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Full Length Research Paper

Combination of high frequency electromagnetic fields with pre heat to inactivate mesophil microorganisms of flexible packed cooked chick and cooked chick meal

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The effect of high frequency Electromagnetic Induction (EMI) combined with various condition of pre-heating for inactivation of mesophil microorganisms in multilayer flexible pouches has been studied. All samples were filled in pouches and, have been put in (water bath chamber; different condition of pre heating (80°C 5 min, 80°C 10 min, 80°C 15 min, 85°C 5 min, 85°C 10 min, 85°C 15 min) have been done; and ready for EMI sterilization which discharges square-wave pulses with variable voltage 1-20 kV/cm and frequency 8-10 GHz. The spores of these bacteria (gr+) were practically resistant in electric field; however, pre heat caused spore changed its behavior from passive forms (latent) to active forms (vegetative). If cells are cultivated at higher temperature, increasing tendency which can permanently keep fluidity viscosity of the cell membrane before electromagnetic field so EMI efficiency is increased. The populations of mesophil microorganisms depended on type of treatment type of chick type and type of culture. The death ratio of mesophil microorganisms increasing in chick 14200% more than chick meal, chance of negative mesophile microorganism growth in every treatment compares with last treatment increasing 54%. Negative growth in culture "PCA" is 3.3 degree more than culture "PE 2, in culture "PE 2" is 330% more than culture "Cook meat"; how ere these parameter in various thermal processing without EMI was evaluated positive mesophile microorganism growth increasing in chick meal 1905% more than chick type, and chance of passive mesophile microorganism growth in every treatment compares with last treatment decreasing 41% and type culture have no effect on growth of mesophil bacteria.

Key words: High frequency electromagnetic fields, electromagnetic induction, flexible packaging, mesophil bacteria, thermal processing, cooked chick, cooked chick meal.

INTRODUCTION

During the latter years, consumption of ready to eat food has plenty effect in manner of offering of new food packaging products. This quick change is because of that in lately decades enter variety forms of restorable multi layers of plastic films laminated with aluminum (Datta and Prosetya, 1991) for packaging cooked meat and cooked poultry instead of can (Anonymous, 1999). The meat and poultry industry's efforts at surveillance and intervention, but not eliminated, second microbial contamination of these packed products (Food born Outbreak Response Surveillance Unit, 2000; Food Safety and Inspection Service, 2003) which may be potential

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source of patho-gens microorganism, specially mesophil aerobic and an aerobic bacteria, since the low acidity (pH 4-5) and suitable water activity of these packed meal can favor the growth of their activity (Mortazavi, et al., 1989, 2002) in this package and, consumers continue to have preven-table illnesses and even to die as a result of microbial contamination of foods (Food Safety and Inspection Service, 2003). Although, thermal treatment (120°C, 20 min) effectively destroys these mesophil microorganisms (Mortazavi et al., 1989) has been used widely, proteins and some other physiological substrates are denatured or inactivated, and consequently the flavor, taste, and contents of nutrients in foods are lost (Kasevich, 1998; Mortazavi et al., 2002). “Some of these non thermal techniques were developed, but we have also tested and improved other technologies” Zhang says. “Our work has improved food safety by enabling the food industry to make better decisions about how to reduce or eliminate pathogens microorganism (Food Safety and Inspection Service, 2003). Such treatment is carried out at high tem-perature at which shrinkages and leakages of pouches have been occurred that caused second contamination. For that reason, significant efforts are leading to the development of novel non-thermal processes such as high frequency electromagnetic fields, an alternative pre-servation process that is proving to be able to inactivate mesophil (spoilage) microorganisms without significantly affect nutritional properties of several foods (Jay et al., 2003; Piyasena et al., 2003). This technology involves the application high frequency (2-15 GHZ) in electric field (typically 1-20 kV/cm) to fluid foods placed between two electrodes in batch flow systems using low processing temperatures (near 40°C) and low energy consumption with regard to the thermal treatment (Mudgett and Schwartzberg, 1982; Rowley, 2001). This frequency allocated by Federal Communication Commission (FCC) (Metaxas and Meredith, 1988; Metaxas, 1996; Roussy and Pearce, 1995). It is worthwhile to note outside of USA frequency 43 GHZ are used also (Food Safety and Inspection Service, 2003).The primary advantage of improved uniformity of heating was shown in- package sterilized by this method (Datta and Liu, 1992; Datta et al., 1994; Datta et al, 1992; Wig et al., 1999). However, the effect of high frequency electromagnetic fields treatment on mesophil microorganisms of cooked chick is not adequate (Mortazavi et al.,1989; Nourozi, 1992), in this method vegetative form of microorganism was inactive but spore of these bacteria are too resistance(Hamilton and Sale, 1967; Knorr et al., 1994), mesophil microorganisms of cooked chick with spores are usually gram positives with special cell (Nourozi, 1992) the use of EMI in combination with various pre heating condition caused spore changed its behavior from passive forms (latent) to active forms (vegetative) so inactivate without a significant adverse effect on food properties and taste (Zhang and Datta, 1999; 2000) has been investigated by many researchers (Jayaram and Castle, 1992; Qin et al., 1994; Sinensky,

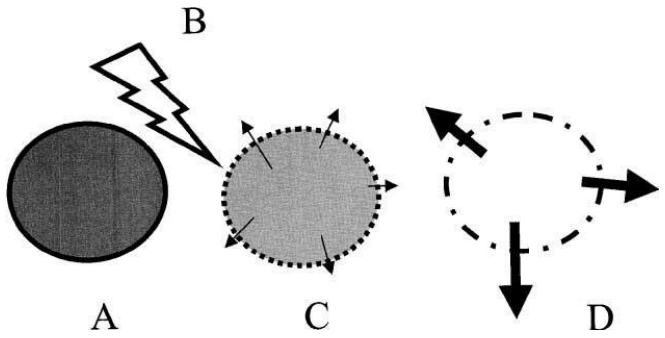

Figure 1. Irreversible electropermeabilization. (A) Intact cells. Their cytoplasm content is pictured in dark grey. (B*) pre heat (B) electromagnetic. (C) Cell membranes are permeabilized. The cytoplasm content leaks out as shown by the light grey colour and the small arrows. (D) The cell membrane is irreversibly permeabilized and cannot be repaired. All the cytoplasmic content leaks out.

1974). Breakdown is well known and can be explained relatively by electromechanical compression (Zimmermann, 1982).This phenomenon causes the formation of trans membrane pores. The size or number of these pores can be varied according to the parameters of electric field. If the total area of induced pores is small in relation to the total surface area of the membrane, the pores are able to close again mainly due to the diffusion of lipid molecules and rearrangement of the proteins (reversible disruption). If the number pores is approaching a critical limit of the membrane, it is no longer able to recover these pores. In this method, the ratio of total pore area becomes unfavorable; the membrane is no longer able to repair these perturbations (irreversible disruption). The effect of system seems to be related to the temperature of medium in which the bacteria are suspended (Jayaram and Castle, 1992; Ohshima et al., 1997; Zhang et al., 1994). On the other hand, bacteria have an optimal temperature for the cultivation or growth, but these can be cultivated at lower or higher temperature than optimal bacteria can vary the fatty acid composition of membrane lipids as a function of the culture temperature (McElhaney and Souza, 1976; Sinensky, 1974).

In this study, we investigate the electromagnetic steri-lization mesophil microorganism in packed cooked chick and packed cooked chick meal that were cultivated at various temperatures which would be primarily attack for bacteria (Figure 1).

MATERIALS AND METHODS

Chick and chick meal preparation

Chicken 1.7 kg weights were chosen for this experiment from local supermarket Mashad- Iran. The chickens were washed and cooked in water with 1% salt (Wig et al., 1999; Zhang et al., 1999). After cooking, cut into sliced (26) two kinds of samples were prepared: (Fakhouri and Ramaswamy, 1993).

1. Pouches contain 50 g chick (Chick)
2. Pouches contain 50 g chick+50 g sauce (pH=4.5, Brix =8, salt=1.5%) (Chick meal)

All pouches were filled hot for pull out oxygen (exhausting) and after sealing pouches different condition of pre heating have been done in bath water; then cool them immediately (T=20°C). The approximate of oxygen in pouches is 2-3% which is measured.

Analytical parameters such as pH (Crison, 2001 pHmeter; Crison Instruments, SA, Barcelona, Spain) soluble solid content (Atago RX-1000 refract meter; Atago Company Ltd., Japan), sealer (Impulse sealer, Manual Instruction, Korea) O_2-measuring cell (Electro-chemical MAT14 Modified Atmosphere Packaging Control, cycobel group, Germany) were measured according to the ISIRI Regulation (Institute of Standards and Industrial Research of Iran (ISIRI), No 2326 (2003a), Institute of Standards and Industrial Research of Iran (ISIRI), No 8758 (2003c)).

Microbial culture

PCA (Peptone from casein 5 g /1000 ml; glucose 1 g /1000 ml, Yeast extract 2.5 g /1000 ml. Agar 14 g /1000 ml, Distillated water 1000 ml) plate count agar is a general media for aerobic for aerobic. RCM (Peptone from casein 10 g/1000 ml; Meat extract 10 g /1000, Yeast extract 3 g /1000 ml, Starch 1 g/1000 ml, glucose 5 g /1000 ml, l- cystein hydrochloride 0.5 g /1000 ml, Sodium acetate 3 g /1000 ml, Sodium chloride 5 g /1000 ml, Agar 12.5 g /1000 ml, Distillated water 1000 ml). Rein Clostridia is a culture media for clostridium. CMM (Beef heart 454 g /1000 ml, Proteose peptone 20 g /1000 ml, glucose 5 g /1000 ml, Sodium chloride 5 g /1000 ml, Sodium hydrochloride ½ 454 g /1000 ml, Distillated water 1000 ml). Cooked meat is enrichment media for aerobic bacteria. PE 2 (Peptone digest of animal extract 20 g /1000 ml, Yeast extract 3 g /1000 ml, 2% alcoholic solution of bromocresol purple 0.04 g /1000 ml, Cicer arietinum L 450 no, Distillated water 1000 ml) . Peptone yeast extract Bromocresol purple is enrichment media for anaerobic bacteria (Institute of Standards and Industrial Research of Iran (ISIRI), No. 2326 (2003a), Institute of Standards and Industrial Research of Iran (ISIRI), No. 3139 (2003b)).

For microbial test each sample chick and chick meal to be treated with or without high frequency electromagnetic fields and pre heating were inoculated 15 day in temperature 37°C for mesophil bacteria growth, after incubation for aerobic growth 1 - 2 g of samples were put in CCM (3 - 4 day) then 1 - 2 g from CCM transfer to PCA after 2 - 5 day for anaerobic growth 1 - 2 g of samples were put in PE 2(3 - 4 day) then 1 - 2 g from PE 2 transfer to RCM after 2 - 5 day. Growth of bacteria in CCM and PE 2 has been showed as positive or negative response (Institute of Standards and Industrial Research of Iran (ISIRI), No. 2326 (2003a), Institute of Standards and Industrial Research of Iran (ISIRI),No. 8758 (2003c) (bad odor discoloration and producing gas) so in this investigation the growth of bacteria in PCA and RCM, CCM, PE 2, have been showed as response (non parametric)

High frequency electromagnetic field and processing parameters

A continuous flow high frequency electromagnetic model pilot-scale (www.ASKco.org; Khabazi, 2005a b, 2006) which discharges square-wave pulses (Hamilton and Sale, 1967) was used to process samples of chick and chick meal. Inner part of system composed electromagnetic induction, water bath, and stainless-steel tube submerged in water bath, variable pump electromagnetic induction containing, capacitor: balance of voltage; fuse: safety of system; diode: safety of system; magnetron: source of frequency transformation: change of voltage 1-20 kV/cm (Hamilton and Sale, 1967) in different frequency (2-15 GHZ) . The packages of chick and chick meal was put between treatment chamber with volume 60 lit (W=40 cm, L =60 cm, H=25 cm) and stainless- steel tube submerged in water bath to maintain the different treatment temperature (80-85°C) during combination thermal processing and electromagnetic induction.

The full intelligent PLC composed 30 memories to choose different programming of voltage and frequency pulse. Total usages of power (7-21 KW) were controlled through of a pulse generator, which the excessive decrease of usage energy in comparison with other system. The flow rate (300 - 400 ml/s) was adjusted by gear pump. Other technological specification is complete isolation system of environment, two intelligent micro processor for controlling electromagnetic induction and critical point of system so the temperature during electro-magnetic induction did not exceed 40°C. The applied residence time in this chamber was calculated according to Yang et al. (2004) as follows:

$$TR = VC/ Fr$$

Vc is the volume of a chamber (cm^3) and Fr flow rate (ml/s) which estimate 3 - 5 min (20 min induction, 20 min rest) 2 pulse per min (Figure 2).

High frequency electromagnetic field and thermal processing

In this study, electromagnetic field in variable voltage 1-20 kV/cm and frequency 8-10 GHz was used according to previous research (Anonymous, 1999; Khabazi 2005, 2006). The effect of each thermal processing combined with electromagnetic field (Zhang and Datta, 1999) for chick (7 treatments) and chick meal (7 treatments) was evaluated (in 3 run).

Thermal processing

Thermal processing was done in water bath chamber for chick (7 treatments) and chick meal (7 treatments without EMI to compare privilege of combination with EMI (in 3 run).

Samples packaging and storage

Unprocessed and processed chick and chick meal were filled (leaving the minimum amount of headspace volume) and packaged in to sterile 2 kind of polymeric flexible pouches (Datta and Liu, 1992; Datta et al., 1994, 1992). Finally, packaged chick and chick meal were put at room temperature (Table 1).

Statistical analysis

Multilevel factorial design was carried out in chick and chick meal samples inoculated in different condition with usage EMI or not, different thermal processing and combination of EMI with different thermal processing in different packages, at the end we can find a model for relationship between type of chick and type of culture and type of treatments.

We have described this variable (mesophil Microorganism) with frequency tables; cross tables and relative diagrams so for deduction these variables have been used "logistic regression" and "add ratio". Total positive number of microorganisms in different thermal processing is too high so suspected positive growth of microorganisms in enrichment culture evaluate negative to obtain model of logistic regression in thermal processing; which has showed in Figures 3 and 4.

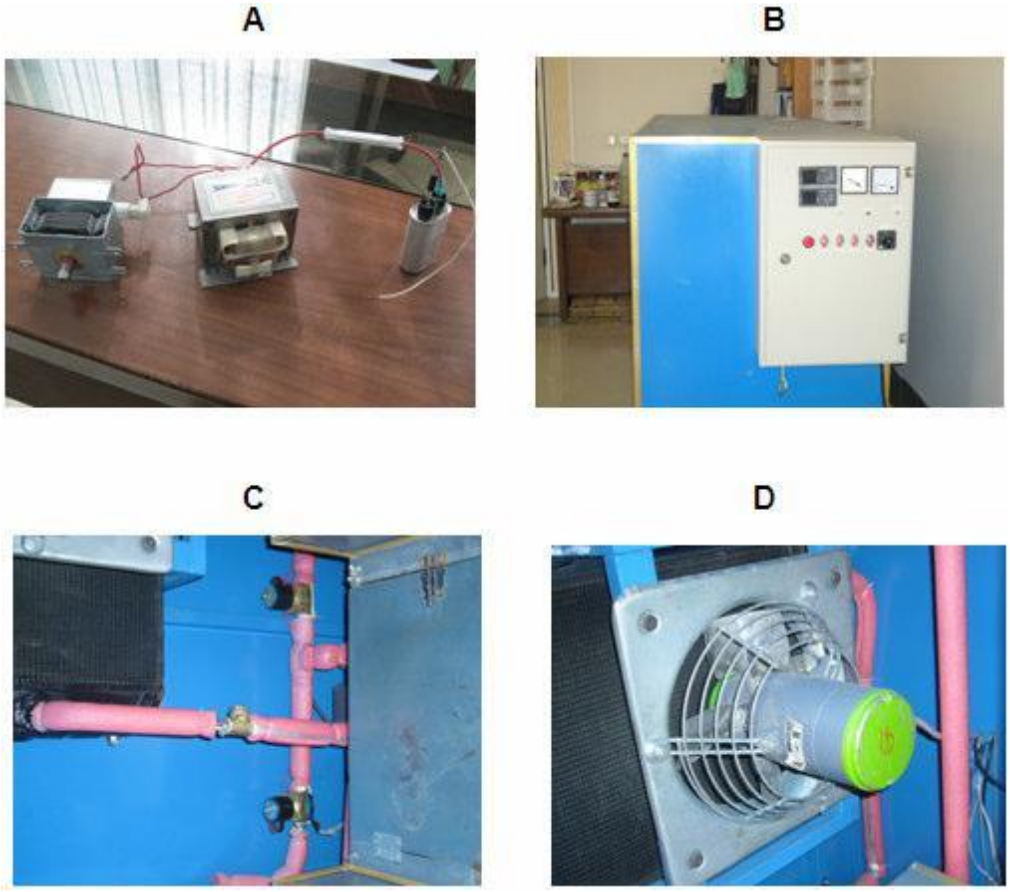

Figure 2. Electromagnetic field(A), 2-panel control(B), 3-inner part(c) 4-inner part "fan" reduce temperature (D).

Table 1. Analytical characteristics of 2 kinds of containers pouch (www.pmaprrintopack.com).

Sample	Layers	Tensile of film	Tensile of sealing film (normal)	O.T.R (ml/m 2.day) Oxygen transition rate	W.V.T.R (g/m 2.day Water transition rate
PET\AL\LLD	12\12\100	93.11	58.88	0	0.11
PET\AL\PET\LLD	12\7\12\100	104.61	61.03	0	0.089

PET; poly ethylene terphetalat, LLD; low density poly ethylene, AL; aluminum.

RESULT

Effect of high frequency electromagnetic field and thermal processing on growth of mesophil bacteria

Total number of mesophile microorganism variable

Total populations of mesophile microorganism in different treatment was showed in (Table 2) when thermal processing treatment was applied to these samples negative number is 39.9% however, effects of combined EMI with thermal processing were found in inactivation mesophil microorganisms 50.6%.

Number of mesophile microorganism variable in culture

Significant effects of different treatment in mesophile microorganism reductions were found when growths of them in different culture were tested (Table 3). Dead rate of mesophil in combination of EMI and thermal processing is higher than other treatment in rcm culture (negative=100%) and has significant level equal to 0.001 between mesophil growth and culture, but there were not found significant level between mesophil growth and culture p-value>0 in thermal processing treatment (Table 4, Figures 5 and 6). Number of mesophile microorganism

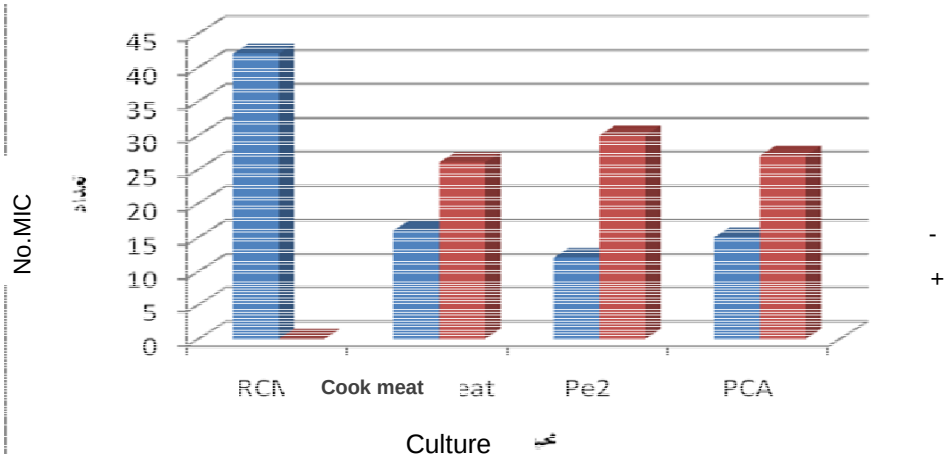

Figure 3. Number of mesophile microorganism variable in culture in EMI+pre heat.

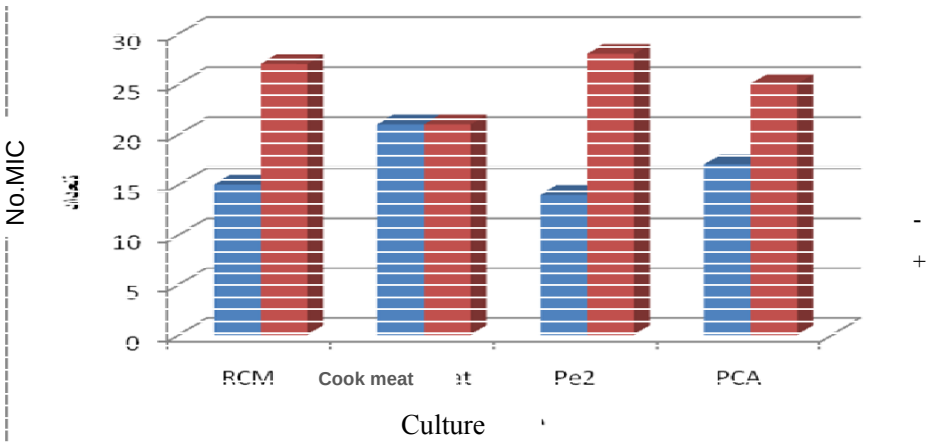

Figure 4. Number of mesophile microorganism variable in culture in various pre heating.

Table 2. Total populations of mesophile microorganism in different treatment.

Treatment	EMI+Pre heat		Pre heat	
Type (Mesophile))	Number	Percent (%)	Number	Percent (%)
Negative	85	50.6	67	32
Positive	83	49.4	101	136
Total	168	100	168	168

variable in different type of chick (Table 5). In general, population of mesophile microorganism in chick meal was more resistant than chick population of mesophile in each treatment (Figures 7 and 8).

Effect of high frequency electromagnetic field and thermal processing on properties of polymeric flexible packaging after EMI and pre heat

Tensile of sealing has been measured in order to see the effect of this sterilization on polymeric flexible pouches, as can be seen in Table 6 and Figure 9.

Conclusions

Effect of combination high frequency electromagnetic field and thermal processing

We have obtained these results with" logistic regression" and "add ratio" for combination high frequency

Table 3. Number of mesophile microorganism variable in culture.

Treatment	EMI+Pre heat (Figure 3)			Pre heat (Figure 4)		
Culture	**Mesophile**	**Number**	**Percent (%)**	**Mesophile**	**Number**	**Percent (%)**
RCM	Negative	42	100	Negative	15	35.7
	Positive	0	0	Positive	27	64.3
Cook meat	Negative	16	38.1	Negative	21	50
	Positive	26	61.9	Positive	21	50
Pe2	Negative	12	28.6	Negative	14	33.3
	Positive	30	71.4	Positive	28	66.7
PCA	Negative	15	35.7	Negative	17	40.5
	Positive	27	64.3	Positive	25	59.9

Table 4. Number of mesophile microorganism variable in different treatment.

Treatment	EMI+Pre heat (Figure 5)			Treatment	Pre heat (Figure 6)		
	Mesophile	**Number**	**Percent (%)**		**Mesophile**	**Number**	**Percent (%)**
Chick (control)	Negative	3	25	Chick (control)	Negative	0	0
	Positive	9	75		Positive	12	100
Chick (80°C + 5 min)+EMI	Negative	3	25	Chick (80°C + 5 min)	Negative	0	0
	Positive	9	75		Positive	12	100
Chick (80°C + 10 min)+EMI	Negative	5	41.5	Chick (80°C + 10 min)	Negative	5	41.5
	Positive	7	58.3		Positive	7	58.3
Chick (80°C + 15 min)+EMI	Negative	12	100	Chick (80°C + 15 min)	Negative	0	0
	Positive	0	0		Positive	12	100
Chick (85°C + 5 min)+EMI	Negative	3	25	Chick(85°C + 5 min)	Negative	0	0
	positive	9	75		positive	12	100
Chick (85°C + 10 min)+EMI	Negative	9	75	Chick (85°C + 10 min)	Negative	0	0
	Positive	3	25		Positive	12	100
Chick (80°C + 15 min)+EMI	Negative	12	100	Chick (85°C + 15 min)	Negative	0	0
	Positive	0	0		Positive	12	100
Chick Meal (control)+EMI	Negative	3	75	Chick Meal (control)	Negative	0	0
	Positive	9	25		Positive	12	100
Chickmeal (80°C + 5 min)+EMI	Negative	3	25	Chick meal (80°C + 5 min)	Negative	0	0
	Positive	9	75		Positive	12	100
Chickmeal (80°C + 10 min)+EMI	Negative	3	25	Chick meal (80°C + 10 min)	Negative	0	0
	Positive	9	75		Positive	12	100
Chickmeal (80°C + 15 min)+EMI	Negative	10	83.3	Chick meal (80°C + 15 min)	Negative	0	0
	Positive	2	16.7		Positive	12	100
Chickmeal (85°C + 5 min)+EMI	Negative	3	25	Chick meal (85°C + 5 min)	Negative	10	0
	Positive	9	75		Positive	2	100

Table 4. Contd.

Chickmeal (85°C + 10 min)+EMI	Negative	4	33.3	Chick meal (85°C + 10 min)	Negative	0	0
	Positive	8	66.7		Positive	12	100
Chickmeal (80°C + 15 min)+EMI	Negative	12	100	Chick meal (85°C + 15 min)	Negative	4	33.3
	Positive	0	0		Positive	8	66.7

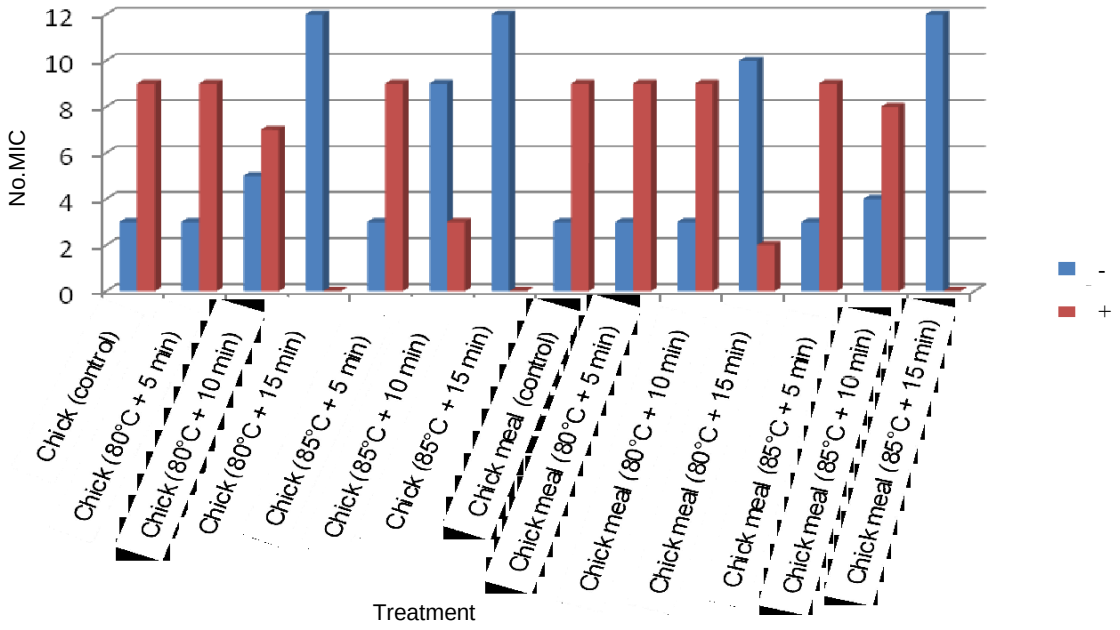

Figure 5. Number of mesophile microorganism variable in different treatment in EMI+Pre heat.

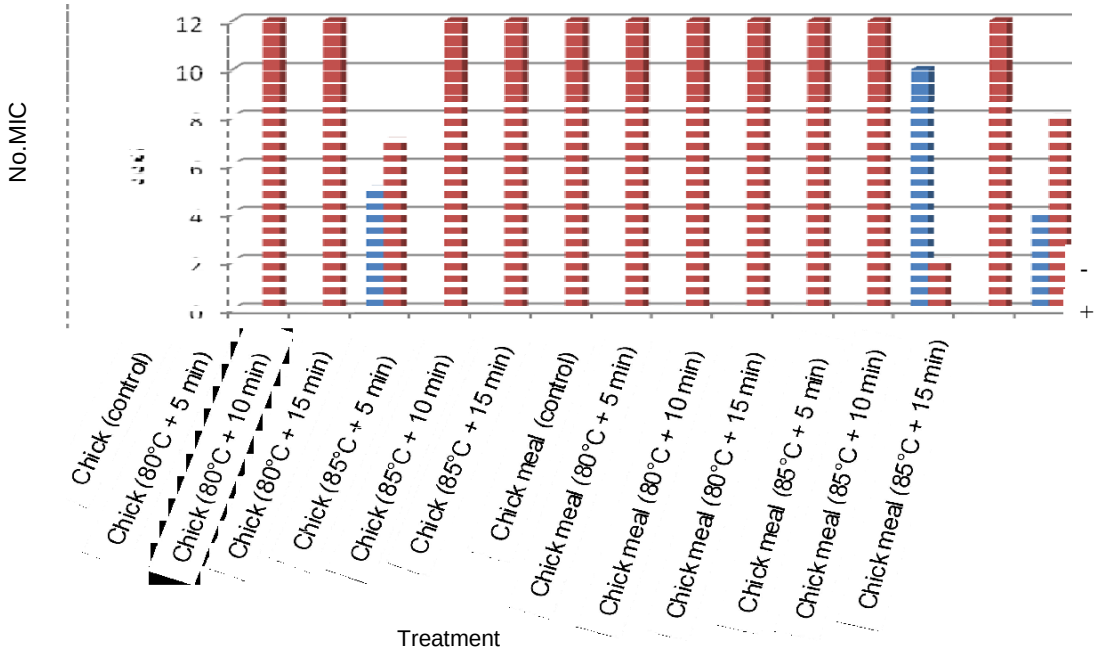

Figure 6. Number of mesophile microorganism variable in different treatment in various pre heating.

Table 5. Number of mesophile microorganism variable in different type of chick.

Treatment	EMI+Pre heat (Figure 7)			Pre heat (Figure 8)		
Culture	**Mesophile**	**Number**	**Percent (%)**	**Mesophile**	**Number**	**Percent (%)**
Chick	Negative	47	56	Negative	41	48.8
	Positive	37	44	Positive	43	51.2
Chick meal	Negative	38	45.2	Negative	26	31
	Positive	46	54.8	Positive	58	69

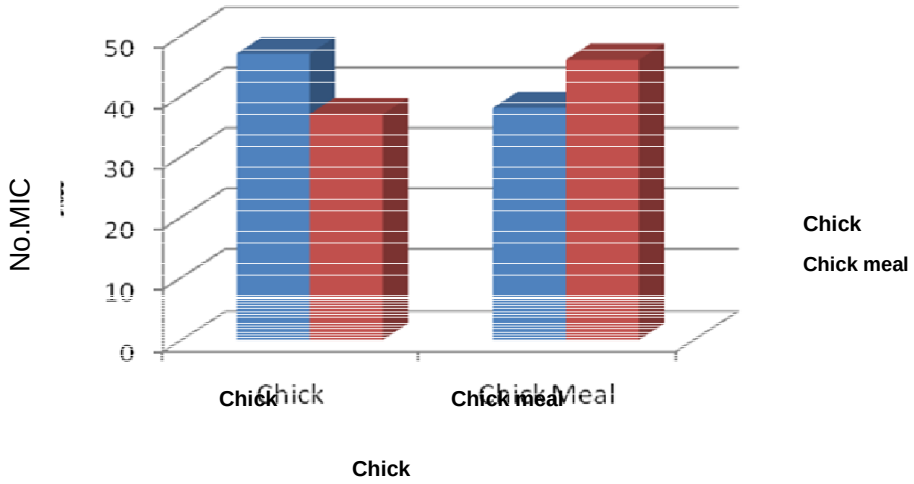

Figure 7. Number mesophile microorganism variable in different type of chick in EMI+pre heat.

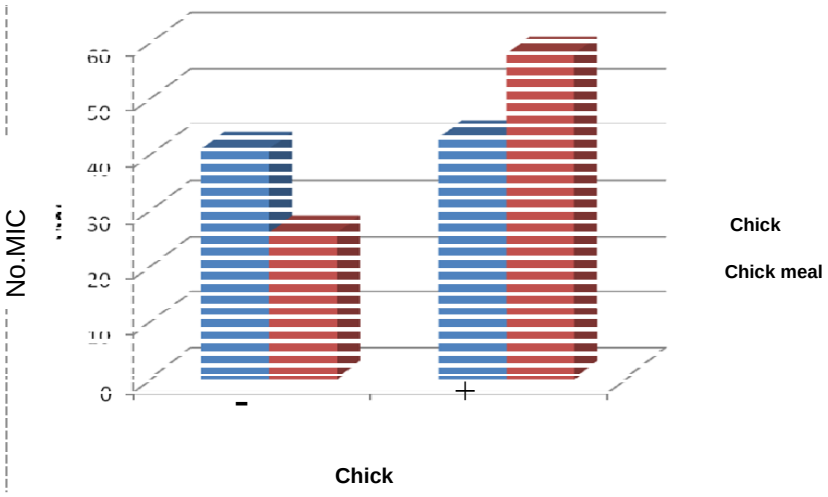

Figure 8. Number mesophile microorganism variable in different type of chick in various pre heating.

electromagnetic field and thermal processing (Table 7). According to “Wald test”, effect of p-value for type of chick type of treatment and type of culture has significant level (0.001). Other hand chance of negative mesophile microorganism growth increasing in chick 14200% more than chick meal and has significant level equal to 0.001 between mesophil growth and type of chick, and chance of negative mesophile microorganism growth in every

Table 6. Properties of polymeric flexible packaging after EMI and pre heat (32).

Sample	Tensile of sealing film (after 80°C, 5 min+EMI)	Tensile of sealing film (after 80°C, 10 min+EMI)	Tensile of sealing film (after 80°C, 15 min+EMI)	Tensile of sealing film (after 85°C, 5 min+EMI)	Tensile of sealing film (after 85°C, 10 min+EMI)	Tensile of sealing film (after 85°C, 15 min+EMI)	Tensile of sealing film (after 80°C, 5 min)	Tensile of sealing film (after 80°C, 10 min)	Tensile of sealing film (after 80°C, 15 min)	Tensile of sealing film (after 85°C, 5 min)	Tensile of sealing film (after 85°C, 10 min)	Tensile of sealing film (after 85°C, 15 min)
12/12/100	47.404	45.164	42.916	47.404	45.164	42.916	47.824	45.477	43.129	47.824	45.477	43.129
12/7/12/100	60.109	59.709	59. 424	60.109	59.709	59.42	60.193	59.836	59.509	60.193	59.836	59.509

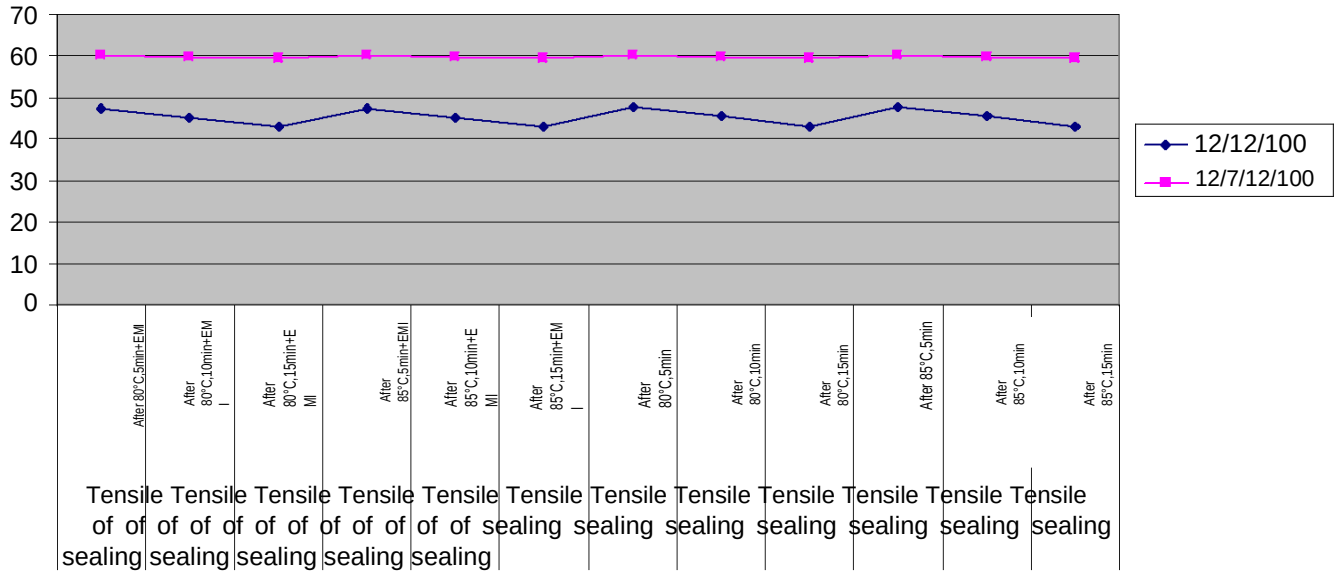

Figure 9. Tensile of sealing in 2 kind of polymeric flexible pouches after EMI and pre heat (32).

treatment compares with last treatment increasing 54% (negative mesophile growth from up to down increasing 54%) so has significant level equal to 0.001 between mesophil growth and type of treatment. And chance of negative mesophile microorganism growth in culture "PCA" is 3.3 degree more than culture "PE 2" and chance of negative mesophile microorganism growth in culture "PE 2" is 330% more than culture "Cook meat" so it has significant level equal to 0.001 between mesophil growth and culture.

Effect of thermal processing

We have obtained these results with "logistic regression" and "add ratio" for thermal processing too (Table 8). According to “Wald test”, effect of p-value for type of chick type of treatment has significant level (p-value=0.001). Other hand chance of passive mesophile microorganism growth increasing in chick meal 190500% more than chick and has significant level (p-value=0.001) between mesophil growth and type of chick, and

Table 7. Results of mesophil growth for combination high frequency electromagnetic field and various thermal processing.

	Coefficient	Statistic	Degree of freedom	P-value (Sig)	(Chance) add ratio
Constant	-5.94	31.27	1	0.00	-
Type of chick	4.95	26.88	1	0.00	141.87
Type of treatment	-0.61	27.16	1	0.00	0.544
Culture	1.32	32.60	1	0.00	3.37

Model of logistic regression is written: Log it (be negative) = -5.94 + 4.95 (Type of chick) - 0.61 (type of treatment) + 1.23 (culture).

Table 8. Results of mesophil growth for various thermal processing.

	Coefficient	Statistic	Degree of freedom	P-value (Sig)	(Chance) add ratio
Constant	-3.91	16.67	1	0.00	0.02
Type of chick	7.55	42.20	1	0.00	1904.91
Type of treatment	-0.90	43.49	1	0.00	0.41
Culture	0.06	0.008	1	0.927	1.02

Model of logistic regression is written: Log it (be positive) =-3.91+ 7.55 (Type of chick) -0(culture)-0.9 (type of treatment).

and chance of passive mesophile micro-organism growth in every treatment compares with last treatment decreasing 41% (passive mesophile growth from up to down decreasing to 41%) so it has significant level of (p-value=0.001) between mesophil growth and type of treatment, but there is no significant level between mesophil growth and culture (p-value>0).

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