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EFFECT OF MAGNETIC FIELD TREATMENT ON THE MILK CHARACTERISTICS WITH THE PRESENCE OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS RHAMNOSUS G*G BACTERIA

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Abstract

Two types of bacteria strains was used in this work, *Lactobacillus rhamnosus* GG and *Lactobacillus plantarum* were exposed to different exposure time 0,24,48 and 72 hours with different magnetic strength field 0.35 and 0.55 tesla. Results were significant gradually decreasing in coagulation time in exposed milk with increasing of time and strength exposure was at 0.35 tesla from 21 at zero time to 15.30 at 72 hour for *Lactobacillus rhamnosus* GG and decreased from 22 to 17 for *Lactobacillus plantarum* at zero time and after 72 time exposure. Respectively. At 0.55 tesla strength exposure Coagulation time was decreased from 13.30 to 10.30 when for *Lactobacillus rhamnosus* GG after 24 hour exposure time and from 16 to 12 for *Lactobacillus plantarum* after 72 hour. pH, Protein, Fat and Lactose content decreased significantly with decreasing of time and magnetic strength field exposure. Microbial content for *Lactobacillus rhamnosus* GG increased from $73.0x10^7$ to $77.0x10^8$ after 24 and 72 hour respectively, and from $34x10^7$ to $59.6x10^8$ with 0.35 tesla and after zero and 72 hour respectively, and at 0.55 tesla *Lactobacillus rhamnosus* GG increased from $36.0x10^8$ to to $95x10^8$ after 24 and 72 hour, respectively and from $98x10^7$ after 24 hour to $81x10^8$ after 72 hour for *Lactobacillus plantarum*.

Key words : Lactic acid bacteria, magnetic field, milk components.

Introduction

Milk is a natural source containing different nutrients, it is a white liquid produced from mammals and considered a primary source for infant mammals including humans. It contains antibodies from the mother which are able to reduce and prevent the risk of being infected by several diseases (Pehrsson *et al*., 2000). World wide, it is estimated that more than six to seven billion people consume milk and its products (Hemme and Otte, 2010). The energy contained in milk comes from its compositions which include protein, fat, carbohydrates as lactose, which is considered a problematic exception especially to people who lack the ability to produce lactase, so by converting the milk to other products will result in decreasing the amount of lactose (Curry, 2013). Milk contains an approximate of 87% water, which is a major component in the body and considered significant in human metabolism. pH of the milk is ranges from 6.4 to 6.8. Regarding carbohydrates, milk contains approximately 4.8-5.0% of carbohydrate in the form of lactose which is known to be a source for energy. Fat is another energy source with an amount of 3.4-3.5%. It is the main energy source used by the body during activities and prolonged exercises. The fatty acids in milk include 65% saturated, 29% monounsaturated, and 6% polyunsaturated fatty acids. Other milk content is protein, which is in the amount of approximately 3.3-3.5% and contains all the essential amino acids. The milk protein consists of both casein with an amount of 82% and whey at approximately 18%, both these elements is present in ice cream and yoghurt. As for vitamins, several essential vitamins are present such as (A, B, C and D). Lactic acid bacteria (LAB) are potential microorganisms used in food fermentation and are considered safe organisms. They are also known for their ability in enhancing food safety and increasing health benefits (Panesar, 2011; Widyastuti *et al*., 2014). These bacteria are considered the most extensive microorganisms used for milk fermentation which enhance products with good quality (Maragkoudakis *et al*., 2006).

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Large-scale manufactured fermented milk products have become an essential industrial application of LAB as starter cultures. LAB has the ability to produce acid which is considered a preservative agent in generating the flavor of the products. The presence of lactic acid bacteria in milk during fermentation has the ability to produce several compounds, which converts α- or β-lactose to D or L lactic acids. This conversion causes the decrease in milk pH to 4.6 (Lopez *et al*., 2006). *Lactobacillus plantarum* is a lactobacillus bacteria found in fermented food products such as cheese. It is known to be very flexible and has the ability to grow between 15-45°C at a pH of 3.2 and higher. It is also known to develop probiotics (de Vries *et al*., 2006). *Lactobacillus plantarum* has the ability to survive gastric transit. Several studies have observed the use of *Lactobacillus plantarum* an ingestible living vaccine (de Vries *et al*., 2006). Regarding *Lactobacillus rhamnosus* GG, this probiotic bacterium is found in yoghurt, fermented and un-pasteurized milk and also in semi-hard cheese. Regarding growth conditions, it has the ability to grow between 15-40°C at a pH level of 4.5 to 6.4 (Valik *et al*., 2008). Clinical experiments with *Lactobacillus rhamnosus* GG observed a resistance to respiratory and gastrointestinal infections also fever decrement, also healing effects to atopic eczema (Mattila-Sandholm *et al*., 1999). Despite the importance of these bacteria in food production, recently a developing interest in fermented milk products for other beneficial purposes such as health purposes and prevention of toxins produced by harmful bacteria that enters the human body (Ali, 2010; Sharma *et al*., 2012) such as *Staphylococcus aureus*, was observed (Meng–Xiang *et al*., 2005) due to this magnetic field exposure was used as a new developed method to eliminate unwanted microorganisms and to preserve food quality (Miyakoshi, 2005). The magnetic field is a force that is created by moving electric currents and magnetic dipoles. An exposure to this field causes an energy transfer which causes a production of a stream of ions that are capable of exposing at a high velocity (Hofmann, 1985). The application of lactic acid bacteria in milk with the presence of a magnetic field has received great attention in recent years (Deegan *et al*., 2006; Glvez *et al*., 2007). This also depends on the type of food, preparation and the type of LAB present. It was observed that exposure to the magnetic field caused an increase in the permeability of ion channels in the cytoplasm membrane and formation of free radicals, and also cell wall dissociation (Cabisco *et al*., 2010; Inhan-Garip *et al*., 2011). Recent studies showed that this field has the ability to affect the pathogenic potential harmful microorganisms (Fojt *et al*., 2009). Other reported the effect of a magnetic field in causing an increase in the growth and cell activity for some bacteria e.g. *E.coli* and a decrease in cell activity with the presence of *A. baumannii* (Fijałkowski *et al*., 2015). Several studies have also observed that the rotating magnetic field's frequency can modulate functional parameters of different species of bacteria (Fijałkowski *et al*., 2015). In general, Use of magnetic field has a remarkable impact on the bacterial growth of LAB as compared with other harmful bacteria; such influence relies on the cell type, exposure time, magnetic intensity and frequency. The aim of this study was to investigate the effect of magnetic field strength on exposure milk composition and growth of two types of bacteria.

Materials and Methods

Bacteria strain

The strain *Lactobacillus plantarum*, obtained from the laboratories of the College of Agriculture, University of Baghdad was used for this study. After several diagnostic tests to ascertain its identity, the bacterium was developed on MRS broth media with the presence of 0.5% lactose. The isolates were then activated using a sterile MRS broth media only, this procedure was repeated three times. *Lactobacillus rhamnosus GG* (L.GG), obtained from the PRO Thera Klari LAB's division factor 1 USA, was used also in the study. Its purity was determined by observing the colonies under the light microscope and after dyeing them with gram stain. The isolates of the second type of bacteria were also activated using a sterile MRS broth media only, this procedure repeated was repeated three times also.

Starter setup

Dried sorted milk → Recovery of 12% → Sterilize at 121°C for 10 minutes
↓
Incubation at 37°C ← Vaccination with bacteria by 5%
↓
Re-process 3 times for activation purpose

Electromagnet preparation

The electric magnet was prepared using a wire role (the number of rolls is known) through which an electric current passes through. The heart of the coil is iron. Limit 0.55 and 0.35 Tesla was obtained by changing the current. The current was determined according to the following equation.

$$B - \frac{\mu NI}{2r}$$

r = Radius

I = Current intensity

B = Magnetic field strength

N = Number of user file rolls

Preparation of milk for the magnetic current and manufacture of fermented milk

Cow milk was taken from the dairy lab of the College of Agriculture, University of Baghdad. The milk was then poured into glass containers of 50 cm^3, then sealed and sterilized at 121°C for 10 minutes. The glass containers were subjected to different magnetic fields (0.55, 0.35) Tesla respectively in (73, 48, 24) hours. 5% (size/volume) of the activated starter was added and incubated at 37°C until full coagulation. Glass containers containing fermented milk were stored and several test were performed.

Chemical analysis

a. Fat Content

The percentage of fat was estimated according to Egan *et al*. (1981).

b. Determination of lactose sugar

This procedure was determined by calculating the difference between the ingredients (Pearson, 1976) for the milk sample.

c. Total protein content

Determination carried out by micro-Keldal method according to AL-Rawi (1985).

d. Determination of acidic function

The acidic function of the milk sample (Egan *et al*., 1981) was estimated using a pH- meter device. The total number of bacteria and the time of product clotting were calculated by placing a flask of sterilized milk containing a starter of 5% (volume/volume) in a water bath at 37°C. The time of thrombosis was established.

Calculation of the total number of survive bacteria

The total number of live bacteria in the starter and product was calculated by taking 1 cm^3 of the model for both starter and product and creating a sequence of decimals. The method of (Pour-Plate) (Speak, 1984) was applied using MRS medium, incubating the dishes at 37°C for 48 hrs in anaerobic conditions. The number of developing colonies was calculated using colony counter.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results and Discussion

Magnetic field exposure impact on milk in the presence of both *Lactobacillus plantarum* and *Lactobacillus rhamnosus GG* bacteria.

The magnetic field is a field of force that is created by movement of electrical currents and magnetic dipoles which exerts forces on the other close electrical currents and magnetic dipoles. In this procedure, milk was exposed to a magnetic current of 0.35 and 0.55 Tesla, respectively for 24, 48 and 72 hours. After these exposure times, both lactic acid bacteria were applied to the milk. The application of *Lactobacillus plantarum* to 24 hrs magnetic field exposed milk observed a coagulation time for only (20.30) hrs, compared to the control milk, which the coagulation time was after 22.00 hrs. This was also observed when applying the mentioned bacterium to a (48) hrs magnetic field exposed milk, which also showed less coagulation period of 18.00 hrs and only (17.00) hrs of a coagulation time to a 72 hrs magnetic field exposed milk (table 1).

This experiment was done by using (0.35) Tesla. By comparing the results mentioned above with the results during the use of 0.55 Tesla, observes remarkable decrease in the coagulation time using the same conditions mentioned previously which showed time increment from (16.00) hrs for the (24) hrs magnetic field exposed milk to gradually (14.30) hrs for the (48) hrs magnetic field exposed milk to a (12.00) hrs for the (72) hrs magnetic field exposed milk. These results show the effect of magnetic field exposure to the milk and its positive effect in decreasing the coagulation period, the higher the exposure, the lower the coagulation time. These results were also observed in the presence of *Lactobacillus rhamnosus GG* when using a magnetic field of (0.55) Tesla (Table 1), which observed a remarkable coagulation time of (10:30) hrs for the (72) hrs magnetic field exposed milk, compared to the result in the presence of *Lactobacillus plantarum.* By increasing numbers of pulses, total energy input was increased (Ulmer *et al*., 2002). The magnetic field exposed milk at (48) hrs also showed a decrease in the coagulation time of (11:00) hrs as for the magnetic field exposed milk at (24) hrs showed a coagulation period of (13:30) hrs. These results show an obvious difference between the effect of both bacteria in the exposed milk which indicates the highly influence of the magnetic field exposure in decreasing the coagulation time in the presence of *Lactobacillus rhamnosus GG* compared to *Lactobacillus plantarum*. These results were also noticed when using lower magnetic field strength of (0.35) Tesla with the presence

Table 1 : shows the time of selected milk exposed to a magnetic field strength (0.35, 0.55) Tesla and at different hours (73, 48, 24) hours using *Lactobacillus plantarum* and *Lactobacillus rhamnosus GG* bacteria.

Coagulation time (hour)		Exposure time(hour)	Magnetic field strength (Tesla)
Lactobacillus rhamnosus GG	***Lactobacillus plantarum***		
21.00	22.00	0	0.35
19.00	20.30	24	
17.30	18.00	48	
15.30	17.00	72	
13.30	16.00	24	0.55
11.00	14.30	48	
10.30	12.00	72	
3.695 *	4.813 *	LSD	

* $(P<0.05)$.

Table 2 : Effect of strength of magnetic field and different time on chemical milk composition.

Magnetize field (Tesla)	Magnetize field (time)	pH	Protein %	Fat %	Lactose %
0	0 min	5.22	3.45	3.18	4.27
0.35	24 h	5.10	3.31	3.09	4.11
	48 h	4.82	3.23	2.79	3.89
	73 h	4.44	3.10	2.55	3.65
0.55	24 h	5.01	3.11	3.00	4.01
	48 h	4.63	3.03	2.53	3.74
	73 h	4.33	2.95	2.31	3.32
LSD value		0.794*	0.388*	0.763*	0.702*

* $(P<0.05)$.

Table 3 : Microbial content of milk after magnetic field treatment for different period's exposure time.

Lactobacillus rhamnosus GG	*Lactobacillus plantarum*	Magnetize field(time)	Tesla
73.0×10^{7}	34.0×10^{7}	0 min	0.35
98.9×10^{7}	73.0×10^{7}	24 h	
25.2×10^{8}	17.0×10^{8}	48 h	
77.0×10^{8}	59.6×10^{8}	72 h	
36.0×10^{8}	98.0×10^{7}	24 h	0.55
67.0×10^{8}	39.6×10^{8}	48 h	
95.0×10^{8}	81.0×10^{8}	72 h	
22.069 *	19.573*		LSD

* $(P<0.05)$.

of *Lactobacillus rhamnosus GG*, which from the (24) hrs magnetic field exposed milk, the coagulation time observed to be (19.00) hrs comparing the result to the control milk which shows a coagulation time of (21.00) hrs. Regarding the (48) hrs magnetic field exposed milk, the coagulation period observed was (17.30) hrs; and the (72) hrs magnetic field exposed milk showed a coagulation period of (15:30) hrs (table 1).

Effect of magnetic field on different milk compositions

Table 2 showed the milk chemical compositions after being exposed to a magnetic field for different periods of time. pH was decrease when milk was exposed for longer time, It was 5.22% at zero time and decreased to 5.10, 4.82 and 4.44 after 24, 48 and 73 hr. of exposure and at 0.35 Tesla magnetic field respectively. Simpson (1993) observed that increasing of pH values in his study increased the antimicrobial activity of hop extracts, more ever this will be decrease of growth of *Lactobacillus Plantarum* (Gänzle *et al.*, 2001). Other studies mentioned that the actual pH of the medium has negative effect on *Lactobacillus plantarum* (Wouters *et al.*, 1999).

Fat percentage showed a gradual previous decrease, it was decreased from 3.18% at zero time to 3.09, 2.79 and 2.55% after 24,48 and 73 hr. exposure treatment at 0.35 tesla. magnetic field, respectively. The reason was due to adhesion of some fat molecules on the pipes walls of magnetic device. Since milk went through pipes at room temperature (25C), fat molecules were solid and not be homogenized. Other studies observed a decrease in fat percentage from 3.14% at zero point to 2.69% after one hour of magnetic field exposure (Ali *et al.*, 2015). Milk also showed a percentage decrement from 2.92% to 2.69% at the similar exposure period. This decrement might be associated to fat precipitation during exposure (Korolenko *et al.*, 1995). Milk protein showed slight decreases in percent from 3.45% at zero time to 3.10% after 73 hr. when milk exposed with 0.35 tesla of treatment. This may be due to association of protein with fat precipitated on pipes walls (Korolenko *et al.*, 1995). Lactose concentrate was decreased from 4.27% at zero time to 4.11, 3.89, 3.65% after 24, 48, and 73 hr. respectively when milk was exposed with 0.35 tesla of treatment.

Effect of magnetize milk on microbial content

Table 3 showed significant increasing (P<0.05) in *Lactobacillus rhamnosus GG* content of magnetize milk after different exposure periods. It increased from 73.0×10^7at zero time to 77.0×10^8 after 72 hr. of exposure by using 0.35 tesla treatment. On the other hand, *Lactobacillus plantarum* content increased significantly (P<0.05) after different exposure periods. It was 34.0×10^7 cfu/g when milk exposed on 0.35 tesla at zero time to 59.6×10^8 cfu/g after 72hr treatment.

Rustles showed that the calculated of survived bacteria counts in milk had been increased with increasing time of exposure of magnetic field. On the other hand, when *Lactobacillus rhamnosus GG* exposed milk with 0.55 tesla results showed significant increased with increasing of magnetic treatment was increased from 36.0×10^8 to 95.0×10^8 tesla after 72 hr. exposure. Same effective was detected in *Lactobacillus plantarum* content was increased significant (P<0.05) with increasing magnetic treatment from 34.0×10^7 at zero time to 59.6×10^8 at 72 hr. exposure and to 81.0×10^8 after 72 hr. and 0.55tesla exposure.

The viability of cells observed to be affected by the magnetic current treatment, which resulted that cell metabolic activity and membrane integrity were increased with magnetic field current strength and energy input (Ulmer *et al.*, 2002). Regardless of both bacterial growth numbers, *Lactobacillus rhamnosus GG* has shown a highly significant number in bacterial growth compared to *Lactobacillus plantaru*m which shows the different bacteria affected by the presence of magnetic field exposure and its ability to grow and survive.

References

Ali, A. A. (2010). Beneficial Role of Lactic Acid Bacteria in Food Preservation and Human Health. *Research Journal of Microbiology*, **5(12)** : 1213-1221. http://dx.DOI.org/10.3923/jm.2010.1213.1221.

Ali, H. I., A. R. S. Al-Hilphy and A. K. Al-Darwash (2015). The effect of magnetic field treatment on the characteristics and yield of Iraqi local white cheese. *IOSR Journal of Agriculture and Veterinary Science*, **8(9) II** : 63-69.

Cabisco, E., J. Tamarit and J. Ros (2010). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology*, **3(1)** : 3-8.

Curry, A. (2013). Archaeology: The milk revolution. *Nature*, **500 (7460)** : 20–22. DOI:10.1038/500020a.

de Vries, M. C., E. E. Vaughan, M. Kleerebezem and W. M. de Vosa (2006). *Lactobacillus plantarum*–survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, **16** : 1018–1028. DOI:10.1016/j.idairyj.2005.09.003.

Deegan, L. H., P. D. Cotter, C. Hill and P. Ross (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. *International Dairy Journal*, **16** : 1058–1071. DOI:10.1016/j.idairyj.2005.10.026.

Egan, H., R. S. Kirk and R. Sawyer (1981). Pearson's chemical analysis of food. 8th Edition .Reprinted Longman scientific and technical, UK.

Fijałkowski, K., P. Nawrotek, S. Struk, M. Kordas and R. Rakoczy (2015). Effects of rotating magnetic field exposure on the functional parameters of different species of bacteria. *Electromagn Bioloy and Medicine*, **34 (1)** : 48–55. https://DOI.org/10.3109/15368378.2013.869754.

Fojt, L., P. Klapetek and A. K. L. Stras (2009). 50 Hz magnetic field effect on the morphology of bacteria. *Micron.*, **40** : 918–922. DOI: 10.1016/j.micron.2009.06.009.

Glvez, A., H. Abrioue, R. L. López and N. B. Omar (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiol*, **120** : 51–70. DOI:10.1016/j.ijfoodmicro.2007.06.001.

Hemme, T. and J. Otte (2010). Status and Prospects for Smallholder Milk Production: A Global Perspective. Food and Agriculture Organization of the United Nations.

Hofmann, G. A. (1985). Deactivation of microorganisms by an oscillating magnetic field. *U.S. Patent*, **4** : 524,079.

Inhan-Garip, A., B. Aksu, Z. Akan, D. Akakin, A. N. Ozaydin and T. San (2011). Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria. *Int J Radiat Biol.*, **87 (12)** : 1155-61. https://DOI.org/10.3109/09553002.2011.560992.

Lopez, C., C. Bourgaux, P. Lesieur, A. Riaublanc and M. Ollivon (2006). Milk fat and primary fractions obtained by dry fractionation 1. Chemical composition and crystallisation properties. *Chemistry and Physics of Lipids*, **144** : 17-33.

Maragkoudakis, P. A., C. Miaris, P. Rojez, N. Manalis, F. Magkanari, G. Kalantzopoulos and E. Tsakalidou (2006). Pro duction of Traditional Greek Yoghurt Using *Lactobacillus* Strains with Probiotic Potential as Starter Adjuncts. *International Dairy Journal*, **16 (1)** : 52-60. http://dx.DOI.org/10.1016/j.idairyj.2004.12.013.

Mattila-Sandholm, T., S. Blum, J. K. Collins, R. Crittenden, W. de Wos, C. Dunne, R. Fondén, G. Grenov, E. Isolauri, B. Kiely, P. Marteau, L. Morelli, A. Ouwehand, R. Reniero, M. Saarela, S. Salminen, M. Saxelin, E. Schiffrin, F. Shanahan,

E. Vaughan and A. von Wright (1999). Probiotics: towards demonstrating efficacy. *Trends in Food Science and Technology*, **10** : 393–399.

Meng-xiang, G., M. Le and G. Kangquan (2005). Intense pulsed magnetic field on the bactericidal effect of milk and its nutritional composition studies of the impact of Sterilization effect of pulsed magnetic field and its influence on milk nutrient components journals. *Journal of Agricultural Engineering core Transactions of The Chinese Society Of Agricultural Engineering*, **03**.

Miyakoshi, J. (2005). Effects of static magnetic fields at the cellular level. *Prog Biophys Mol*. DOI:10.1016/j.pbiomolbio.2004.08.008.

Panesar, P. S. (2011). Fermented Dairy Products: Starter Cultures and Potential Nutritional Benefits. *Food and Nutrition Sciences*, **2(1)** : 47-51. http://dx.DOI.org/10.4236/fns.2011.21006.

Pearson, D. (1976). The chemical analysis of food, 7th Edition. Churchill living stone, Edinburgh, London and New York.

Pehrsson, P. R., D. B. Haytowitz, J. M. Holden, C. R. Perry and D. G. Beckler (2000). USDA's National Food and Nutrient Analysis Program: Food Sampling. *Journal of Food Composition and Analysis*, **13(4)** : 379–389. DOI:10.1006/jfca.1999.0867.

SAS (2012). *Statistical Analysis System*, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sharma, R., B. S. Sanodiya, D. Bagrodia, M. Pandey, A. Sharma and B. S. Bisen (2012). Efficacy and Potential of Lactic Acid Bacteria Modulating Human Health. *International Journal of Pharma and Bio Sciences*, **3(4)** : 935-948.

Simpson, W. J. (1993). Studies on the sensitivity of lactic acid bacteria to hop bitter acids. *Journal of the Institute of Brewing*, **99** : 405–411.

Speak, M. (1984). *Compendium of method for the microbiological examination for food*. 2nd Edition. Washington, D.C. USA.

Ulmer, H. M., V. Heinz, M. G. Gänzle, D. Knorr and R. F. Vogel (2002). Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. *Journal of Applied Microbiology*, **93** : 326–335. DOI:10.1046/j.1365-2672.2002.

Valik, L., A. Medveddova and D. Liptakova (2008). Characterization of the growth of *Lactobacillus rhamnosus* GG in milk at suboptimal temperatures. *Journal of Food and Nutrition Research*, **47(2)** : 60-67.

Widyastuti, Y., Rohmatussolihat and A. Febrisiantosa (2014). The Role of Lactic Acid Bacteria in Milk Fermentation. *Food and Nutrition Sciences*, **5** : 435-442. DOI:10.4236/fns.2014.54051.

Wouters, P. C., N. Dutreux, J. P. P. M. Smelt and H. L. M. Lelieveld (1999). Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. *Applied and Environmental Microbiology*, **65** : 5364–5371.