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Combination of high frequency electromagnetic induction with thermal processing for shelf life prolongation of packed vegetables meal

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ABSTRACT

The combination of high frequency electromagnetic induction, with different condition of thermal processing (80- 85 ^{o}c) has been studied for sterilization of vegetables meal and also the effect of this treatment evaluated on sensory properties of these meals. In this method all samples were filled in pouches (retort multilayer flexible films). EMI treatment which discharges square-wave pulses with variable voltage 1-20 kV/cm , with different frequency(2-3GHz ,3-4 GHz ,4-5GHz. 5-6 GHz) have been done in step one . The usage of high frequency electromagnetic on clostridium and bacillus was 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 meal ,and type of culture ,so the death ratio of mesophile microorganisms increasing in vegetables meal(without spice) 14466 percent more than vegetables meal (with spice). However, in every conditions of this process growth of thermophile microorganism has not been reported.During the period of this experiment (2 - 12 months) appearance, color , texture , odor, as oranoleptic properties of these samples in various conditions , had no significant differences and also the taste and mouth feel of vegetables meal (without spice) during (2 - 12 months) had no significant differences too, but in last months (10 -12) these properties in vegetables meal (with spice) was unpleasant, so we can estimate shelf life of meal 1 two month more than meal 2 in ambient temperature.

Key words: electromagnetic induction (EMI); retort flexible films, mesophile bacteria, thermophile bacteria, thermal processing, vegetables meal, sensory properties

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INTRODUCTION

High frequency electromagnetic induction, as you know is a non-thermal method for destruction of cell membrane bacteria (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). During lately decades, consumption of ready to eat meal has plenty effect in manner of offering new food packaging products. This quick change is because of entering new forms of retort flexible multilayer films laminated with aluminum (3-6, 41, 45) for packaging different kind of meals instead of can (1, 45) .These packed meals without a efficient processing are potential source of pathogens microorganism,

specially mesophile and thermophile aerobic and anaerobic clostridium and bacillus, as the low acidity (pH 4-5) and high water activity of these packed meals can favor the growth and activity of bacteria in these kind of meals (26,28). Although, thermal treatment (120 ^{o}C,20 min) destroys these microorganisms (26,29), and also has been used widely, proteins and some other physiological substrates are inactivated, so 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 may be caused second contamination. For these reason, high frequency electromagnetic field (39-44,46) which is proving to be able for inactivation spoilage microorganisms without significantly affect nutritional properties of several foods(16). The usage of high frequency (2-15GHz) with different voltage (1-20kV/cm) (20) for 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) (8 ,23,24,35). The primary advantage of improved uniformity of heating was shown in- package sterilized by this method (3,4,5,6,38,39-44,46) . Packaging materials must 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) which are 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) is not adequate because spore of these bacteria are too resistance(10,21) , so the usage of EMI in combination with various preheating inactivate them without a significant adverse effect on food properties and taste(39-44). This phenomenon causes the formation of trans membrane pores and, the ratio of total pore area becomes unfavorable; the membrane is no longer able to repair these irreversible disruption. (33, 39-42,46,47). In this study, we investigate about the electromagnetic sterilization of packed vegetables meal which can substitute meat meals in future, and also ,effect of EMI sterilization on packed vegetables meal(with spice, without spice) in first step and combination of EMI with different thermal processing in second step(39-42,46,47) as " 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, 46).

MATERIALS AND METHODS

2.1. Preparation of Vegetables Meal

These mixed vegetables meal are prepared from five cooked vegetables (mushroom- Green peas - Corn - Carrots – Potato).These material were bought from local supermarket in Tehran-Iran. These vegetables were washed and cut into slices then cooked under steam condition with sauce (1.5 % salt, PH=4-5, Brix =8) (39-42,46). After cooking, two kinds of samples were prepared (cooked vegetables+ water) (39,40,41-43,46)

1-**Vegetables Meal 1:**Pouches contain 100 g cooked vegetables meal(without spice:1.5% Salt) (46)

2-**Vegetables Meal 2:**Pouches contain 100 g cooked vegetables meal (with spice: 1.5% Salt, 0.5% Pepper, 0.5% Turmeric, 0.5% Cinnamon, Tomato paste) (46)

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) (39.40,41-43).The approximate of oxygen in pouches is 3-5% which is 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)

Finally, all of packed samples (with spice or without spice, unprocessed (control) or processed) were put at room temperature in order to estimate the shelf life of them. Analytical characteristics of this container (retort multilayer flexible pouch) (4,5,6,41,45,46).) and also the best storage time of this pouch was reported, as you see in table 1

Table 1 Analytical characteristics of container (retort multilayer flexible pouch) (32,41, 45)

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)	
						Vegetables Meal 1	Vegetables Meal 2
PET\AL\PET\LLD	**12\7\12\100**	104.61	61.03	0	0.089	12	10

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

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 0. 04g/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 vegetable meals (with spice or without spice) to be combined EMI with pre heating , 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) while 1-2 g from CMM transferred to PCA after 2-5 day. For anaerobic growth 1-2 g of samples were put in PE 2(3-4 day) while 1-2 g from PE 2 transferred to RCM after 2-5 day. Growth of bacteria in CMM and PE 2 has shown 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 shown as a response or non parametric. (39,40,42-44,46)

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 to process samples of packed vegetables meal (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 vegetables meal (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) 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/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 formula (36) as follows:

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

V_c is the volume of a chamber (cm^3) and F_r flow rate (ml/s) which estimate 3-5 min (20 min induction,20 min rest)2 pulse per min (39,40,42-44,46)

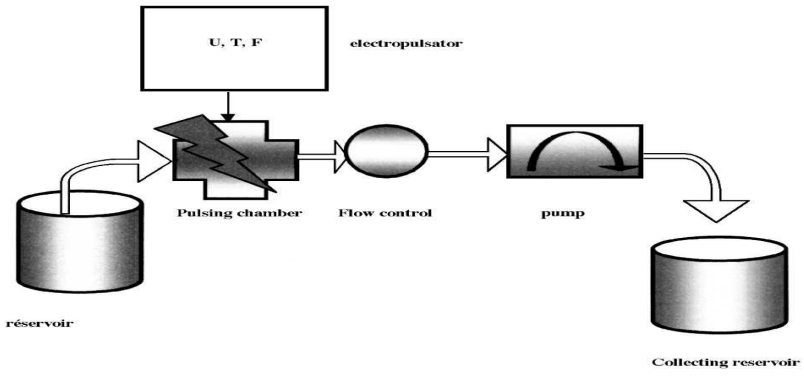

Fig. 1. Electromagnetic field. (A) Cells are taken from the reservoir. (B) The flow through the pulsing chamber where a controlled number of calibrated pulses is applied. The pulsing chamber is connected to the high-power pulse electro pulsation (voltage" U", pulse duration" T", pulse frequency" F")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

3- STATISTICAL ANALYSIS

Multilevel factorial design was carried out in packed vegetables meal(with spice or without spice) inoculated in different condition of combination EMI with different thermal processing , At the end we can find a model for these method ,type of meal and type of culture. (46)

We have described these 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 this treatment, suspected positive growth of microorganisms in enrichment culture evaluate negative ,in order to obtain model of logistic regression, which were showed in table 2 (39,40,42-44,46).

Evaluation of sensory properties (appearance, color , texture , odor taste , mouth feel) has been done according to numerical parameter for each meals. These parameter were shown in table 5 (application form) , have been filled by 100 person, in order to describe these variables. We must designed a model to analysis relationship between type meals, and sensory properties .So comparison of data was performed by the prism test in this part.

RESULTS

Growth of Microorganisms

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). The best result belong to 5-6GHz .So the effect of each thermal processing combined with this frequency of electromagnetic field (39,40,42,46), while for vegetables meal 1 (7 treatment) and vegetables meal 2 (7 treatment) was evaluated, and repeated in 3 run as you see in tables below ,table 2 “Number of mesophile and thermophile microorganisms in combination EMI treatment (5-6GHz) with various thermal processing“(39,40,42,46), table 3 “Number of mesophile microorganism variable in different cultures” , and table 4 “Number of mesophile microorganism variable in different type of meals “

Table 2 Number of mesophile and thermopile microorganisms in combination EMI treatment (5-6GH) with various thermal processing

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

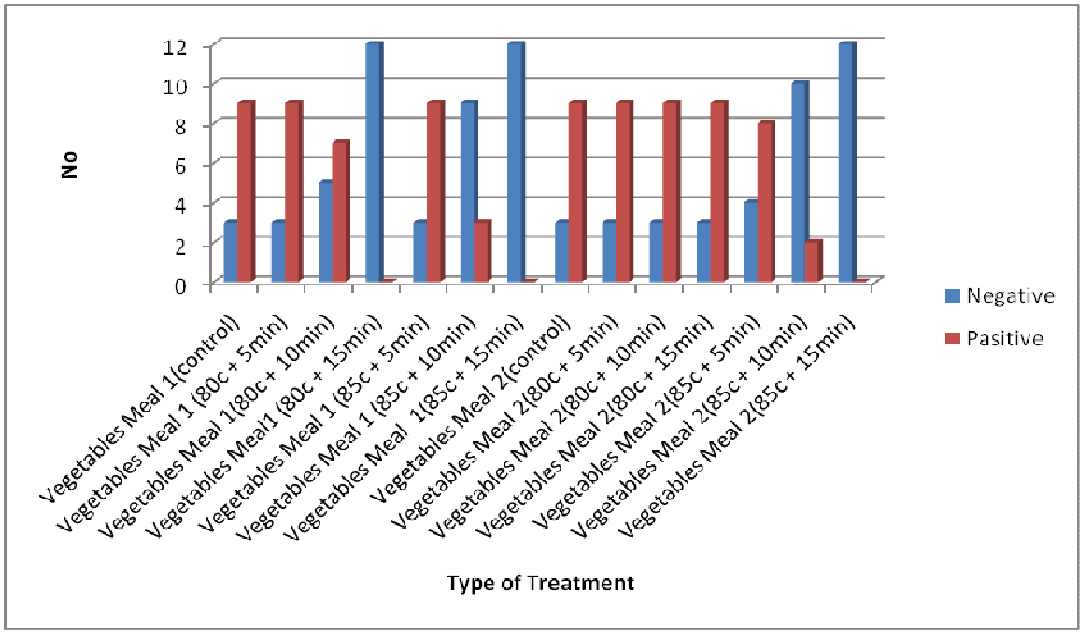

Fig 2 Number of mesophile microorganism in combination EMI treatment (5-6GHz) with various thermal processing

Table 3. Number of mesophile microorganism variable in different cultures

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

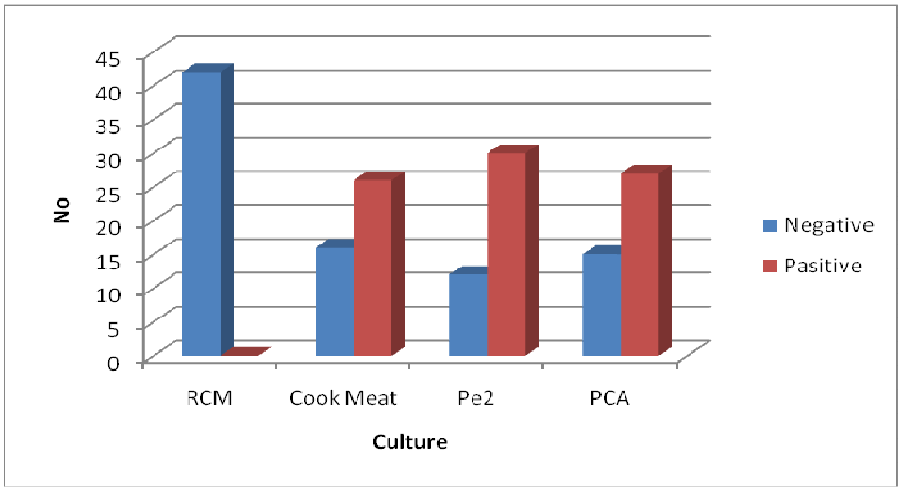

Fig 2. Number of mesophile microorganism variable in different cultures

Table 4 Number of mesophile microorganism variable in different type of meals

Treatment	EMI+Pre heat		
culture	Mesophile	number	Percent (%)
Vegetables Meal	negative	47	56
	positive	37	44
Vegetables Meal	negative	38	45.2
	positive	46	54.8

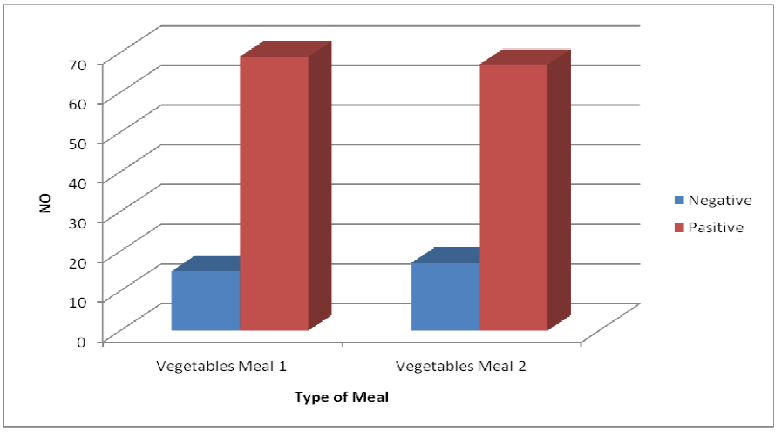

Fig 3. Number of mesophile microorganism variable in different type of meals

Sensory Properties

Evaluation of sensory properties (appearance, color, texture, odor taste, mouth feel) has been designed according to numerical parameter for each meals, as you see in table 5 . These properties have been described with marking system. Best marking system were 1-5 (1-Very Good ; 2-Good ; 3-Medium ; 4- Weak ; 5- Very Weak).

Table 5 .Evaluation of sensory properties (appearance, color , texture , odor taste , mouth feel) of Vegetable Meal

Notes	Organoleptic Properties						Product
	Mouth Feel	Smell	Taste	Texture	Color	Appearance	
							Vegetable Meal 1
							Vegetable Meal 2

Appearance: During the period of this experiment (2 - 12 months); appearance of samples in various conditions, had no significant differences.

Color: During the period of this experiment (2 - 12 months); color of samples in various conditions, had no significant differences.

Texture: During the period of this experiment (2 - 12 months); texture of samples in various conditions, had no significant differences.

Taste: During the period of this experiment (2 - 12 months); taste of meal 1 in various conditions, had no significant differences, however during the last months (10 - 12 months) taste of meal 2 was unpleasant.

Odor: During the period of this experiment (2 - 12 months); odor of samples in various conditions, had no significant differences.

Mouth feel: During the period of this experiment (2 - 12 months) ; mouth feel of meal 1 in various conditions, had no significant differences, however during the last months (10 - 12 months) mouth feel of meal 2 was unpleasant .

CONCLUSION

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

Mesophile

Table 6- Effect of p-value on type of meal , type of treatment and type of culture

(Chance) add ratio	P-value (Sig)	Degree of freedom	Statistic	Coefficient	Condition
-	0,00	1	33.77	-5.22	**Constant**
144.66	0,00	1	27.12	5.33	**Type of Meal**
0.566	0,00	1	23.44	-0.781	**Type of treatment**
3.55	0,00	1	31.77	1.55	**Culture**

Model of logistic regression is written:

Log (be negative) = -5,22 + 5.33(type of meal) -0.7811(type of treatment) + 1.55(culture)
According to" Wald test", effect of p-value on type of meal, type of treatment and type of culture has significant level (0,001).Other hand chance of negative mesophile microorganism growth increasing in meal 1 14466 percent more than meal 2 and has significant level equal to 0,001 between mesophile growth and type of vegetables meal, and chance of negative mesophile microorganism growth in every treatment compares with last treatment increasing 56.6 percent(negative mesophile growth from up to down increasing 56.6 percent) ,so has significant level equal to 0,001 between mesophile growth and type of treatment. And chance of negative mesophile microorganism growth in culture "PCA" is 3.55degree more than culture "Pe2" and chance of negative mesophile microorganism growth in culture "Pe2" is 355 percent more than culture "Cook Meat" so has significant level equal to 0,001 between mesophile grow.

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

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