 360th day. This means that, about 3.47 cycle logarithmic increase during the year (figure 5). Also the average number of coliforms count in the different treatments pasteurized with waves were less than 10 per ml and in blank samples were the 1.15×104 cfu / ml in the 360th day. also the average number of molds & yeasts count in the different treatments pasteurized with waves were less than 30 per ml and in blank samples were the 7.5×103 cfu / ml in the 360th day (table 1). Thus, according to Iran's national standard for limits the number of aerobic mesophilic bacteria's in conventional thermal pasteurized products (3.0×104) and limits for the number of coliforms (less than 10) per ml and limits the number of molds & yeasts (less than 50) per ml and the products shelf life 7 days at(0-4 °C) has been determined. Then Concluded that pasteurized samples with wave after one year is still held. Therefore results of this study, confirmed Sanvo co. reports (2010) that invented commercial system for using waves in egg pasteurization , and announced that

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pasteurization by waves can increase eggs shelf life and protect its functional properties (Colavitti, 2010). Lotfian et.al (2012) showed that; according to Iranian National Standard, in electromagnetic method liquid yolk shelf life has been defined 14 days, which it is very ideal in comparison with thermal pasteurization (7d max) and control sample (2d max). Tang *et al*. (2002) in Washington university by similar studies on bean showed that the products processed by microwave had better color and taste than those pasteurizing by regular method in cans and shelf life of sterile product by microwave will increase (Tang *et al*., 2002).

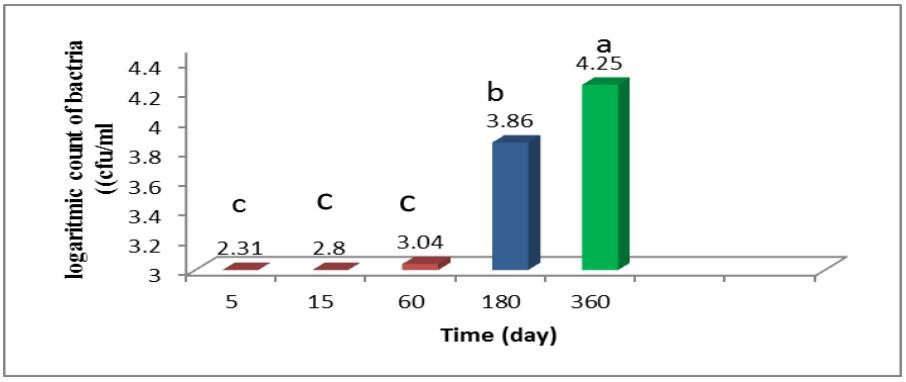

Fig. 4: Effect of different storage time on logarithmic amount of aerobic mesophilic bacteria's in liquid white egg in cold pasteurization procedure.

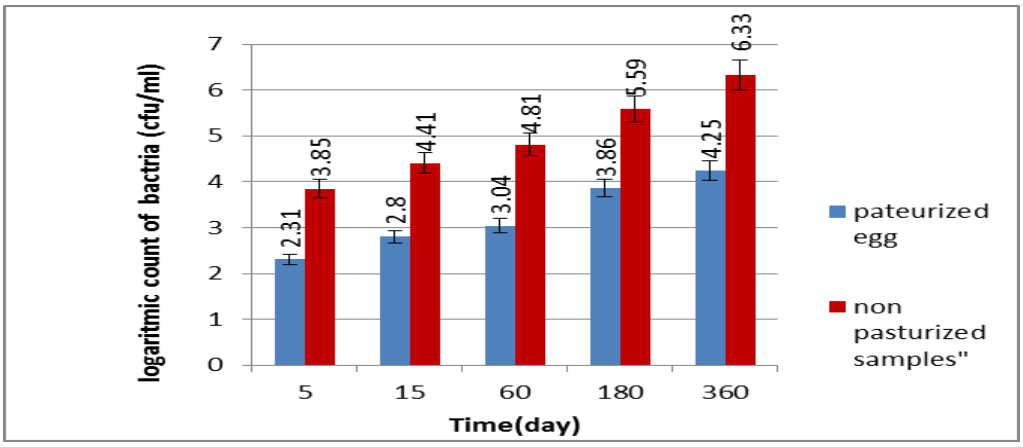

Fig. 5: Effect of different storage time on logarithmic amount of aerobic mesophilic bacteria's in pasteurized & non- pasteurized liquid white egg.

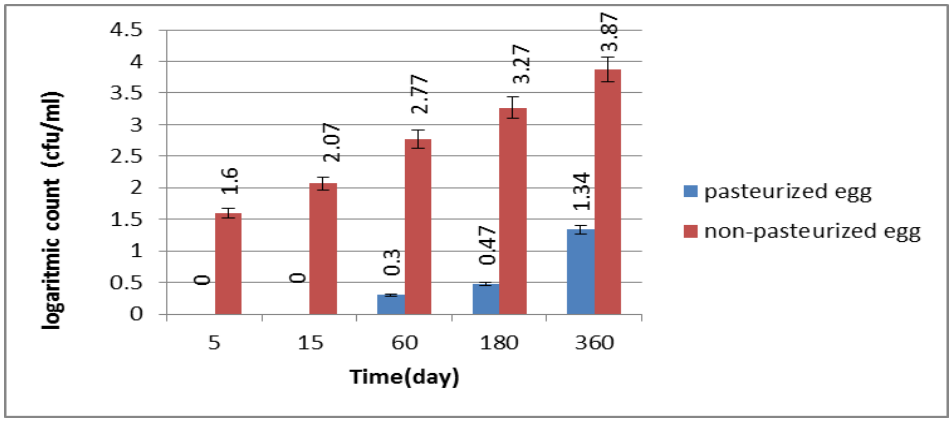

Fig. 6: Effect of different storage time on logarithmic amount of molds & yeasts in pasteurized & non-pasteurized liquid white egg.

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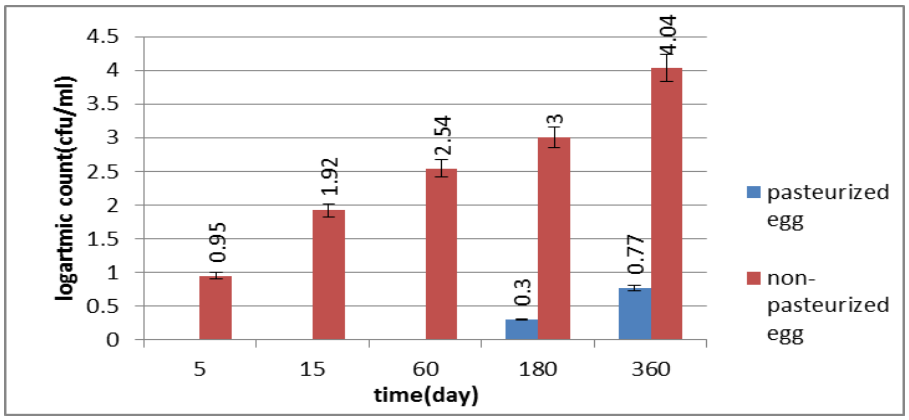

Fig. 7: Effect of different storage time on logarithmic amount of coliform bacteria's in pasteurized & non-pasteurized liquid white egg.

Conclusion:

Liquid white egg cold pasteurization significantly decreased total aerobic mesophilic count & other microorganism in samples. Therefore this method can be used in liquid white egg manufacturing industry. Because the white egg are extremely sensitive to temperature and heating time on protein denaturation and loss of functional properties, it is very effective .So this method can be a safe and high quality process for use in food production. Shelf life of heat pasteurized liquid white egg in the refrigerator temperature is maximum one month and in this new methods 12 times (about one year) increased, then it has economic benefits of reduction in production costs and maintenance and transportation for manufacturers and consumers and less impact of price fluctuations in fresh eggs.

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