Iraqi J. Vet. Med. 34 (2): 20 – 29; (2010).

Influence of mild pulsed electric field conditions on the growth and protease activity of *Streptococcus thermophilus*

Najim Hadi Najim
Dept.of Vet.Public Health,College of Vet.Med.,University of Baghdad.

Accepted – Septemper – 2010

Summary

Pulsed electric field (PEF) processing involves the application of pulses of voltage for less than one second to fluid products placed between two electrodes. *Streptococcus thermophilus* is an important bacterium used for the production of fermented dairy products. Objective of this study was to determine the influence of a mild PEF condition on the growth and protease activity of *Streptococcus thermophilus*. A range of mild pulsed electric field conditions were earlier screened by the author to arrive at an optimum overall mild pulsed electric field condition for various probiotic characteristics. Freshly thawed *Streptococcus thermophilus* was suspended in 0.1% w/v sterile peptone water and treated in a pilot plant PEF system. The treatment was a mild PEF condition of positive square unipolar pulse of 3 µs, pulse period of 0.5 sec. and voltage of 1 kV/cm. Control was run through PEF system but without receiving any pulsed electric field condition. Control and treated samples flow rates were kept constant at 60 ml/min. Samples were individually inoculated in lactobacillus MRS broth. Samples were plated in duplicate. Pour plates were incubated aerobically at 37°C for 3 days. Growth was determined hourly for 20 hours. The extracellular protease activity of *Streptococcus thermophilus* was determined by the o-phthaldialdehyde (OPA) spectrophotometric assay. Experiments were replicated three times.The control and mild PEF treated samples had the same counts of 10.97 (+/- 0.25) log cfu/ml at 0 hour. The mild PEF treated samples reached the log phase an hour earlier than control. Although at most time points, counts were within the same log cfu/ml for the control and treated samples, the mild PEF treated samples had significantly ($p<0.05$) higher counts compared to control for most of the time points over the 20 hours of growth. The mild PEF condition enhanced growth of *Streptococcus thermophilus*. Mild PEF treatment conditions had a significant influence on the protease activity of *Streptococcus.thermophilus* where a significant enhancement in its proteolytic activity compared to the control has been detected.

Key words:protease,streptococcus thermophilus, o-phthaldialdehyde.

Iraqi J. Vet. Med. 34 (2): 20 – 29; (2010).

تاثير المجال الكهربائى النابض والمعتدل على كل من النمو وفعالية انزيم البروتييز لبكتريا *Streptococcus thermophilus*

نجم هادي نجم
فرع الصحة العامة البيطرية / كلية الطب البيطري / جامعة بغداد

الخلاصة

يتضمن عمل المجال الكهربائي النابض تسليط موجات كهربائية نابضة وبجهد (فولتية) معين لمدة زمنية اقل من ثانية على المنتجات الغذائية السائلة اثناء مرورها بين قطبين . تكمن اهمية بكتريا *Streptococcus thermophilus* فى استعمالها لانتاج منتجات الالبان المتخمرة .كان الهدف من هذه الدراسة هو تعيين مدى تاثير المجال الكهربائي النابض والمعتدل على كل من النمو وفعالية انزيم البروتييز لبكتريا *Streptococcus thermophilus* قام الباحث بتجارب غربلة وعلى مدى واسع لمختلف المجالات الكهربائية النابضة من اجل الوصول الى الحالة المثلى لها والمؤثرة على خصائص هذه البكتريا .بعد اذابة بادىء *Streptococcus thermophilus* النقي قد لقح في (0.1 %) من مرق البيتون المعقم وعولج داخل منظومة المجال الكهربائي النابض .تضمنت المعالجة تسليط مجال كهربائي نابض ومعتدل سعة 3 مايكروثانية من نوع احادى القطب الموجب ولمدة نبض 0.5 ثانية وبجهد كهربائي 1 كيلوفولت / سم .قد مررت نماذج السيطرة داخل منظومة المجال الكهربائي النابض ولكن بدون استخدام اي جهد كهربائي نابض عليها .كانت سرعة جريان مرق البيتون الملقح بالبكتريا لكل من نماذج السيطرة والاخرى المعالجة بالمجال الكهربائي النابض هي 60 ملليليتر/ دقيقة .جمعت النماذج بعد مرورها داخل منظومة المجال الكهربائي النابض ولقحت فى مرق MRS لمراقبة نمو البكتريا وكذلك لقحت في الحليب الفرز المعقم لمراقبة فعالية انزيم البروتييز .زرعت نماذج النمو بعد مرور كل ساعة ولمدة 20 ساعة باطباق مزدوجة لكل تخفيف وبطريقة الصب وحضنت بدرجة حرارة 37 م ولمدة ثلاثة ايام تحت الظروف الهوائية .تم تعيين فعالية انزيم البروتييز بطريقة o-phthaldialdehyde للتحليل الطيفى وكررت التجارب لثلاثة مرات فقط .كانت اعداد هذه البكتريا فى كل من نماذج السيطرة والاخرى المعالجة بالمجال الكهربائي النابض هي 10.97 (+/ - 0.25) Log cfu/ml في ساعة الصفر .وصلت النماذج المعالجة بموجات المجال الكهربائي النابض والمعتدل الى الطور اللوغارتمي للنمو بساعة مبكرة عن نماذج السيطرة . بالرغم من كون اعداد هذه البكتريا كانت ضمن نفس Log cfu/ml لكل من نماذج السيطرة والاخرى المعاجة بالمجال الكهربائي النابض لكن كانت اعدادها في النماذج المعالجة بالمجال الكهربائي النابض المعتدل مرتفعة وبصورة معنوية عن مثيلتها من نماذج السيطرة .قد عزز المجال الكهربائي النابض والمعتدل في زيادة اعداد البكتريا خلال النمو وزيادة فعالية انزيم البروتييز لنفس البكتريا .

Introduction

Pulsed electric field (PEF) is a non-thermal treatment that offers the advantage of inactivating microorganisms with minimal impact on quality and nutritional factors (1). Microbial inactivation by PEF is affected by many factors which, in decreasing order of importance, include: electric field strength, treatment time, number of pulses, pulse wave shape, processing temperature, type of microorganism, microbial growth stage, electrical conductivity of the food, viscosity, pH of the food and the presence of antimicrobials (2,3,4and5). Microbial inactivation increase with an increase in the electric field intensity, above the critical transmembrane potential (6) which was found to be in the range of 1V (7) leading to permeabilization of the membrane by pore formation. Below critical values of electric field strength cell damage was reversible and both the metabolic activity and membrane integrity were initially reduced without loss of viability, above the critical values, however, irreversible cell damage occurred (8,9and10). It is well known that most if not all microorganisms are practically unaffected by electric

fields of less than about 4-8 kV/cm (11). Garcia (12) confirmed that the transient reversible pores by mild PEF would mechanically reseal in less than two minutes after removing the electric field and the process may involve a structural reorganization and resealing rather than a synthesis of new components. Several consensus documents have acknowledged the probiotic nature of yogurt cultures and this includes the report of the Joint Food and Agriculture Organization/World Health Organization working group (13), the International Scientific Association for Probiotics and Prebiotics workshop consensus document (14), and the Lancet review on gut flora in health and disease (15). Guarner (16) concluded that the yogurt starter culture clearly fulfill the current concept of probiotics. *Streptococcus.thermophilus* and *L. bulgaricus* were reported to improve digestion absorption and reduce the risk of lactose intolerance symptoms (17,18and19). They reduce antibiotic-associated diarrhea ,produce anti ulcer effects, prevent chronic gastritis, reduce the incidence rate of diseases like colorectal cancer and necrotizing entero colitis (16,20,21,22and23). Yogurt containing these two strains was used in the management of acute diarrheal disorders as recommended by the World Health Organization (24and25). Yogurt consumption enhanced the immune system in the immune compromised people (16,26and27). It was reported that a probiotic mixture containing *Streptococcus. thermophilus* was successful in increasing the immunity of HIV infected children (28). Yogurt bacteria have been detected in faeces of human subjects consuming yogurt (29and30). Most of the published works were focused on microbial inactivation by high intensity pulsed electric field (10-50 kV/cm) (31,and32). However, there is a lack of literature and only limited information available concerning the effects of mild PEF on friendly bacteria and have not received much attention. Accordingly, our objective of the present study then was to understand the influence of mild PEF (1 kV/cm) on the growth characteristics of *Streptococcus salivarius subsp. thermophilus* ST-M5.

Materials and methods

The control and PEF treatment samples were prepared by inoculating 10 ml of freshly thawed pure frozen concentrated stock culture of *Streptococcus thermophilus* ST-M5 (F-DVS ST-M5, Chr. Hansen's Laboratory, Milwaukee, WI, USA) into 990 ml of sterile 0.1% (w/v) peptone water and pumped through while being exposed to the electric field at elevated PEF treatment temperature (40.5ºC). The control run through PEF system but without receiving any PEF condition. A range of mild pulsed electric field conditions were earlier screened by the author to arrive at an optimum overall mild PEF condition for various probiotic characteristics. The treatment was mild PEF condition of positive square unipolar pulse width of 3 µs, pulse period of 0.5 sec., voltage of 1 kV/cm and flow rate of 60 ml/min. The equipment used to apply the different PEF conditions in this study was an integrated continuous fluid handling pilot plant processor (OSU-4M, Columbus, OH, USA). The PEF processor consist of four (2 pairs) co-field tubular treatment chambers, each chamber contains two stainless steel electrodes separated by a gap of 0.29 cm. Prior to testing the PEF equipment was cleaned and sanitized with 5% sodium hypochlorite

solution and then rinsed thoroughly with three liters of sterile distilled water. Growth characteristics analyses was determined hourly throughout 20 hours, where 10 ml of each of control and PEF treated samples were transferred into 90 ml of MRS broth individually and incubated aerobically at 37ºC. 1 ml samples were taken hourly and ten-fold diluted in a sterile 0.1% buffered peptone water and plated in duplicates by using *Streptococcus thermophilus* agar (33). Pour plates were incubated aerobically at 37ºC for 72 hours before enumeration. Experiments were replicated three times. The extracellular protease activity of *Streptococcus salivarius subsp*. *thermophilus* ST-M5 was determined by the o-phthaldialdehyde (OPA) spectrophotometric assay according to the method described by Oberg (34). *Streptococcus salivarius subsp*. *thermophilus* ST-M5 in control and pulsed electric field (PEF) treated samples was inoculated (10% {v/v}) into sterile skim milk (autoclaved at 121°C for 15 min.), and incubated at 37°C for 0, 12, 24, 36, and 48 hours. After incubation, 2.5 ml from each sample was mixed with 1 ml distilled water and transferred into test tubes containing Trichloroacetic acid (TCA) and the test tubes were vortexed at the same time. After setting at room temperature for 10 minutes the acidified samples were filtered through a Whatman Number 2 filter paper .Non-inoculated sterile skim milk was prepared similarly to use as a reference in the assay. Duplicate aliquots from each TCA filtrate were analyzed by the o-phthaldialdehyde (OPA) spectrophotometric assay using an UV-Vis spectrophotometer (Nicolet Evolution 100, Thermo Scientific; Madison, WI, USA). One hundred and fifty ul of each TCA filtrate were mixed with 3 ml of o-phthaldialdehyde final solution in a 3 ml cuvette, and the absorbance at 340 nm was read. The absorbance of the o-ohthaldialdehyde final solution with non inoculated sterile skim milk (reference) was subtracted from each sample reading. The o-phthaldialdehyde final solution was used as a blank to calibrate the UV-Vis spectrophotometer.These experiments were replicated three times.

Results

The growth curve of *Streptococcus salivarius subsp. thermophilus* ST-M5 subjected to the positive square unipolar pulse width of 3 µs for pulse period of 0.5 sec. using voltage of 1kV/cm at elevated PEF treatment temperature (40.5ºC) over the growth curve periods of 20 hours are shown in Figure 1. PEF treatment*hour interaction effect was significant ($p=0.0117$) (Table 1). The control and the mild PEF treated samples had the same counts of 10.97 (+/- 0.25) log cfu/ml at 0 hour. The mild PEF treated samples reached the log phase an hour earlier than the control. From hours 2 to 5, there were significant ($p<0.05$) differences between the control and that subjected to PEF treatment (Table 2). The PEF treatment effect had significant ($p=0.0002$) influence on the growth curve (Table 1). The exponential (log) phase of the growth curve of that subjected to PEF treatment was significantly ($p<0.05$) higher than that of the control. Although at most time points, counts were within the same log cfu/ml for the control and PEF treated samples, the PEF treated samples had non significantly ($p<0.05$) higher counts compared to the control for both the

stationary and decline phases of the growth curve. The mild PEF conditions significantly ($p<0.05$) enhanced the growth of the *Streptococcus salivarius subsp. thermophilus*. The Streptococci counts (log cfu/ml) of both the control and PEF treated samples increased significantly ($p<0.0001$) during the log phase and decreased significantly ($p<0.0001$) during the decline phase (Table 1) .The Optical Density (OD) values of the protease activity subjected to the same PEF conditions over the five time points of 0, 12, 24, 36 and 48 hours are shown in Figure 2. PEF treatment*hour interaction effect was non significant ($p=0.4223$) (Table 1). There were significant differences ($p<0.0001$) in the proteolytic activity of the control samples between 0, 12 and 24 hours of incubation at 37ºC whereas non significant ($p>0.05$) differences were found between 24, 36 and 48 hours of incubation at 37ºC. There were significant ($p<0.01$) difference in the proteolytic activity of the PEF treated samples between 0, 12, 24 and 36 hours of incubation at 37ºC while non significant ($p>0.05$) differences were found between 36 and 48 hours of incubation at 37ºC. At hours 0 and 24, there were no significant ($p>0.05$) differences among the control and the PEF treated samples. At hours 12, 36 and 48 the protease activity subjected to the PEF treatment were significantly ($p<0.05$) higher compared to the control (Table 2). PEF treatment effect had significant ($p=0.0005$) (Table 1) influence by enhancing the protease activity of *Streptococcus salivarius subsp. thermophilus* ST-M5.

Discussion

Some studies reported that electrical treatment could affect cell physiology (35). Garcia (12) confirmed that the transient reversible pore would be mechanically resealed in less than two minutes after removing the electric field and the process may involve a structural reorganization and resealing. The application of moderate electric field (MEF) (1 V/cm) at frequency of 60 HZ have been shown to alter the metabolic activity of the microbial cells (36). The application of MEF at different frequencies and waveforms during fermentation altered some of the growth kinetics of L. acidophilus (37). MEF treatment with purely sinusoidal waveforms at frequencies of 45 and 90 at 30ºC produced a shorter lag phase than conventional (control) fermentation of *L. acidophilus* OSU at the same temperature and the fermentation process was accelerated by applying pure sinusoidal MEF at the early stage of growth and concluded that the MEF fermentation could add economic benefits by decreasing the fermentation time (37). However, recent studies under controlled temperature conditions have shown that MEF accelerate growth in the early stage, but inhibits growth at the late stage of fermentation *of L. acidophilus* (39) and *Lactococcus lactis* (39). In general, enzymes require more severe high intensity PEF treatment than microorganisms to obtain significant inactivation (40&41). Pilar and Antonio (42) suggested that the PEF could originate small conformational changes leading to enhanced proteolytic activity. Bendicho, (41) studied PEF treatment on Bacillus subtilis protease and reported that an enhancement in proteolytic activity was found when the PEF treatment was carried out in milk.

Table 1: Mean square (MS) and Pr>F of PEF treatment, hour, and their interaction for growth and protease activity of *Streptococcus thermophilus*.

Source	Growth		Protease activity	
	MS	Pr > F	MS	Pr > F
PEF treatment	0.1911	0.0002	0.0013	0.0005
Hour	0.6703	<0.0001	0.0075	<0.0001
PEFtreatment*hour	0.0259	0.0117	0.0001	0.4223
Error	0.0125		0.0001	

Table 2: Least square means for growth characteristics and protease activity of *Streptococcus thermophilus* as influenced by pulsed electric field.

Treatment	Growth	Protease activity
	LSMean	LSMean
PEF	11.6164 A	0.0937 A
Control	11.5365 B	0.0804 B

LS Means with the same letter are not significantly different (p>0.05)

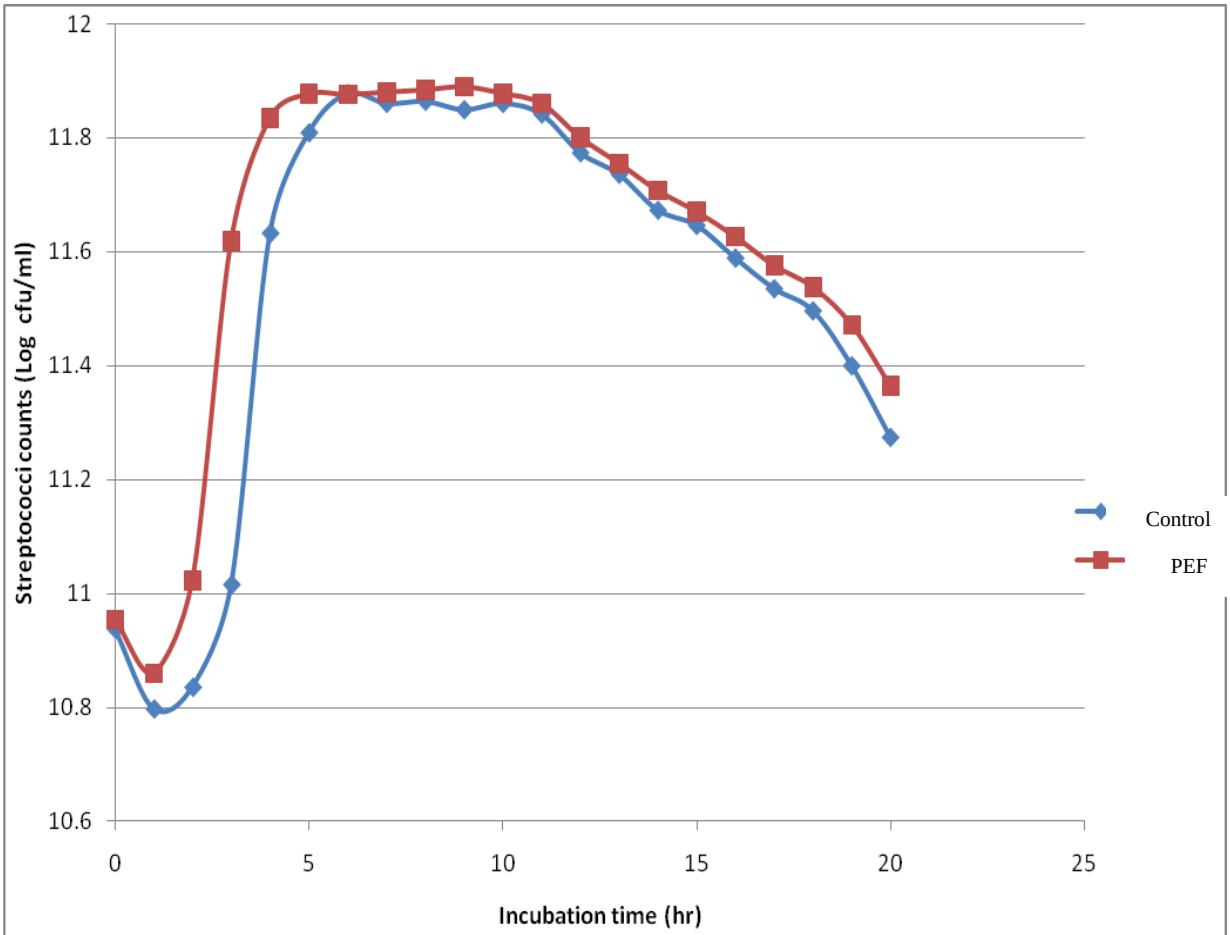

Figure 1 : The influence of mild pulsed electric field (PEF)on the growth characteristics of Streptococcus thermophilus

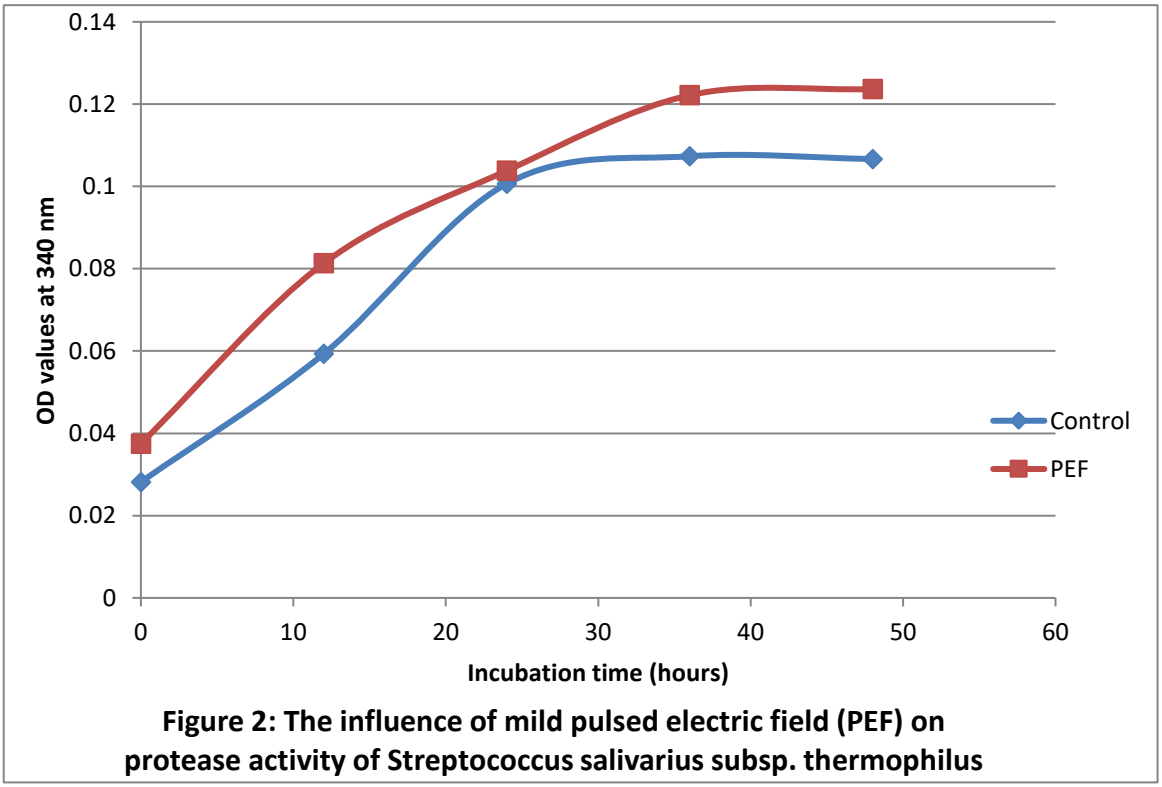

Figure 2: The influence of mild pulsed electric field (PEF) on protease activity of Streptococcus salivarius subsp. thermophilus

References

1-Sampedro FA Rodrigo A Martinez A and Rodrigo D(2005). Quality and safety aspects of PEF application in milk and milk products. Crit Rev Food Sci Nutr. 45:25-47.

2-Hodgins AM Mittal GS and Griffiths MW(2002). Pasteurization of fresh orange juice using low- energy pulsed electric field. J Food Sci. 67:2294.

3-Ho SY and Mittal GS(2000). High voltage pulsed electric field for liquid food pasteurization. Food Rev Int. 16:395.

4-IU J Mittal GS and Griffiths MW(2001). Reduction in levels of E. coli 0157:H7 in apple cider by pulsed electric fields. J Food Protect. 64:964.

5-Smith K Mittal GS and Griffiths MW(2002). Skim milk pasteurization by pulsed electricalfield and antimicrobials. J Food Sci. 67:2304.

6-Qin BL Barbosa-Canovas GV Swanson BG and Pedrow PD(1998). Inactivating microorganismusing a pulsed electric field continuous treatment system. IEEE Trans. Indus. Applic. 34(1):43- 49.

7-Zimmermann U and Neil GA(1996). Electromanipulation of cells. Boca Raton, Florida, U.S., CRC Press

8-Heinz V and Knorr D(2000). Effect of pH, ethanol and high hydrostatic pressure on the inactivation of *Bacillus subtilis* by pulsed electric fields. Innovative Food Science and Emerging Technologies. 1:151-159

9-Jayaram SH(2000). Sterilization of liquid foods by pulsed electric fields. IEEE Electrical Insulation Magazine. 16:17-25

10-Ulmer HM Heinz V Ganzle MG Knorr D. and Vogel RF(2002). Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. J Appl.Microbiol. 93:326-335.

11-Castro AJ Barbosa-Canovas GV and Swanson BG(1993). Microbial inactivation of foods by pulsed electric fields. J Food Process Pres. 17:47-73

12-Garcia D Mana P Gomez N Raso J and Pagan R(2006). Biosynthetic requirements for the repair of sublethal membrane damage in E. coli cells after pulsed electric fields. J Appl. Microbiol. 100: 428-435.

13-Joint Food and Agriculture Organization/World Health Organization working group (2002). Guidelines for the evaluation of probiotics in food. http://www.fao.org./es/esn/food/.

.14-Reid G Sanders ME Gaskins HR Gibson GR Mercenier A Rastall R Roberfroid M Rowland I Cherbu C and Klaenhammer TR(2003). New scientific paradigms for probiotics and prebiotics. J Clin Gastroenterol. 37:105-118.

15-Guarner F and Malagelada JR(2003). Gut flora in health and disease. Lacet. 361:512-519.

16-Guarner F Perdigon G Corthier G Salminen S Koletzko B and Morelli L(2005). Should yogurt cultures be considered probiotics. British J Nutr. 93:783-786.

17-Labayen L Forga L Gonzalez A Lenoir I and Martinez A(2001). Relationship between Lactose disgestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. Ailment pharmacol. Ther. 15:543-549.

18-Pelletier X Laure-Boussuge S and Donazzolo Y(2001). Hydrogen excretion upon ingestion of dairy products in Lactose-intolerant male subjects; Importance of the live flora. Eur J Clin Nutr. 55:509-512

19-Rizkalla SW Luo J Kabir M Chevalier A Pacher N and Slama G(2000). Chronic consumption of fresh but not heated yogurt improves breath-hydrogen status and short-chain fatty acid profiles; a controlled study in healthy men with or without lactose maldigestion. Am J Clin Nutr. 72:1474-1479

20-Baricault L Denariaz G Houri JJ Bouley C Sapin C and Trugnan G(1995). Use of HT-29 a cultured human colon cancer cell line, to study the effect of fermented milk on colon cancer cell growth and differentiation. Carcinogenesis. 16:245-252.
21-Bin-Nun A Bromer R Wilschanski M Kaplan M Rubensy B Caplan M and Hammerman C (2005). Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. The J Pediatrics. 147:192-196.
22-Rodriguez C Medici M Rodriguez AV Mozzi F and Font de Valdez G(2008). Prevention of chronic gastritis by fermented milk
23-Senesse P Boutron-Rault MC Faivre J Chatelain N and Belghiti C(2002). for colorectal adenomas; a case-control study in Burgundy (France). Nut Cancer. 44:7-15.
24-WHO/CDR/95.3 10/95(1995). The treatment of diarrhea. A manual for physicians and other senior health workers.
.25-Kligler B HanawayP Cohrssen A(2008). Probiotics in children. Pediatric Clinics of North America. 54(6):949-96made with exopolysaccharide producing *strept. thermophilus* strains. J Dairy Sci. 92:2423-2434.
26-Isolauri E Rautava S Kalliomaki M Kirjavainen P and Salminens S(2002). Role of probiotics in food hypersensitivity curr. opin. Allergy Clin Immunol. 2:263-271.
27-Miettinen M Lehtonen A Julkunen I and Matikanen S(2002). Lactobacilli and Streptococci activate NF-Kappa B and STAT signally pathways in human macrophages. J Immunol. 164: 3733-3740.
28-Trois L Cardoso EM and Miurac E(2008). Use of probiotics in HIV-infected children. J. Tropical Pediatrics. 54(1):19-24
29-Brigidi P Swennen E Vitali B Rossi M. and Matteuzzi D(2003). PCR detection of *Bifidobacterium* and *Streptococcus thermophilus* in feces of human subjects after oral bacteriotherapy and yogurt consumption. Int J Food Microbiol. 81:203-209.
30-Callegari ML Morelli L Ferrar SL Cobo Sanz JM. and Antoine JM(2004). Yogurt symbiosis survived in human gut after ingestion. FASEB. J. 18:1158.
31-Qin BL Pothakamury UR Barbosa-Canovas GV and Swanson BG(1996). Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. Critical Review in Food Sci Nutr. 36:603-67.
32-Vega-Mercado H Powers JR Martin-Belloso O Luedecke L Barbosa-Canovas GV and Swanson BG(1997). Effect of pulsed electric fields on the susceptibility of proteins to proteolysis and inactivation of an extracellular protease from P. fluorescens M 3/6, In; Engineering and Food at ICEF7:Part 1, Sheffield Academic Press, Sheffield, U.K., pp. c73-c76.
33-Dave RJ and Shah NP(1996). Evaluation of media for selective enumeration of *Strept. thermophilus*, L. *bulgaricus, L. acidophilus*, and *bifidobacteria*. J Dairy Sci. 79:1529-1538.
34-Oberg CJ Weimer BC Moyers LV Brown RJ and Richardson GH (1991). Proteolytic characterization of *L. bulgaricus* strains by the o-phyhaldialdehyde Test and Amino Acids Analysis.J. Dairy Sci. 74:398-403.
35-Aronsson K Lindgren M Johansson BR and Ronner U (2001). Inactivation of microorganisms using pulsed electric fields: The influence of process parameters on E. coli, *L. innocua, L. mesentreroides* and *S. Cerevisiae*. Innonative Food Science and Emerging Technologies.2:41-54
36-Loghavi L Sastry SK Yousef AE(2007). Effect of Moderate Electric Field on the metabolic activity and growth kinetics of *L. acidophilus*. Biotechnol Bioeng. 98(4):872-881

37-Loghavi L Sastry SK and Yousef AE(2008). Effect of Moderate Electric Field frequency on growth kinetics and metabolic activity of *Lactobacillus acidophilus*. Biotechnol. Prog. 24(1):148-153.

38-Cho HY Yousef AE and Sastry S(1996). Growth kinetics of L. acidophilus under ohmic heating. Biotechnol Bioeng. 49:334-340.

39-Unal R(Foods as risk factors 2000). Interaction of microorganisms with electricity; Growth kinetics of Lactococcus lactis subsp. lactis ATCC 11454 under sublethal ohmic heating. PhD Dissertation. The Ohio State University. Columbus, OH.

40-Bendicho S Barbosa-Canovas GV and Martin O(2002). Milk processing by high intensity pulsed electric fields. Trends Food Sci. Techol. 13:195-204.

41-Bendicho S Barbosa-Canovas GV Martin O(2003). Reduction of protease activity in milk by continuous flow high-intensity pulsed electric field treatments. J Dairy Sci

42-Pilar M and Antoinio V(2006). Effect of PEF on enzymes and food constituents, in Pulsed Electric Fields Technology for food industry. Fundamentals and Applications by Javier Raso and Volker Heinz. U.S.