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European Journal of Experimental Biology, 2013, 3(2):598-607

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Sterilization of packed mushroom meal by high frequency electromagnetic field

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ABSTRACT

The effect of high frequency electromagnetic field, and combination of it with various condition of pre-heating (80-85 ^{o}c) for sterilization of different mushroom meals has been studied. All samples of mushroom meal were filled in pouches (retort multilayer films). EMI treatment which discharges square-wave pulses with variable voltage 1-20 kV/cm ,and different frequency(2-3GHz ,3-4 GHz ,4-5GHz, 5-6 GHz) have been done in step one . The effect of high frequency electromagnetic on clostridium and bacillus is not adequate because spore of these bacteria are practically resistant in electric fields, so pouches have been put in water bath chamber ,and different condition of pre heating (80^{o}c 5min;80^{o}c 10min;80^{o}c 15min;85^{o}c 5min;85^{o}c 10min;85^{o}c 15min) have been done ,so the effect of each thermal processing combined with best result of electromagnetic field for these meals which belonged to 5-6 GHz. If cells are cultivated at higher temperature, this increasing tendency which can permanently keep fluidity viscosity of the cell membrane before electromagnetic field thus EMI efficiency was increased. The populations of mesophile microorganisms depended on type of treatment, type of mushroom meal ,and type of culture ,so the death ratio of mesophile microorganisms increased in mushroom meal (without spice) 14466 percent more than mushroom meal(with spice), but in every conditions of this process growth of thermophile microorganism has not been reported. However, the chances of passive mesophile microorganisms in various EMI treatment without preheating was evaluated in mushroom meal(with spice) as 180133 percent more than mushroom meal(without spice)while the chance of positive thermophile microorganism growth in various EMI treatment without preheating decreased in mushroom meal(with spice) by 73.33 percent more than mushroom meal(without spice) .

Key words: high frequency electromagnetic field, electromagnetic induction (EMI), flexible packaging, mesophile bacteria, thermophile bacteria, thermal processing, mushroom meal(with spice), mushroom meal(without spice)

INTRODUCTION

High frequency electromagnetic induction (EMI) is useful for various research. However, it is well known that there is a non-thermal method for inactivation bacteria due to physical destruction of cell membrane(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 (HACCP)" 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(9,34), 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 retort multi layers flexible films laminated with aluminum for packaging different meals instead of can (1).These products without a efficient processing are potential source of pathogens microorganism ,specially mesophile and thermophile aerobic and aerobic clostridium and bacillus, since the low acidity (pH 4-5) and suitable water activity of these new packed meal can favor the growth of them activity(26,28)in these packages. Although, thermal treatment (120 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, 39-44).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 , which is proving to be able to inactivate spoilage microorganisms without significantly affect nutritional properties of several foods (15, 47). This method involves the usage high frequency (2-15GHz) in electric field (typically 1-20kV/cm)(8, 16,20) to fluid foods placed between two electrodes in batch flow systems using low processing temperatures (near 40^{o} 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 (3-6,38). 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. (30,31,41,45) , 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) these kind of meal(with or without spice) is not adequate because spore of these bacteria are too resistance(10,21) so the usage of EMI in combination with various pre heating inactivate them without a significant adverse effect on food properties and taste (39-42,46,48) which can be explained by electromechanical compression (39,40,42,43,49). 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, and 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 mushroom meals (with spice, without spice) in first step and combination of EMI with different thermal processing in second step(39,40,42)

MATERIALS AND METHODS

2.1. Preparation of mushroom meals (with spice, without spice)

Mushrooms (10 kg) were bought for this experiment from local supermarket Tehran-Iran. Mushrooms were washed and cooked in water with 1.5 % salt, PH=4.5,Brix =8 (38,40,41). After cooking, two kind of mushroom meals (mushroom+ water) were prepared : (39,40,42,43)

1-**Meal 1 :**Pouches contain 100 g mushroom meal (without spice "1.5% Salt")
2-**Meal 2:**pouches contain 100 g mushroom meal (with spice"1.5% Salt, 0.5% Pepper, 0.5% Turmeric, 0.5% Cinnamon, Tomato paste")

All pouches were filled hot for pulling out oxygen(exhausting) and after sealing pouches, different condition of pre heating have been done in bath water; then cool them immediately ($T=20^{o}c$) (40,41,43,44).The approximate of oxygen in pouches is 2-4% which was measured by O2-measuring cell. Analytical parameters such as pH (Crison 2001 Ph meter; 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 5g/1000 ml; glucose 1g/1000 ml, Yeast Extract 2.5 g/1000 ml, Agar 14g/1000 ml ;Distillated water 1000 ml) plate count agar is a general media for aerobic for aerobic, RCM(Peptone from casein

10g/1000 ml; Meat Extract 10g/1000 ml; Yeast Extract 3g/1000 ml; Starch 1g/1000 ml, glucose 5 g/1000 ml, l-cystein hydrochloride 0.5g/1000 ml;Sodium acetate 3g/1000 ml, Sodium chloride 5 g/1000 ml, Agar12 g/1000 ml; Distillated water 1000 ml)Rein Clostridia is a culture Media for clostridium.CMM(Beef heart 454g/1000ml ; Proteose peptone 20 g/1000ml; glucose 5 g/1000ml;Sodium chloride 5 g/1000ml;Sodium hydrochloride ½ 454 g/1000ml; Distillated water 1000 ml)Cooked Meat is enrichment media for aerobic bacteria.PE 2(Peptone digest of animal extract 20 g/1000ml; Yeast Extract 3 g/1000ml;2%Alcoholic solution of bromocresol purple o.o4 g/1000ml, Cicer arietinum L 450 no;Distillated water1000 ml) Peptone Yeast Extract Bromocresol Purple is enrichment media for anaerobic bacteria(12,13)

For microbial test each samples of packed mushroom meals (with spice or without spice) to be combined EMI with or without pre heating .we were incubated 15 day in temperature 37^{o} c for mesophile bacteria growth and 7 day in temperature 55 ^{o} c for thermophile bacteria, After incubation for aerobic growth 1-2 g of samples were put in CMM (3-4 day) then 1-2 g from CMM 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 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) (39,40,42,43)

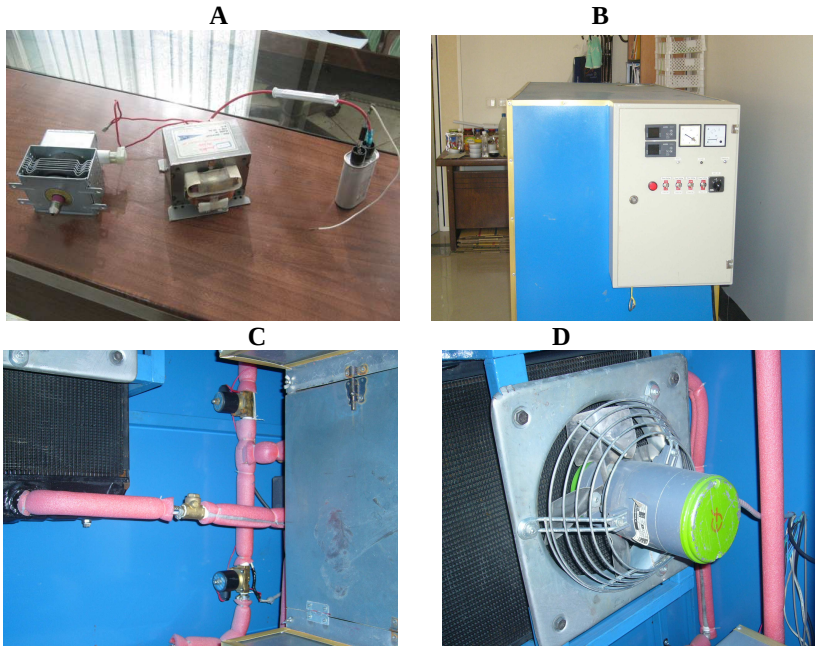

Fig 1. (A)-Electromagnetic field, (B)-panel control, (c).inner part, (D)inner part"fan"reduce temperature"

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(Hamilton) was used to sterile samples of packed mushroom meals(with spice or without spice) (11).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). Packed mushroom meals (with spice or without spice) was 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 (80-85^{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 so 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:

$$\mathbf{TR = V_C / Fr}$$

V_c 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

2. 4-Samples packaging and storage

Unprocessed (control) and processed packed mushroom meals(with spice or without spice) were filled (leaving the minimum amount of headspace volume) and packaged in to 3 kind of retort multilayer flexible pouches (3-6,33). Finally, all packaged samples (different mushroom meals) were put at room temperature in order to estimate the shelf life of these packed meals. Analytical characteristics of these containers (3 or 4 layer) and the best storage time for each pouch were reported, as you see in table 6 (39, 40, 42, 43)

Table 1. Analytical characteristics of three multilayer flexible pouches (32, 46)

Sample	Layers	Tensile of film	Tensile of sealing film (normal)	O.T.R (ml/m 2.day)	W.V.T.R (gr/m 2.day)	Shelf life (month)	
						Meal 2	Meal 1
PET\AL\LLD	**12\7\100**	60.83	45.88	0	0.5	*6*	*7-8*
PET\AL\LLD	**12\12\100**	93.11	*58.88*	*0*	*0.11*	*8*	*9-10*
PET\AL\PET\LLD	**12\7\12\100**	104.61	61.03	0	0.089	10	11-12

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

3- STATISTICAL ANALYSIS

Multilevel factorial design was carried out in packed mushroom meals (with spice or without spice) inoculated in different condition with EMI or, combination EMI with different thermal processing ,thus we can find a model for these method and type of meal and type of culture. We have described this variables (mesophile and thermophile microorganisms)with frequency tables; cross tables and relative diagrams so for deduction this variable have been used "logistic regression" and "add ratio" as a large amount of positive number of microorganisms in EMI treatment and different thermal processing combined with EMI, suspected positive growth of microorganisms in enrichment culture evaluate negative ,in order to obtain model of logistic regression, which were showed in tables 2, 3 (39,40,42,43)

RESULTS

In this study, electromagnetic field in variable voltage 1-20 kV/cm and frequencies(2-3GHz, 3-4GHz ,4-5GHz,5-6GHz) was used according to previous research(1,17,18,19), for meal 1 (5 treatment) and meal 2(5 treatment) was evaluated(in 3 run)as you see in table 2.The best result belonged to 5-6GHz , so The effect of each thermal processing combined with this frequency of electromagnetic field (44,47,49) for meal 1 (7 treatment)and meal 2 (7 treatment) was evaluated(in 3 run)as you see in table 3**.** (39, 40, 42, 43)

Table 2. Number of mesophile and thermophile microorganisms in EMI treatment

treatment	Response	Mesophile	Thermophile
Meal 1 (control)	**negative**	0	0
	positive	12	12
Meal 1 (2-3GHz)	**negative**	0	0
	positive	12	12
Meal 1 (3-4GHz)	**negative**	0	6
	positive	12	6
Meal 1 (4-5GHz)	**negative**	0	12
	positive	12	0
Meal 1 (5-6-GHz)	**negative**	3	12
	positive	9	0
Meal 2 (control)	**negative**	0	0
	positive	12	12
Meal 2 (2-3GHz)	**negative**	0	0
	positive	12	12
Meal 2 (3-4GHz)	**negative**	0	6
	positive	12	6
Meal 2(4-5GHz)	**negative**	0	6
	positive	12	6
Meal 2 (5-6GHz)	**negative**	2	0
	positive	10	12

Table 3.Number of mesophile and thermopile microorganisms in combination EMI treatment (5-6GH) with various thermal processing

treatment	Response	Mesophile	Thermophile
Meal 1(control) +EMI	**negative**	3	0
	positive	9	12
Meal 1 (80^0c 5min)+EMI	**negative**	3	0
	positive	9	12
Meal 1 (80^0c 10min)+EMI	**negative**	5	0
	positive	7	12
Meal 1 (80^0c 15min)+EMI	**negative**	12	6
	positive	0	6
Meal 1 (85^0c 5min)+EMI	**negative**	3	0
	positive	9	12
Meal 1 (85^0c 10min)+EMI	**negative**	9	3
	positive	3	9
Meal 1 ($85\ ^0$c 15min)+EMI	**negative**	12	6
	positive	0	6
Meal 2 (control)+EMI	**negative**	3	0
	positive	9	12
Meal 2 (80^0c 5min)+EMI	**negative**	3	0
	positive	9	12
Meal 2 (80^0c 10min)+EMI	**negative**	3	0
	positive	9	12
Meal 2 (80^0c 15min)+EMI	**negative**	10	6
	positive	2	6
Meal 2 (85^0c 5min)+EMI	**negative**	3	0
	positive	9	12
Meal 2 (85^0c 10min)+EMI	**negative**	4	5
	positive	8	7
Meal 2 (85^0c 15min)+EMI	**negative**	12	6
	positive	0	6

4-1- Effect of EMI and combination of EMI with thermal processing on growth of mesophile bacteria

4-1-1.Total number of mesophile microorganism variable

Table 4 .Total number of mesophile microorganism variable

Treatment	EMI+Pre heat		EMI	
Type(Mesophile))	**number**	**percent**	**number**	**percent**
negative	85	50.6	5	4.2
positive	83	49.4	115	95.8
Total	168	100	120	100

4-1-2.Number of mesophile microorganism variable in culture

Table 5. Number of mesophile microorganism variable in culture

Treatment	EMI+Pre heat			EMI		
culture	Mesophile	number	Percent (%)	Mesophile	number	Percent (%)
RCM	negative	42	100	negative	5	16.6
	positive	0	0	positive	25	83.4
Cook Meat	negative	16	38.1	negative	0	0
	positive	26	61.9	positive	30	100
Pe2	negative	12	28.6	negative	0	0
	positive	30	71.4	positive	30	100
PCA	negative	15	35.7	negative	0	0
	positive	27	64.3	positive	30	100

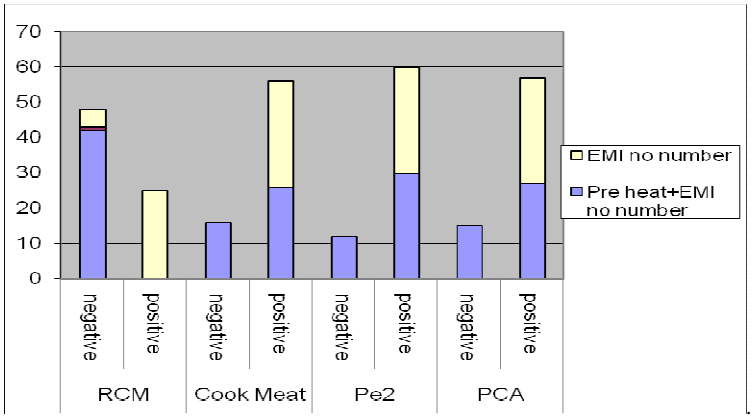

Fig 2.Number of mesophile microorganism variable in culture

4-1-3- Number of mesophile microorganism variable in different type of mushroom meals

Table 6. Number of mesophile microorganism variable in different type of mushroom meals

Treatment	EMI+Pre heat			EMI		
culture	Mesophile	number	Percent (%)	Mesophile	number	Percent (%)
Meal 1	negative	47	56	negative	3	5
	positive	37	44	positive	57	95
Meal 2	negative	38	45.2	negative	2	3
	positive	46	54.8	positive	58	97
Total	negative	85	51	negative	5	4
	positive	83	49	positive	115	96

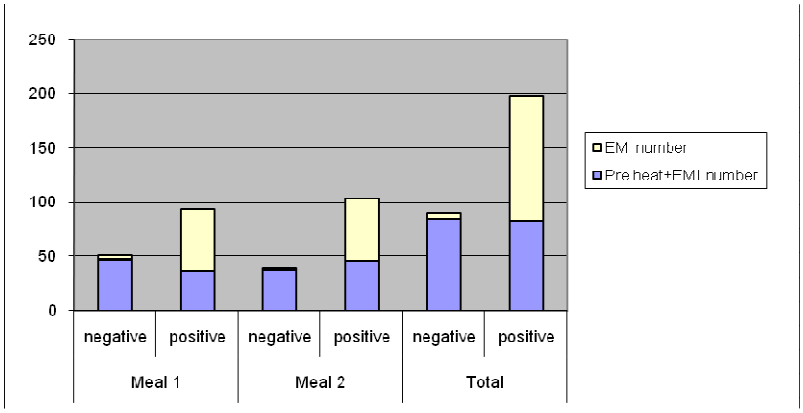

Fig 3.Number of mesophile microorganism variable in different type of mushroom meals

4-2- Effect of EMI and combination of EMI with thermal processing on growth of thermophile bacteria

4-2-1.Total number of thermophil microorganism variable

Table 7.Total number of thermophil microorganism variable

Treatment	EMI+Pre heat		EMI	
Thermophile	**number**	**percent**	**number**	**percent**
negative	168	100	54	45
positive	0	0	66	55
Total	168	100	120	100

4-2-2.Number of thermophile microorganism variable in culture

Table 8. Number of thermophile microorganism variable in culture

Treatment	EMI+Pre heat			EMI		
Culture	**Thermophile**	**number**	**Percent (%)**	**Thermophil**	**number**	**Percent (%)**
RCM	**negative**	42	100	**negative**	18	60
	positive	0	0	**positive**	12	40
Cook Meat	**negative**	42	100	**negative**	9	30
	positive	0	0	**positive**	21	70
Pe2	**negative**	42	100	**negative**	18	60
	positive	0	0	**positive**	12	40
PCA	**negative**	42	100	**negative**	9	30
	positive	0	0	**positive**	21	70

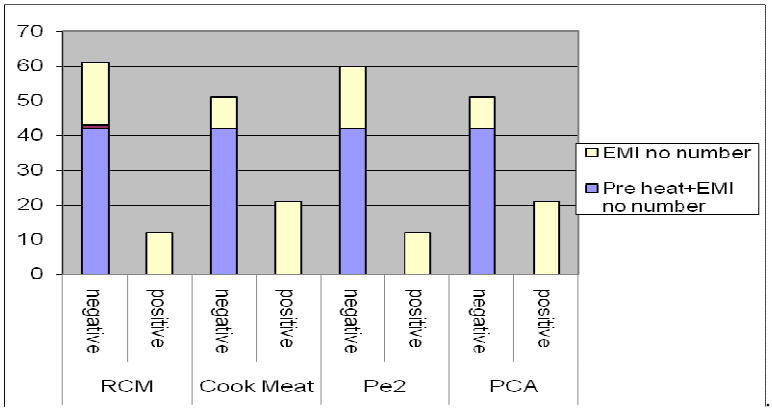

Fig 4.Number of thermophile microorganism variable in culture

4-2-3- Number thermopile microorganism variable in different type of mushroom meals

Table 9.Number thermophile microorganism variable in different type of mushroom meals

Treatment	EMI+Pre heat			EMI		
culture	Thermophile	number	Percent (%)	Thermophile	number	Percent (%)
Meal 1	negative	84	100	negative	30	50
	positive	0	0	positive	30	50
Meal 2	negative	84	100	negative	24	40
	positive	0	0	positive	36	60
Total	negative	168	100	negative	54	45
	positive	0	0	positive	66	55

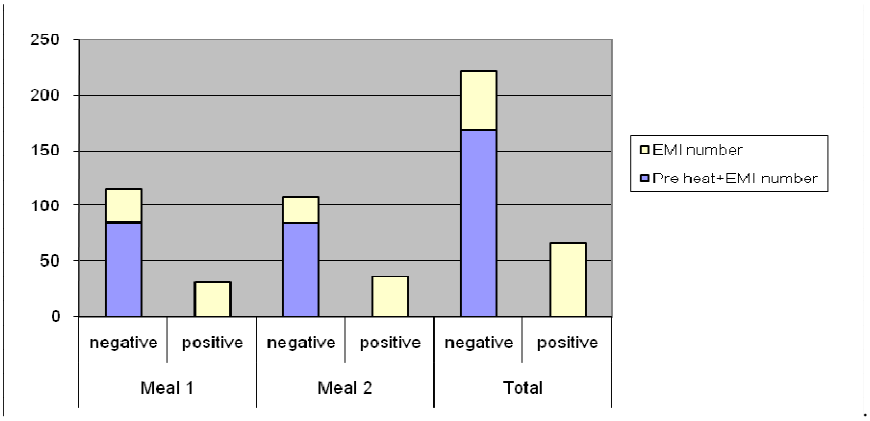

Fig 5.Number thermophile microorganism variable in different type of mushroom meals

CONCLUSION

We have obtained these results with" logistic regression" and "add ratio" for electromagnetic induction and combination of EMI with thermal processing

5-1 Effect of combination EMI and thermal processing

5-1-1-Mesophile

Table 10.Results of mesophile growth for combination EMI and various thermal processing

Ratio(Chance) Add	P-value (Sig)	Degree of freedom	Statistic	Coefficient	Condition
144.66	0.00	1	27.12	5.33	**Type of meal**
3.55	0.00	1	31.77	1.55	**Culture**

According to" Wald test', chance of negative mesophile microorganism growth increasing in meal 114466 percent more than meal 2 and has significant level equal to 0.001 between mesophile growth and type of meal. And chance of negative mesophile microorganism growth in culture "PCA"is 3.55 degree more than culture "PE 2" And chance of negative mesophile microorganism growth in culture "PE 2"is 355 percent more than culture "Cook Meat" so has significant level equal to 0.001 between mesophile growth and culture

5-1-2-Thermophile

In every conditions growth of thermophile microorganism has not been reported so type of meal; cultures; treatments do not have any effect.

5-2- Effect of EMI

5-2-1-Mesophile

Table 11. Results of mesophile growth for EMI treatment

Add Ratio(Chance)	P-value (Sig)	Degree of freedom	Statistic	Coefficient	Condition
1801.33	0.00	1	41.37	6.85	**Type of meal**
1.04	0.91	1	0.007	0.07	**Culture**

According to" Wald test', chance of passive mesophile microorganism growth increasing in meal 2 180133 percent more than meal 1 and has significant level (p-value=0.001) between mesophile growth, , but there is no significant level between mesophile growth and culture(p-value>0).

5-2-2-Thermophile

Table 12. Results of thermophile growth for EMI treatment

Add Ratio(Chance)	P-value (Sig)	Degree of freedom	Statistic	Coefficient	Condition
73.33	0.00	1	24.29	1.22	**Type of meal**
0.652	0.02	1	0.304	-0.55	**Culture**

According to" Wald test' chance of positive thermophile microorganism growth decreasing in meal 27333 percent more than meal 1 and has significant level (p-value=0.001) between thermopile growth and type of meal 1, and chance of positive thermophile microorganism. And chance of positive thermophile microorganism growth in culture "PE 2"is 65.2 percent more than culture "PCA" And chance of positive thermopile microorganism growth in culture " Cook Meat "is 65.2 percent more than culture " PE 2" so has significant level (p-value=0.05) between thermophile growth and culture

Acknowledgements

The author would like to thank, Eng. Mostafa Emampour (Logistic Studies and Research Center, Tehran-Iran), PMA Com (Plastic Machine Alvan, Tehran-Iran) ,and Ask Com (Afra Sanat Kimia Engineering , Mashhad-Iran) for supplying equipments and materials

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