



STUDIES ON PHOSPHATE AS CHEMICAL PRESERVATIVES IN SOME MEAT PRODUCTS.

Saad, S.M. Edris, A.M., Ibrahim, H.M., Hassanin, F.S. and Nayel, M.S.

Food hygiene Department, Fac. Vet. Med., Benha University.

ABSTRACT

A total of one hundred and twenty random samples of different meat products (Beef burger, Luncheon, Canned corned beef, Sausage, Pastrami and Kofta) (20 of each) were collected from different supermarkets in Menoufia governorate. The samples were subjected to chemical determination of Phosphate content (ppm) in the examined meat products. The levels of phosphate (%) in the examined meat products that the mean value of phosphate was 0.43 ± 0.02 for beef burger, 0.41 ± 0.02 for luncheon, 0.43 ± 0.019 for sausage and 0.38 ± 0.029 for kofta. Phosphate failed to be detected in the examined canned corned beef and pastrami samples while failed to be detected in 10%, 60%, 20% and 20% in the examined beef burger, luncheon, sausage and kofta samples, respectively. The detectable samples above the permissible limit were 75%, 35%, 75% and 55% for beef burger, luncheon, sausage and kofta samples respectively.

(BVMJ-24(1): 289-297, 2013)

1. INTRODUCTION:

Phosphates are slightly bacteriostatic as it slows down the growth of some Gram-positive bacteria. Phosphates are not considered as direct preservatives. They only can impart some desirable properties when used as acidulants or in combination with other food ingredients such as nisin, EDTA, NaCl, nitrites, erythorbate, etc; can inhibit Gram-positive bacteria such as *Leuconostoc carnosum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus stearothermophilus*, *Bacillus brevis*, *Bacillus subtilis*, *Bacillus sphaericus*, *Bacillus sp.*, *Micrococcus luteus* and *Corynebacterium glutamicum*; and have a little effect on Gram-negative bacteria such as *Salmonella typhimurium*, *Salmonella enteritidis* and *Escherichia coli* (1), (2) and (3). Also sodium pyrophosphate may have significant bacteriostatic activity against *C. perfringens* and may provide processed meat with a

degree of protection against this microorganism particularly if employed in conjunction with acidic pH, high salt concentration and adequate refrigeration (4). Addition of Phosphate caused an increase in water-holding of about 30-40 g water per 100 g meat (5) and improve the yield (6) so phosphate is applied for improving sausage emulsion, water holding capacity and stability (7). While the addition of salts together with phosphates at the same time to a meat product lead to the muscular protein becomes soluble and solubilized, or activated; and the solubilized protein can immobilize high levels of added water as well as emulsify a large amount of fat (8). Although there are at least four functional properties for phosphates that can be used in meat products: cleaving the actomyosin bond, increasing the ionic strength, changing pH, and binding to divalent cations (9), but Foods which contain phosphate additives have a phosphorus content nearly 70% higher than the samples which did not

contain additives. This creates a special concern because this extra amount of phosphorus is almost completely absorbed by the intestinal tract. These hidden phosphates and worsen phosphate balance control as well as increase the need for phosphate binders and related costs (10) leading to elevated serum phosphorus which is a major, preventable etiologic factor associated with the increased cardiovascular morbidity and mortality of dialysis patients (11) and hyperphosphatemia is a risk factor for the development of several different complications of chronic kidney disease (CKD), including secondary hyperparathyroidism and cardiovascular complications, due to the formation of calcium-phosphate deposits (12). Chronic kidney failure is associated with high levels of phosphate and calcium, accelerated atherosclerosis, calcification of various parts of the cardiovascular system, and increased morbidity and mortality from CVD(13). There is association between phosphate intake and cancer and reported on a link between low phosphorus intake and a reduced risk of prostate cancer and also reported that high intake of phosphates may increase the risk for and proliferation of lung cancer (14). High dietary phosphate intake reduced growth, skeletal material, and structural properties and decreased bone strength in growing male rats. Adequate calcium could not overcome this (15), while in adult rats excessive intake of dietary phosphate without the accompany of calcium caused rise in concentration of serum parathyroid hormone and hindered mineral deposition into cortical bone, leading to lower bone mineral density (16). In vivo hyperphosphatemia is associated with alterations of calcium and vitamin D levels, both of which are known to alter the parathyroid hormone (PTH) release independently (17). So that the aim of this work is to determine the level of phosphate in some meat products (luncheon, Pastrami,

Sausage, Beef burger, Kofta and Corned beef) and experimental work.

2. MATERIAL AND METHODS:

2.1. Samples

A total of 120 random samples of meat products represented by luncheon, Pastrami, Sausage, Beef burger, Kofta and Corned beef (20 of each) were collected from different supermarkets in Menofya governorate for determination of their phosphate content (%). The collected samples were directly transferred to the laboratory without undue delay and examined as rapidly as possible. All collected samples were subjected to the chemical examination to estimate the phosphate content (%). to evaluate the acceptability of such meat products according to the specification stipulated by Egyptian Standard Specification or Egyptian Organization for Standardization and Quality Control (18) and compare the content on the label with the obtained results. Also the effect of this phosphate on lab animal was determined to measure the margin of safety on the consumer. Accordingly, the following examinations were carried out;

2.2. Determination of Phosphate (19) :

Accurately, 3 gm of the sample were digested in 20 ml nitric acid & 5 ml sulphauric acid. Heating and complete digestion by drop wise addition of nitric acid until colourless were carried out. The heating was continued until appearance of white fumes. After cooling, 15 ml distilled water was added and boiled for 10 minutes was done. Accordingly, the determination of phosphates was occurred by the colourimetric procedure. To 25 ml of the prepared solution, 5 ml molybdate solution and 2 ml hydrazine sulphate solution were added in 50 ml graduated flask. The mixture was then diluted with distilled water to the mark and boiling in a water bath for 10

minutes. The absorbance was measured at 680 nm against the blank reagent. The phosphate content was determined by aid of a calibration graph prepared from measuring of the standard solutions of potassium dihydrogen phosphate (0, 2.5, 10, 20, 30, 40 and 50 mg).

2.3. Experimental part

The present study was carried out on 10 white male albino rats of 3 months old and weighed between 100- 115 gm. Animals were kept at a constant environmental and nutritional condition throughout the period of investigation (2 weeks) and housed in a clean separate steel cages and fed on balanced diet throughout the period of the experiment. Water was offered ad-libitum.

2.4. Experimental design;

The rats were divided into two groups, placed in individual cages and classified as follows

Group 1 (Control group); Composed of 5 rats were fed on cooked meat free from any preservatives (used as control for all the experimental groups). Group 2 (Phosphate supplemented group at the higher level detected during chemical estimation); Included 5 rats were fed on cooked meat with phosphate where phosphate was added in a dose equal to the highest level detected during chemical estimation (0.62%).

2.5. Sampling

Blood samples were taken from all groups at the end of experimental period and at overnight fasting, rats were weighted and heparinized blood samples (20 IU/ ml) were collected from the median canthus of the eye. Each sample was centrifuged at 3000 r.p.m. for 10 minutes for plasma separation. The clean and clear plasma was separated and received in dry sterile sample tube using sterilized pipette and processed directly for determination of Glutamic Oxaloacetic Transaminase (GOT) Glutamic Pyruvic Transaminase (GPT), Alkaline phosphatase, Urea, Serum creatinine and Uric acid. Tissue specimens for histopathological finding. (20); Immediately after blood sampling, the animals were sacrificed and the liver, kidneys, spleen and heart animals were carefully removed immediately, washed with saline and blotted between filter papers. Small specimens of liver 1x1.5 and 5mm in thickness, half of the kidney, whole spleen and half of the heart were immediately fixed in 10% neutral buffered formalin. A paraffin section of 5-7 micron-thick were prepared and stained with routine H&E stain for histopathological finding.

3. Results

Table (1) Statistical analytical results of phosphate levels (%) in the examined meat products samples (n= 20 of each).

Meat products	Phosphate			Samples above permissible limit		Non detected samples	
	Min.	Max.	Mean \pm SE	No.	%	No.	%
Beef burger	0.27	0.61	0.43 \pm 0.02	15	75	2	10
Luncheon	0.25	0.60	0.41 \pm 0.0 2	7	35	12	60
Canned corned beef	-	-	-	-	-	20	100
Sausage	0.30	0.62	0.43 \pm 0.019	15	75	4	20
Pastrami	-	-	-	-	-	20	100
Kofta	0.17	0.60	0.38 \pm 0.029	11	55	4	20

Studies on phosphate as chemical preservatives in some meat products.

Table (2) Serum analysis of *control group (group 1)

Serum	Group	Group 1(Control)		Mean \pm SE
		Min.	Max.	
GOT		134	170	148.8 \pm 6.04
GPT		59	80	71.8 \pm 3.62
Alkaline phosphatase		581	791	705 \pm 48.86
urea		25	40	31.6 \pm 2.48
Uric acid		1.97	2.31	2.11 \pm 0.06
Serum creatinine		0.79	0.92	0.84 \pm 0.02

Table (3) the effect of phosphate (0.62%) on liver and kidney functions.

Serum	Group	Group 4(feed on Phosphate 620 ppm) for 2 weeks		
		Min.	Max.	Mean \pm SE
GOT		179	320	223.8 \pm 28.48
GPT		24	39	38.8 \pm 2.26
Alkaline phosphatase		561	661	601.8 \pm 17.96
urea		52	61	57 \pm 1.73
Uric acid		2.9	3.91	3.42 \pm 0.19
Serum creatinine		1.38	2.11	1.78 \pm 0.12

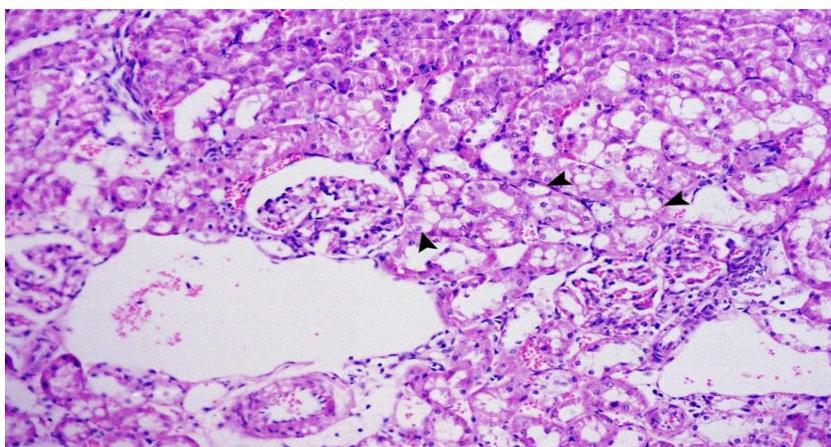


Fig. (1): Kidney of phosphate treated rat (Group 4) showing vacuolar and hydropic degeneration (arrow head) of the lining epithelium of some proximal and distal convoluted tubules in renal cortex.. H&E stain x 200

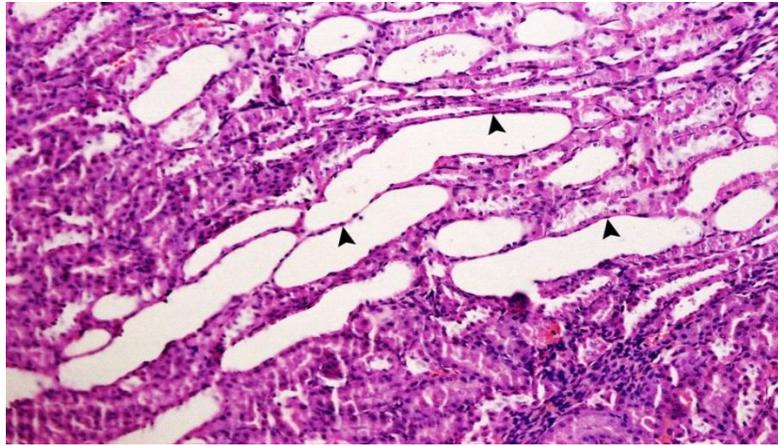


Fig. (2): Kidney of phosphate treated rat (Group 4) showing cystic dilatation of moderate numbers of renal tubules lined by attenuated epithelium (arrow head). H&E stain x 200

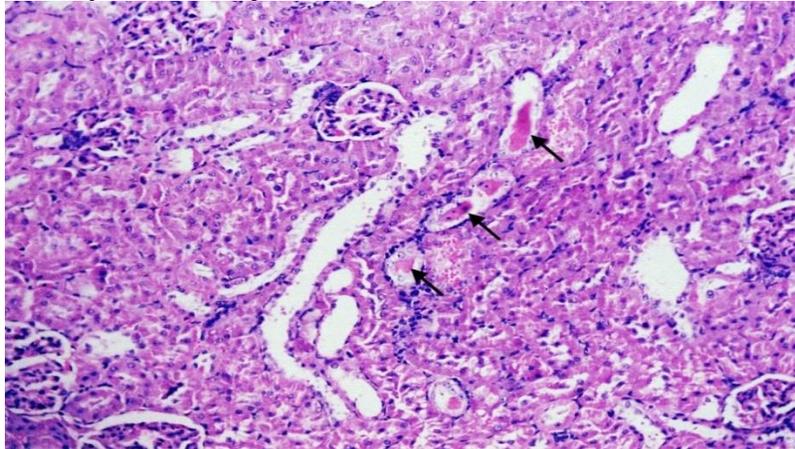


Fig. (3): Kidney of phosphate treated rat (Group 4) showing few numbers of attenuated tubules contained hyalinized eosinophilic casts (arrow) in their lumen. H&E stain x 200

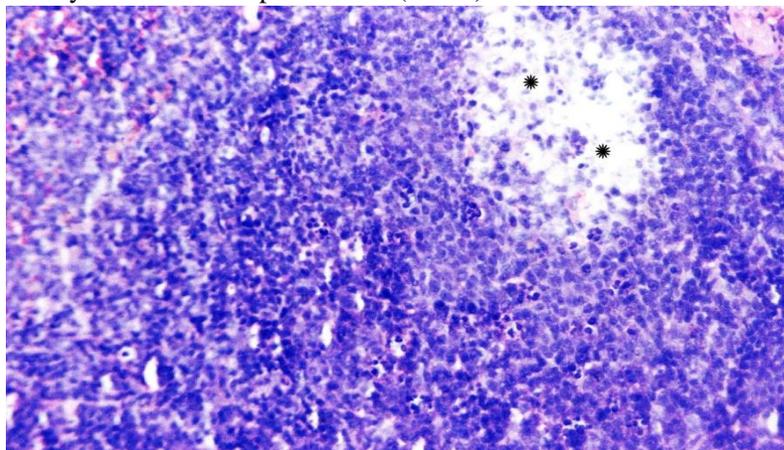


Fig. (4): Spleen of phosphate treated rat (Group 4) showing focal lymphoid depletion (asterisk) of white pulp characterized by reduced cellular density of mature small lymphocyte. H&E stain x 400.

4. DISCUSSION

phosphates are used in meat products for several reasons such as changing and/or stabilizing the pH value, increasing water holding capacity in order to lead higher yields, decreasing losses of weight in cooking, improving texture and sensory properties (tenderness, juiciness, color and flavor), extending shelf-life, etc. In addition, phosphates in meat products are also sources of the supply of phosphorus for consumers through diet, which is an essential mineral for the life of humans (21). The results recorded in table (1) discussed the levels of phosphate (%) in the examined meat products where the mean value was 0.43 ± 0.02 for beef burger, 0.41 ± 0.02 for luncheon, 0.43 ± 0.019 for sausage and 0.38 ± 0.029 for kofta. Phosphate failed to be detected in all examined canned corned beef and pastrami samples while failed to be detected in 10%, 60%, 20% and 20% in the examined beef burger, luncheon, sausage and kofta samples, respectively. The detectable samples above the permissible limit were 75%, 35%, 75% and 55% for beef burger, luncheon, sausage and kofta samples, respectively. This disagrees with (22) who reported that phosphate concentration is below the maximum permitted levels in examined sausages and various meat products and (23) who recorded that the mean concentration of tripolyphosphate (STPP) in different types of meat products varied from 39 mg P_2O_5 /100 mg to 219 mg P_2O_5 /100 mg, these values are below the legal requirements. Although NaCl is recognized as a pro-oxidant in meat, the addition of phosphates is particularly advantageous in those products where rancidity could quickly develop (24) in addition to one of the major advantage of phosphate is the opportunity to reduce the NaCl content in cured meats while retaining the water-binding capacity of the higher salt concentrations that in cooked sausage salt

content could be reduced to about 1.4% with addition of phosphate (25). Using of appropriate amount and mixture of phosphate can lead to the improvement of some properties of final products, such as moisture retention, water holding, color protection, slowing down of oxidation, extension of shelf-life, stabilizing and enhancing structure of final products. Under European legislation, phosphates are not permitted in fresh meat, but could be added to meat products. The permitted levels of phosphates in meat and meat products is 5g /kg expressed as phosphorus peroxide P_2O_5 individually or in combination in the finished product (21). Sensory properties of products should be taken into account while choosing appropriate phosphate mixture content. Phosphate flavor is usually considered as unpleasant. The concentration of 0.3 to 0.5% could lead to products with unacceptable bitter taste (26) so food phosphate, used in meat and meat products, must be manufactured according to good manufacturing practices (GMP) (27). Experimental work: Biochemical analysis could help to identify target organs of toxicity as well as the general health status of animals. It may also to provide an early warning signal in stressed organism (28). The plasma transaminase GOT&GPT as well as alkaline phosphatase (entering the blood after the cell necrosis of certain organs) can be used to establish the tissue damage of the liver and kidney (29) and (30). Rises in the serum level of urea and creatinine is indicator of renal failure and rises in GOT is indicator of hepatocellular injury as reported by (31). In addition, elevated GOT can be used to diagnose myocardial infarction, arrhythmias and severe angina of heart. So that, table (3) discussed the effect phosphate 0.62% (Group 4) on liver and kidney functions in comparison to control group in table(2) that was fed on cooked minced meat without preservatives. Concerning the recorded in table (3) revealed high level of

the mean values of GOT, urea, uric acid and serum creatinine for examined rats (group 2) treated with 0.62% of phosphate for 2 weeks in comparison to control one, 223.8 ± 28.48 , 57 ± 1.73 , 3.42 ± 0.19 and 1.78 ± 0.12 were the mean values of GOT, urea, uric acid and serum creatinine for group (4) and 148.8 ± 6.04 , 31.6 ± 2.48 , 2.11 ± 0.06 and 0.84 ± 0.02 as mean values of GOT, urea, uric acid and serum creatinine for control group (1). While lower mean values of GPT and alkaline phosphatase for this group were reported 38.8 ± 2.26 and 601.8 ± 17.96 in comparison with 71.8 ± 3.62 and 705 ± 48.86 as the mean values of GPT and alkaline phosphatase in the control group. These results may be attributed to calcification of the kidneys as a result of high phosphorus level in the blood and also due to calcium phosphate deposits in the body's tissues that damage organs and calcification of various parts of the cardiovascular system and may lead to chronic kidney failure (12) and (13) and supported by histopathological changes in this study in which the kidney of rats treated with 0.62% phosphate in the food for 15 successive days revealed congestion of some renal blood vessels and intertubular capillaries. Multifocally, the lining epithelium of some proximal and distal convoluted tubules of renal cortex were occasionally swollen and exhibited vacuolar and hydropic degeneration characterized by pale vacuolated cytoplasm (Fig.1). Moreover, moderate numbers of renal tubules revealed cystic dilatation and were lined by attenuated epithelium (Fig. 2); while few numbers of these attenuated tubules contained hyalinized eosinophilic casts in their lumen (Fig.3). Also the histopathological examination revealed that The heart showed congestion of myocardial blood vessels and inter-muscular capillaries. Rarely, the spleen showed focal lymphoid depletion of white pulp characterized by reduced cellular density of mature small lymphocyte (Fig.4).

5. REFERENCES

- 1- Buňková, L.; Pleva, P.; Buňka, F.; Valášek, P. and Kráčmar, S. (2008): Antibacterial effects of commercially available phosphates on selected microorganisms. *Acta. Univ. Agric. et Silv. Mendel. Brun.* 56: 19-24.
- 2- Feiner, G. (2006): Meat products handbook - Practical science and technology. Woodhead Publishing Limited, Cambridge, England.
- 3- Lampila, L.E. and Godber, J.P. (2002): Food Phosphates. In A.L. Branen, P.M. Davidson, S. Salminen and J.H. Thorngate III (Eds.), *Food Additives - 2nd edition (Chapter 25)*. Marcel Dekker, Inc., New York.
- 4- Juneja, V.K.; Marmer, B. S.; Phillips, J.G. and Palumbo, S.A. (1996): Interactive effects of temperature, initial pH, sodium chloride and sodium pyrophosphate on the growth kinetics of *C. perfringens*. *J. Food Prot.*, 59(9):963-968.
- 5- Puolanne, E.J.; Ruusunen, M.H. and Vainionpää, J.I. (2001): Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. *Meat Science*, 58: 1-7.
- 6- Peng, W.; Xing-lian, X. and Guang-hong, Z. (2009): Effects of Meat and Phosphate Level on Water-Holding Capacity and Texture of Emulsion-Type Sausage during Storage. *Agricultural Sciences in China*, 8(12): 1475-1481.
- 7- Zayas, J.F. (1997): *Functionality of proteins in food*. Springer-Verlag, Berlin Heidelberg.
- 8- Fernández-López, J.; Sayas-Barberá, E.; Pérez-Alvarez, J.A. and Aranda-Catalá, V. (2004): Effect of sodium chloride, sodium tripolyphosphate and pH on color properties of pork meat. *Color Res. Appl.*, 29: 67-74.

Studies on phosphate as chemical preservatives in some meat products.

- 9- Han, M. Y.; Li, Q. L. and Chen, H. Y. (2004): The application of compound phosphates in food. *China Food Additives*, (3): 93-96.
- 10- Benini, O., D'Alessandro, C.; Gianfaldoni, D. and Cupisti, A.(2011): Extra-phosphate load from food additives in commonly eaten foods: a real and insidious danger for renal patients. *J. Ren. Nutr.*, 21(4):303-308.
- 11- Uribarri, J. and Calvo, M. S. (2003): Hidden Sources of Phosphorus in the Typical American Diet: Does it Matter in Nephrology?. *Seminars in Dialysis*, 16(3):186-188.
- 12- Larsson, T.; Nisbeth, U.; Ljunggren, Ö.; Juppner, H. and Jonsson, K. (2003): Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. *Kidney International*, 64: 2272–2279.
- 13- Striker, G.E. Grosjean, F. and Vlassara, H. (2011): Phosphate and CVD. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31: 1951-1952.
- 14- Kapur, S. (2000): Phosphates in Soda, Meats May Increase Skin Cancer Risk. *Cancer Investigation*, 18(7): 664-669.
- 15- Huttunen, M.M.; Tillman, I.; Viljakainen, H.T.; Tuukkanen, J.; Peng, Z.; Pekkinen, M. and Lamberg-Allardt, C. J. E. (2007): High Dietary Phosphate Intake Reduces Bone Strength in the Growing Rat Skeleton. *Journal of Bone and Mineral Research*, 22(1): 83-92.
- 16- Huttunen, M.M.; Pietilä, P.E.; Viljakainen, H.T. and Lamberg-Allardt, C. J.E. (2006): Prolonged increase in dietary phosphate intake alters bone mineralization in adult male rats. *J. Nutr. Biochem.*, 17: 479-484.
- 17- Nielsen, P. K.; Feldt-Rasmussen, U. and Olgaard, K. (1996): A direct effect in vitro of phosphate on PTH release from bovine parathyroid tissue slices but not from dispersed parathyroid cells. *Nephrol. Dial. Transplant.* 11 (9): 1762-1768.
- 18- E.S.S. (2005): Egyptian Standard Specification or Egyptian Organization for Standardization and Quality Control for meat products.
- 19- Pearson, A.M. (1984): *Processed meats*, 2nd ed. AVI publishing company. INC. pp: 46-67.
- 20- Druy, R. A. and Walling, E. A. (1976): *Carleton, S. Histological technique. Fel. Bed.* ; 114-118 London. Oxford Univ. Press. New York Toronto
- 21- Long, N.H.B.S.; Robert, G. and František, B. (2011): Use of phosphates in meat products. *African Journal of Biotechnology*, 10(86): 19874-19882.
- 22- Skrókki, A. (1995): Additives in finnish sausages and other meat products. *Meat Science*, 39(2): 311–315.
- 23- Jastrzebska, A. (2006): Determination of sodium tripolyphosphate in meat samples by capillary zone electrophoresis with on-line isotachophoretic sample pre-treatment. *Talanta*, 69(4): 1018-1024.
- 24- Vasavada, M.N.; Dwivedi, S. and Cornforth, D.P (2006): Evaluation of Garam Masala Spices and Phosphates as Antioxidants in Cooked Ground Beef. *Journal of Food Science*, 71, C292-C297.
- 25- Ruusunen, M. and Puolanne, E. (2005): Reducing sodium intake from meat products. *Meat Science*, 70, 531-541.
- 26- Ranken, M.D. (2000): *Handbook of meat product technology*. Blackwell Science Ltd., Oxford.
- 27- Code of Federal Regulations (2003): Title 21. Food and drugs. Parts 182. Office of the Federal Register, National Archives, and Records Administration, Washington, D.C Available on http://www.access.gpo.gov/nara/cfr/waisidx_03/21cfr182_03.html, 3:456-468.

- 28- Folmar, L.C. (1993): Effects of Chemical Contaminants on Blood Chemistry of teleost fish: A bibliography and synopsis of selected effects. *Environ. Toxicol. Chem.*, 12(2): 337-375.
- 29- Nemcsok, J.; Benedeczky, I.; Boross, B.; Asztalos, B. and Orban, L. (1981): Subcellular localization of transaminase enzyme in fishes and their significance in the detection of water pollution. *Acta. Biol. Szegediensis*, 27: 9-15.
- 30- Nemcsok, J. and Boross, L. (1982): Comparative studies on the sensitivity of different fish species to metal pollution. *J. Acta. Biol. Acad. Sci. Hung.*, 33: 23-27.
- 31- Ruetten, H. and Thiemermann, C. (1996): Effect of selective blockade of endothelin ETB receptors on the liver dysfunction and injury caused by endotoxaemia in the rat. *Br. J. Pharmacol.*, 119(3): 479-486.