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The Usage of High Frequency Electromagnetic Field For Sterilization of Different Packed Meal

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ABSTRACT

The effect of high frequency electromagnetic induction (EMI) and combination of EMI with various condition of pre-heating for sterilization of different packed meal has been studied. All samples were filled in pouches then EMI sterilization which discharges square-wave pulses with variable voltage 1-20 kV/cm and different frequency (2-4GHz ,4-6 GHz ,6-8GHz. 8-10 GHz) have been used in step one . The effect of EMI on clostridium and bacillus is not adequate because spore of these bacteria were practically resistant in electric field's, so pouches have been put in water bath chamber ,different condition of pre heating(80 ^{0}c 5min, 80^{0}c 10;min,80^{0}c 15min,85^{0}c 5min ,85^{0}c 10min ,85^{0}c 15min) have been combined with 8-10GHz .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 mesophile microorganisms depended on type of culture, type of treatment and type of meal .The death ratio of mesophile microorganisms increases in cooked chick and cooked meat 13500 -14200 % more than cooked chick meal and cooked meat meal .Other hand chance of negative mesophile microorganism growth in every treatment compares with last treatment increased 46-54 %.But in every conditions growth of thermophile microorganism have not been reported.

Key words: high frequency electromagnetic field, electromagnetic induction (EMI), flexible packaging, mesophile bacteria, thermophile bacteria, thermal processing, cooked chick, cooked chick meal ,cooked meat, cooked meat meal

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INTRODUCTION

High frequency electromagnetic induction (EMI) is useful for various research ,and industrial fields, it is well known that there is a non-thermal lethal method for bacteria due to physical destruction of cell membrane(10,11).However there is no degradation of flavor and taste with heat denaturation of objectives (7,27). In the future, the demand of EMI sterilization must be widely expanded in food industrial packaging because legal restrictions of sanitary management for a variety of foods have been enhanced internationally on the basis of hazard analysis and critical control point (9), Consumption of ready to eat food has plenty effect in manner of offering new food packaging products in lately decades and enter variety forms of restorable multi layers of polymers and plastic films laminated with aluminum for packaging cooked meat and poultry in stead of can(1).These products without a efficient processing are potential source of pathogens microorganism ,specially mesophile and thermophile aerobic and anaerobic clostridium and bacillus, since the low acidity (pH 4-5) and high water activity of these packed meals can favor the growth of them activity(26,28) in this package. Although, thermal treatment (120 ^{o}C,20 min) effectively destroys these microorganisms (26,29), has been used widely, proteins and some other physiological substrates are inactivated, and consequently the flavor, taste, and contents of nutrients in foods are lost(20,22,27).Other hands such treatment is carried out at high temperature 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 processing such as high frequency electromagnetic fields (39,40,41), which is proving to be able to inactivate spoilage microorganisms without significantly affect nutritional properties of several foods(16). This method involves the usage high frequency (2-15GHZ) in electric field (typically 1-20kV/cm)(20) to fluid foods placed between two electrodes in batch flow systems using low processing temperatures (near 40 ^{0}c) and low energy efficiency for sterilization with regard to the thermal treatment(25). This frequency allocated by federal communication commission (FCC)(23,24,35). The primary advantage of improved uniformity of heating was shown in- package sterilized by this method (3,4,5,6,38) packaging materials need to be microwave transparent and have a high melting point, packages with some metal component can considerably change the food temperatures (critical process factor). The most common packages that have been tried are individual pouches made of microwave transparent rigid films such as polyethylene(LLD) ,ethylene vinyl alcohol (EVOH) and polyethylene terephtalate (PET) is barrier film. (30,31) ,and metallic components present in a package, such as aluminum foil and can dramatically influence on heating rates of the packaged food (3,34). The effect of high frequency electromagnetic on clostridium (37) and bacillus(15) that are existing in packed cooked meal is not adequate because spore of these bacteria are too resistance(10,21) ,so the use of EMI in combination with various pre heating inactivate them without a significant adverse effect on food properties and taste (40,41,43,44,45),which can be explained by electromechanical compression (46). This phenomenon causes the formation of Trans membrane pores so, the ratio of total pore area becomes unfavorable; the membrane is no longer able to repair these irreversible disruption. On the other hand, bacteria have an optimal temperature for the cultivation or growth, so lower or higher temperature than optimal growth can vary the fatty acid composition of membrane lipids so increase effect of EMI (33, 39, 42) by primarily attack with pre heating. In this study, we investigate the electromagnetic sterilization of packed cooked meal in first step and combination of EMI with different thermal processing in second step (40,41)

MATERIALS AND METHODS

2.1. Cooked meal preparation

Chicken 1.7-2 kg weight were chosen for this experiment from local supermarket Tehran -Iran. The chickens were washed and cooked in water with 1-1.5 % salt (38, 40,41,44). After cooking ,cut into sliced(26).Two kind of cooked chick samples were prepared : (8,40,41)

1- pouches contain 50 gr cooked chick
2- pouches contain 50 gr cooked chick + 50 gr sauce (PH=4.5,Brix =8,salt=1.5%,Tomato paste) (cooked chick meal)

Cow meats (5 kg weight) were chosen for this experiment from local supermarket Tehran -Iran. Those meat were washed and cooked in water with 1-1.5 % salt (38, 40,41,44). After cooking ,cut into sliced(26).Two kind of cooked meat samples were prepared : (8,40,41)

1-pouches contain 50 gr cooked meat
2-pouches contain 50 gr cooked meat + 50 gr sauce (PH=4.5, Brix =8, salt=1.5%, Tomato paste) (cooked meat meal)

All pouches were filled hot in order pulling out oxygen(exhausting) and after sealing pouches different condition of pre heating have been done in bath water; then cool them immediately (T=20 ^{0}c) ,The approximate of oxygen in pouches is 2-3% which was measured by O2-measuring cell . 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)O2-measuring cell(Electro-chemical MAT14 Modify ed Atmosphere Packaging Control, cycobel group ,Germany) were measured according to the ISIRI Regulation (12,14)

2.2. Microbial culture

PCA(Peptone from casein 5gr/1000 mlit; glucose 1gr/1000 mlit,Yeast Extract 2.5 gr/1000 mlit,Agar 14gr/1000 mlit,Distillated water 1000 mlit),plate count agar is a general media for aerobic for aerobic,RCM (Peptone from casein 10gr/1000 mlit; Meat Extract 10gr/1000, Yeast Extract 3gr/1000 mlit,Starch 1gr/1000 mlit, glucose 5 gr/1000 mlit,l- cystein hydrochloride 0.5gr/1000,Sodium acetate 3gr/1000 mlit, Sodium chloride 5 gr/1000, Agar12.5gr/1000 mlit, Distillated water 1000 mlit)Rein Clostridia is a culture Media for clostridium.CMM(Beef heart 454gr/1000 ,Proteose peptone 20 gr/1000, glucose 5 gr/1000 mlit, Sodium chloride 5 gr/1000, Sodium hydrochloride ½ 454 gr/1000, Distillated water 1000 mlit).Cooked Meat is enrichment media for aerobic bacteria.PE 2(Peptone digest of animal extract 20 gr/1000m, Yeast Extract 3gr/1000 mlit ,2%Alcoholic solution of bromocresol purple o.o4gr/1000 mlit,Cicer arietinum L 450 no, Distillated water1000 milt) Peptone Yeast Extract Bromocresol purple is enrichment media for anaerobic bacteria(12,13)

For microbial test each samples of to be treated with or without high frequency electromagnetic fields and pre heating were inoculated 15 day in temperature 37 ^{0}c for mesophile bacteria growth and 7 day in temperature 55 ^{0}c for thermophile bacteria, After incubation for aerobic growth 1-2 gr of samples were put in CMM (3-4 day) then 1-2 gr from CMM transfer to PCA after 2-5 day.

For anaerobic growth 1-2 gr of samples were put in PE 2(3-4 day) then 1-2 gr from PE 2 transfer to RCM after 2-5 day. Growth of bacteria in CMM and PE 2 has been showed as positive or negative response(12,14) (bad odor, discoloration and producing gas)so in this investigation , the growth of bacteria in PCA and RCM ,CMM, PE 2,have been showed as response (non parametric: negative or positive)(40,41)

2.3. High frequency electromagnetic field and processing parameters

A continuous flow High frequency electromagnetic model pilot-scale (2,17,18,19),which discharges square-wave pulses was used for sterilization of different packed meal (11,40,41).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 1-20kV/cm(11) in different frequency(2-15 GHZ). The packages of cooked chick and cooked meat were put between treatment chamber with volume 60 lit (W=40cm, L =60cm, H=25 cm) and stainless-steel tube submerged in water bath to maintain the different treatment temperature (80^0c -85 ^{0}c), during combination thermal processing and electromagnetic induction .The full intelligent PLC composed 30 memories to chose different programming of voltage and frequency pulse. Total usage of power (7-21 KW) was controlled through of a Pulse generator, which the excessive decrease of usage energy in comparison with other system; the flow rate (300-400 ml/sec) 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 (fig 1). The temperature during electromagnetic induction did not exceed 40 ^{0}C. The applied residence time in this chamber was calculated according to Yang et al. (36) as follows:

TR = VC/ Fr

Vc is the volume of a chamber (cm3) and Fr flow rate (ml/s)which estimate 3-5 min (20 sec induction,20 sec rest) 2 pulse per min

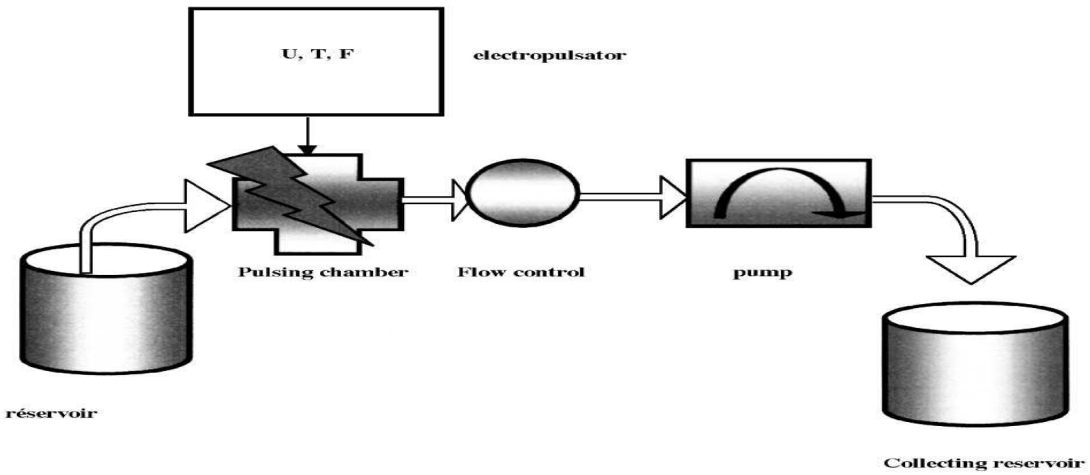

Fig. 1. Flow electropulsation. (A) Cells are taken from the reservoir. (B) They flow through the pulsing chamber where a controlled number of calibrated pulses is applied. The pulsing chamber is connected to the high-power pulse electropulsator where the voltage U, the pulse duration T and the pulse frequency F are under control. (C) The flow Q is obtained by a pump and controlled. (D) Pulsed cells are collected and processed in a collecting reservoir

2. 4-Samples packaging and storage

Unprocessed and processed different cooked meal (chick or meat) were filled (leaving the minimum amount of headspace volume) and packaged into one multilayer flexible pouch (4, 5,6, 39,40,41). The property of this container (4 Layers) was shown in table 1 .Finally, packed meal were put at room temperature. Effect of high frequency electromagnetic field and thermal processing on properties of polymeric flexible packaging after EMI and Pre heat was shown in table 2.Tensile of sealing has been measured in order to see the effect of this sterilization on polymeric flexible pouches, as you see in table 2 (39,40,41)

Table 1- Analytical characteristics of container (32)

Sample	Layers	Tensile of film	Tensile of sealing film	O.T.R(ml/m ^{2}.day) Oxygen Transmission Rate	W.V.T.R(gr/m ^{2}.day) Water Transmission Rate
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

Table 2-Properties of polymeric flexible packaging after EMI and pre heat (32)

Sample	Tensile of sealing film											
	80 ^{0}c ,5min+EMI	80 ^{0}c ,10min+EMI	80^0 c ,15min+EMI	85^0 c ,5min+EMI	85 c ,10min+EMI	85 ^{0}c ,15min+EMI	80 ^{0},5min	80 ^{0}c 10min	80^{0}c,15min	85 ^{0}c,5min	85 ^{0}c ,10min	85^{0}c,15min
12/7/12/100	*60.109*	*59.78*	*59.42*	60.11	59.71	59.42	*60.19*	*59.84*	*59.51*	60.19	59.84	59.51

3- Statistical analysis

Multilevel factorial design was carried out for cooked chick and cooked meat samples which inoculated in different condition with EMI or without EMI ,and combination with different thermal processing ,so we must find a model for relationship between type of chick or meat , type of culture, and type of treatment .We have described this variables (mesophile and thermophile microorganisms) with frequency tables; cross tables and relative diagrams ,and for deduction of this variable have used "logistic regression" and "add ratio"; as a large amount of positive number of microorganisms in EMI treatment and different thermal processing treatment suspected positive growth of microorganisms in enrichment culture evaluate negative ,in order to obtain model of logistic regression, which were showed in table 3 (40,41)

RESULTS

In this study, electromagnetic field with variable voltage 1-20 kV/cm and frequencies (2-4GHz, 4-6GHz ,6-8GHz,8-10GHz) were used according to previous research(1,17,18,19,40,41) in first step .The best result for inactivation of microorganism belong to 8-10GHz (39,40,41). The effect of each thermal processing combined with electromagnetic field (40,41,43)for cooked chick (7

treatments which were renamed A1 –A7) and cooked chick meal (7 treatments which were renamed B1-B7)and for cooked meat (7 treatment which were renamed C1-C7) and cooked meat meal (7 treatments which were renamed D1-D7) in second step and was repeated in 3 run(39,40,41)**,**as you see in table 3 ,fig 2,and fig 3

In general population of thermophile microorganism in both kind of samples and each treatment were zero. The population of mesophile microorganism in chick meal and meat meal was more than the population of mesophile cooked chick in each treatment ,as you see in table 4,fig 4

Table 3-The Effect of Combination thermal processing and EMI for inactivation of Microorganism of cooked chick, cooked chick meal, cooked meat and cooked meat meal

Treatment	Response	Mesophile	Thermopile	Treatment	Response	Mesophile	Thermopile
Chick(control)+EMI	negative	3	12	Meat(control) +EMI	negative	3	12
	positive	9	0		positive	9	0
Chick(80c + 5min)+EMI	negative	3	12	Meat (80c + 5min)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick(80c + 10min)+EMI	negative	5	12	Meat (80c + 10min)+EMI	negative	6	12
	positive	7	0		positive	6	0
Chick(80c + 15min)+EMI	negative	12	12	Meat (80c + 15min)+EMI	negative	9	12
	positive	0	0		positive	3	0
Chick(85c + 5min)+EMI	negative	3	12	Meat (85c + 5min)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick(85c + 10min)+EMI	negative	3	12	Meat (85c + 10min)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick (80c + 15min)+EMI	negative	12	12	Meat (85c + 15min)+EMI	negative	10	12
	positive	0	0		positive	2	0
Chick Meal(control)+EMI	negative	3	12	Meat Meal(control)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick Meal(80c + 5min)+EMI	negative	3	12	Meat Meal(80c + 5min)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick Meal(80c + 10min)+EMI	negative	3	12	Meat Meal(80c + 10min)+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick Meal(80c + 15min)+EMI	negative	10	12	Meat Meal(80c + 15min)+EMI	negative	6	12
	positive	2	0		positive	6	0
Chick Meal(85c + 5min)	negative	3	12	Meat Meal(85c + 5min))+EMI	negative	3	12
	positive	9	0		positive	9	0
Chick Meal(85c + 10min)+EMI	negative	4	12	Meat Meal(85c + 10min)+EMI	negative	5	12
	positive	8	0		positive	7	0
Chick Meal(80c + 15min)+EMI	negative	12	12	Meat Meal(80c + 15min)+EMI	negative	12	12
	positive	0	0		positive	0	0

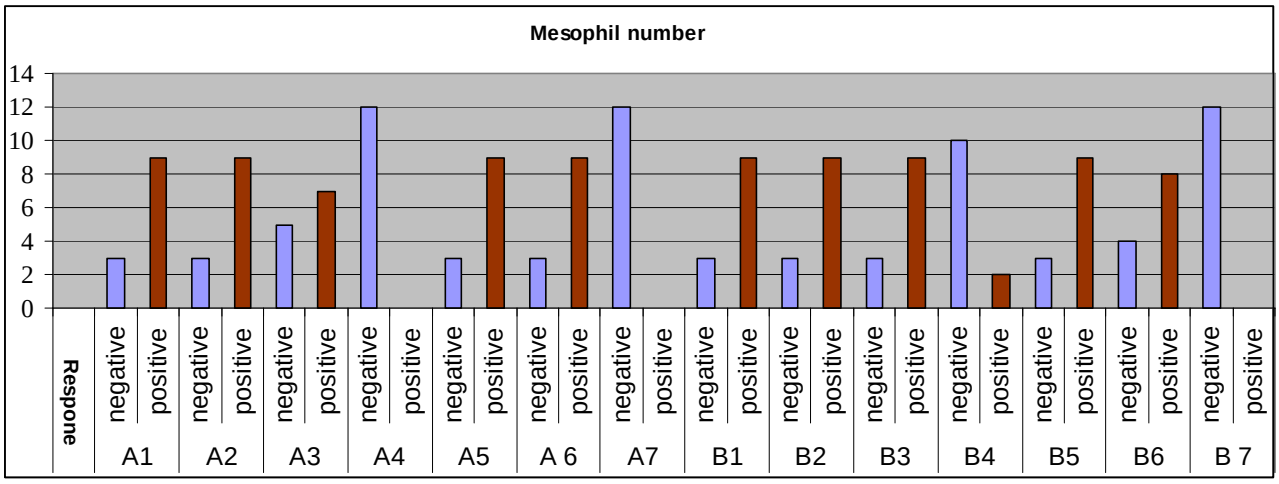

Fig 2-The Effect of Combination thermal processing with EMI for inactivation microorganism of cooked chick, cooked chick meal

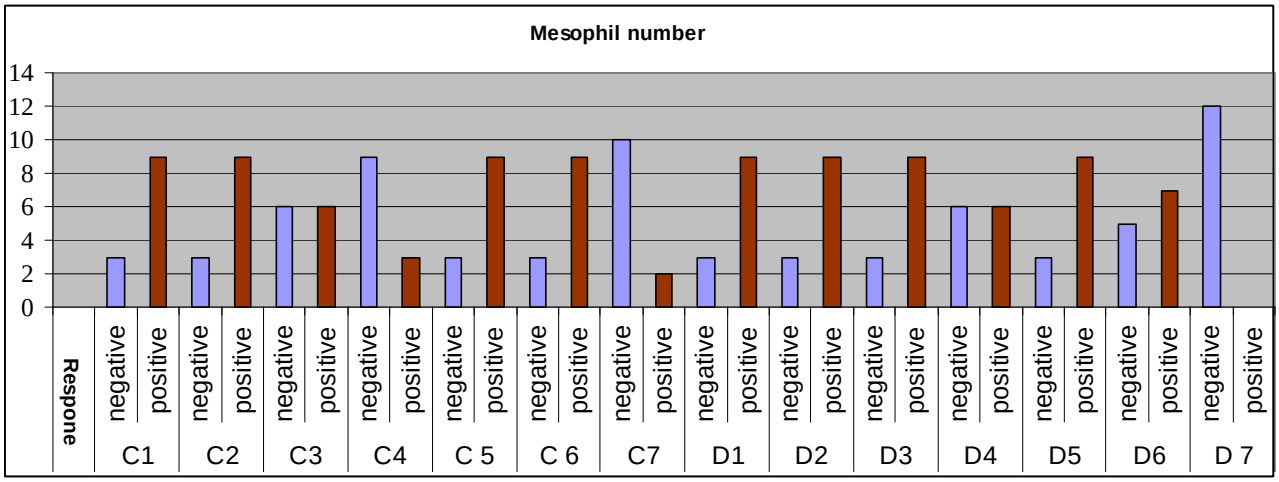

Fig 3-The Effect of Combination thermal processing with EMI for inactivation microorganism of cooked meat and cooked meat meal

Table 4- Number of mesophile microorganism variable in different type meal

Treatment 1	EMI+Pre heat		Treatment 2	EMI+Pre heat	
culture	Mesophile Response	number	culture	Mesophile Response	number
Cooked Chick	negative	47	Cooked meat	negative	37
	positive	37		positive	47
Cooked Chick Meal	negative	38	Cooked meat meal	negative	35
	positive	46		positive	49

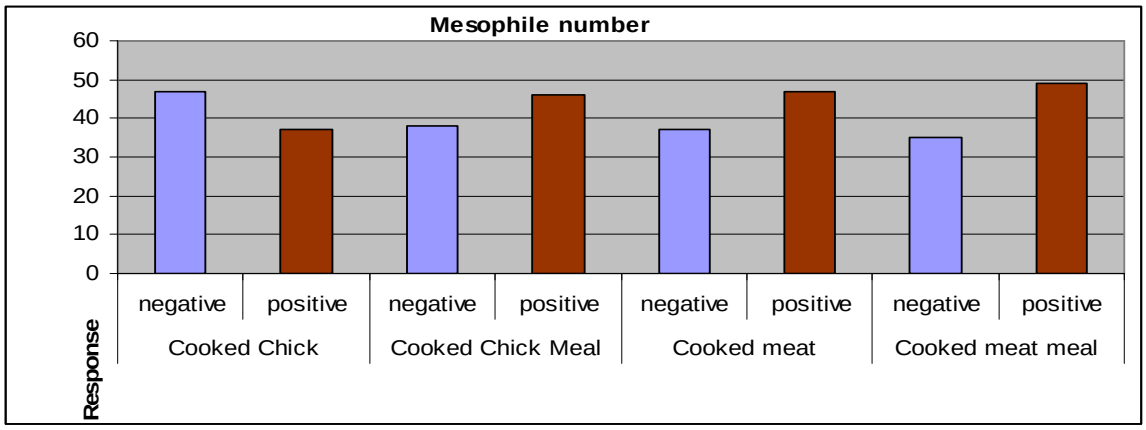

Fig 4 -Number of mesophile microorganism variable in different type meal

5- Determination of T.V.N (Total Volatile Nitrogen)

We have determined Total Nitrogen Volatile (T.V.N) of these samples by Kjeldal method, so there were obtained data, which were according to this range (T.V.N of chick=2.1, T.V.N of meat=2.3):

$$1.113 < T.V.N < 5.4 \text{ mgr/gr}$$

This can prove the protein of product mainly safe during the technologies of sterilization by EMI, however T.V.N of those samples have been sterilized by autoclave ($120^{o}C$, 20 min) increase 20%.From mentioned above follows, that privileges should be given to these kind of packed meal in comparison with other meat products. While nitrogen determination by Kjeldal method data corresponding to range 1.113-5.4 mgr/gr was get, which proves that main part of meat's protein was intact.

CONCLUSIONS

We have obtained these results with" logistic regression" and "add ratio" for combination high frequency electromagnetic field and thermal processing , as you see in table 5 and table 6

Table 5-Effect of combination high frequency electromagnetic field and thermal processing for cooked chick and cooked chick meal

Condition	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

Model of logistic regression is written

Logit (be negative) = -5.94 + 4.95(Type of Chick) -0.61(type of treatment)

According to" Wald test', the 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 cooked chick 14200 percent more than cooked chick meal and has significant level equal to 0.001 between mesophile growth and type of chick,

and chance of negative mesophile microorganism growth in every 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 mesophile growth and type of treatment. (40, 41)

Table 6-Effect of combination high frequency electromagnetic field and thermal processing for cooked meat and cooked meat

Condition	Coefficient	statistic	Degree of freedom	P-value (Sig)	(Chance) add ratio
constant	-7.11	31.27	1	0.00	-
Type of meat	3.32	26.88	1	0.00	141.87
Type of treatment	-0.84	27.16	1	0.00	0.544

Model of logistic regression is written

Logit (be negative) = -7.11 + 3.32(Type of Meat) -0.84(type of treatment)

According to" Wald test', the effect of p-value for type of cooked meat , type of treatments and type of have significant level (0.001).Other hand chance of negative mesophile microorganism growth increasing in cooked meat 13500 % more than cooked meat meal ,and has significant level equal to 0.001 between mesophile growth and type of meat , and chance of negative mesophile microorganism growth in every treatment compares with last treatment increasing, 46 % (negative mesophile growth from up to down increasing 46 %) so has significant level equal to 0.001 between mesophile growth and type of treatment. (40, 41)

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