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DETECTION OF PHOSPHATES AND HYDROXYPROLINE IN SOME MEAT PRODUCTS

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A B S T R A C T

Sixty samples of meat products (beef burger, kofta and canned sausage) (20 of each) were collected to determine phosphates and hydroxyproline content. The mean values of phosphate % were 0.4 ± 0.019, 0.41 ± 0.025 and 0.53 ± 0.020 for beef burger, kofta and canned sausage, respectively. The number of examined samples exceeding the permissible limit recommended by EOS (2005) for phosphates content was 16(80%) and 20(100%) in beef burger and canned sausage samples respectively while it was 4(20%), 5(25%) and 14(70%) in the examined beef burger, kofta and canned sausage samples, respectively according to FAO (2007). The mean values of hydroxyproline contents % in the examined beef burger, kofta and canned sausage samples were 1.16± 0.153, 1.57± 0.192 and 0.603± 0.092, respectively. The public health hazard of increased phosphates level and the indication of high % of hydroxyproline in some meat products were discussed. .

Key words: phosphates, hydroxyproline, beef burger, kofta, canned sausage

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1. INTRODUCTION

Increasing populations and increased demand per capita together with moderately rapid to rapid income growth lead not only to an increased demand for staple foods but also for preferred foods including, particularly, meat products which are concentrated sources of high quality protein and their amino acid composition usually compensates for shortcomings in the staple food. They supply easily absorbed iron and assist the absorption of iron from other foods as well as zinc, and are rich sources of some of the vitamins in the B group (1). Using of food additives has become more prominent in recent years as Consumers need high quality, natural, nutritious, fresh appearance and convenient meat products with natural flavor and taste and an extended shelf life (2 and 3). In living muscles or directly after slaughtering, proteins fix water and meat are compact and juicy. The adenosine triphosphate (ATP) which is present in meat allows the proteins of meat to keep opened structure. A few days after slaughtering, the muscles are contracted and meat becomes exudative, water retention capability and organoleptic properties are altered (4). Hence, in the processing of meat and meat products, food grade phosphates are one of the food additives. They are essential for several reasons such as, increasing pH, increasing water holding capacity (WHC; structure of muscle protein is opened) in order to lead to higher yields and stabilized meat emulsions, decreasing cooking losses of weight, improving texture and sensory properties (tenderness, juiciness, color, flavor). In addition, extending shelf life, etc. (5, 6 and 7). Phosphates used in meat processing industries are the salts of

phosphoric acid and sodium or potassium. They are polyelectrolytes able to change the ionic charges distributions. Thus, the addition of phosphate increases the ionic strength of the meat and consequently, an increased ionic strength leads to a more severe degree of swelling of the muscle fibers and activation of protein. Enhanced levels of activated and swollen protein support the immobilization of the water added to meat products and the emulsification of fat (8, 9, 10, 11 and 12). Recently, a high-normal serum phosphate concentration has also been found to be an independent predictor of cardiovascular events and mortality in the general population. Therefore, phosphate additives in food are a matter of concern, and their potential impact on health may well have been underappreciated (13). Meat consists of connective tissue proteins, which decrease tenderness of meat. Collagen is the most common protein found in connective tissue. For the manufacture of processed-meat products, meat with high levels of collagen can result in detrimental effects on end-product quality, such as gelatin formation, grainy and brittle texture, which are all undesirable. The nutritive value of the product decreases, as connective tissue is deficient in essential amino acids (14). Hydroxyproline, a non-essential amino acid constituent of proteins, is mainly found in connective and bone tissue and forms up to 10% of collagen molecules (15). The addition of low value meat is generally considered to be the most frequent adulteration of meat –based products, such as sausage, hamburger, etc. A suitable marker for identifying the quality of raw material used for meat – based preparation is collagen content which can be calculated by the concentration of the amino acid 4-hydroxyproline (16). Therefore, the aim of this work is to determine phosphates and hydroxyproline content in frozen (beef burger, kofta and canned sausage).

2. MATERIAL AND METHOD

2.1. Collection of samples

60 random samples of meat products represented by frozen (beef burger, kofta and canned sausage) (20 of each) were collected from different supermarkets in Menufia governorate and the following examinations were carried out:

2.2. Determination of Phosphates (17)

Accurately, 3 gm of the sample were digested in 20 ml nitric acid & 5 ml sulphuric acid. Heating and complete digestion by drop wise addition of nitric acid until colorless 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 colorimetric 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. Determination of Hydroxyproline (17)

The following method is widely used for determination of hydroxyproline in meat products. In a clean flask, 4 gm of the sample were mixed with 100 ml tin solution and 6 ml HCl and left for 16 hours. However, the mixture was filtered 3 times through filter paper. Thus, 25 ml of the filtrate was adjusted to pH 8± 0.2 also 4 ml of the solution was pipette to a test tube containing 2 ml chloramines- T- solution and left for 20 minutes. Then 2 ml of color reagent

(dimethylamine benzaldehyde in 35 ml per acetic acid) were added and thoroughly mixed with the solution, which was heated at 60 ^{o}C for 20 minutes. After cooling, the absorbance of the solution was measured at 558 nm. The calibration curve was prepared with 4 ml aliquots of hydroxyproline solutions containing 0.5, 1.0, 1.5 and 2.0 mg / ml, respectively. Thus, the hydroxyproline % in the examined samples was determined based on the calibration graph.

2.4. Statistical analysis procedure:

The data were statistically evaluated by analysis of variance (ANOVA) according to the procedures reported by **(18).**

3. RESULTS AND DISCUSSION

The processing of meat by different ways cannot overcome the potential hazard arising from the bad and illegal use of some curing additives and the use of low quality meat, thus, the quality of raw material (meat) as well as additives and final products are very important for public health. Therefore, the use of low quality ingredients in the processing yields low quality meat products (19). Phosphates are not considered as direct preservatives. They only can impart some desirable properties when used as acidulates or in combination with other food ingredients (20). One of its major advantages is the opportunity to reduce the sodium chloride (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 (21). NaCl is recognized as a pro-oxidant in meat, so that the addition of phosphates is particularly advantageous in those products where rancidity could quickly develop (22). The obtained data in table (1) discussed the statistical analytical results of phosphates content % in the examined meat products samples that the mean values of phosphate % were 0.4 ± 0.019, 0.41 ± 0.025 and 0.53 ± 0.020 for beef burger, kofta and canned sausage, respectively. Nearly similar result for phosphates % in examined beef burger samples was recorded by (23) with mean value 0.399± 0.020 while higher result was reported by (24) with mean values 0.45± 0.02, 0.49± 0.01, 0.54± 0.01 and 0.56± 0.01 for examined beef burger samples related to different processing factories. At the same time, these results were slightly higher than those reported previously (23) for phosphates % in examined canned sausage samples reporting mean value 0.414± 0.0245. In addition, table (1) summarized the number of examined samples exceeding the permissible limit recommended by (25) and (26) for phosphates content that 16(80%), and 20(100%) of beef burger and canned sausage samples exceeded (25) limit and 4(20%), 5(25%) and 14(70%) of beef burger, kofta and canned sausage, respectively exceeded (26) limit. 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, and 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 (20). As phosphate usage is limited to 0.5% in countries such as the United States and Canada, it is totally prohibited in Germany for meat products (27) . At concentration of 0.3 to 0.5% could lead to products with unacceptable bitter taste (28) so food phosphate, used in meat and meat products, must be manufactured according to good manufacturing practices (GMP) (29).

Inorganic phosphate in food additives is effectively absorbed and can measurably elevate the serum phosphate concentration in patients with advanced CKD (13). The dietary intake of phosphate and the serum phosphate concentration are important matters not just for persons with renal disease,

Table (1). **)** Statistical analytical results of phosphates content % in the examined meat products (n =20)

Phosphate % / Products	Minimum	Maximum	Mean ± SE	Samples over permissible limits according to	
				EOS(2005)	FAO(2007)
Beef burger	0.28	0.56	0.40±0.019[a]	16(80%)	4(20%)
kofta	0.21	0.60	0.41±0.025[a]	-	5(25%)
Canned sausage	0.37	0.63	0.53±0.020[b]	20(100%)	14(70%)

* The maximum permissible limit for phosphates (0.3%) according to EOS**.** No. (1688 and 1971 / 2005**)** for beef burger and canned sausage while EOS.No. (1973 / 2005**)** did not have limit for addition of phosphates to kofta. ** The maximum permissible limit for phosphates (0.5%) according to FAO (2007) for meat products. **(**a, b) insignificantly different between similar litter using multiple range test for comparative of means.

Table (2) Analysis of variance (ANOVA) of phosphate content in the examined meat products samples

Source of variance	SS	DF	MS	F. value	P value	
Between products	0.195	2	0.097	10.29	0.0002	***
Within one product	0.542	57	0.009			
Total	0.737	59				

*** = High significant differences between three products ($P < 0.01$). DF = Degree of freedom SS = Sum squares, MS = Mean squares

Table (3). **Statistical analytical results of** Hydroxyproline contents % in the examined samples of meat products (n = 20)

products / Hydroxyproline %	Beef burger	kofta	Canned sausage

Minimum	0.13	0.09	0.083
Maximum	2.34	2.95	1.25
Mean ± SE	1.16 [b] ± 0.153	1.57 [c] ± 0.192	0.603 [a] ± 0.092

(a,b,c) insignificantly different between similar litter using multiple range test for comparative of means

Table (4) Analysis of variance (ANOVA) of Hydroxyproline content in the examined meat products samples

Source of variance	SS	DF	MS	F. value	*P* value
Between products	9.494	2	4.747	10.34	0.0001***
Within one product	26.17	57	0.459		
Total	35.66	59			

******* = High significant differences between 3 products (P< 0.01) **DF** = Degree of freedom

SS = Sum squares **MS** = Mean squares

but for the public as well. It has recently been determined that phosphate additives in food may harm the health of persons with normal renal function (30) and (31). Table (2) revealed that there were high significant differences for phosphate percentage between three products at ($p < 0.01$). Meat tenderness is therefore considered as one of the most important meat qualities attributes (32) and (33). Despite many changes in consumer attitude towards red meat consumption, one expectation has remained constant namely that meat should be consistently tender (34). Meat quality is becoming increasingly important to meat processors and consumers (35). The determination of hydroxyproline in meat products is an often used as parameter for the evaluation of meat quality so table (3) discussed the hydroxyproline contents % in the examined beef burger, kofta and canned sausage samples with mean values 1.16± 0.153, 1.57± 0.192 and 0.603± 0.092, respectively. This result was slightly higher than that recorded by (23) who scored 0.108±0.0024, 0.277±1.057 and 0.116±0.003 as mean values of hydroxyproline % in beef burger, luncheon and sausage, respectively, while higher result 3.04, 2.85 and 2.29 was the mean values of hydroxyproline % in the examined beef burger, kofta and sausage samples, respectively was reported by (36). Table (4) revealed that there were high significant differences for hydroxyproline percentage between three products at (*P*< 0.01). Chemical procedures have high accuracy and suggested that it should be used for control quality in practice to determine connective tissue content and to detect adulteration (37). Presence of high amount of hydroxyproline is an indication that raw material with cheap quality was used in which food business operators (Meat-processing industry) are

trying to lower the cost of their meat products using the lower categories of meat (less biological and nutritional value), with a higher content of connective tissue (38). Although of further research is warranted to develop effective strategies of dietary supplementation with proline or hydroxyproline to benefit health, growth, and development of animals and humans (39) proline promoted the formation of *N*-nitrosopyrrolidine. It could also be confirmed that the higher the temperature of the meat processing procedure and the higher the sodium nitrite amounts added, the higher were the yields of the respective *N*-nitrosamines (40).

We can concluded that some of the examined meat products especially canned sausage exceeded the permissible limit recommended by **EOS(2005)** and **FAO(2007)** for phosphates % and some examined meat products as beef burger and kofta showed higher % of hydroxyproline (nonessential amino-acid) which is used as a measure of connective tissue of meat . which means that there is a replacement of high biological animal proteins with low price constituents. Therefore the following recommendation must be taken in consideration: - High quality raw meat and additives should be used: - Strict hygienic measures during different stage of processing, handling,

- Food safety management (ISO 22000) should be applied to meat processing plants.

- Educational programs should be given for processors to improve the quality of meat products and ensure a maximum of safety to the consumer

- Food control authorities must be played a large role on different factories to produce a very good product storage, transportation and marketing.

REFERENCES

1. FAO (1992) .Food and Agriculture Organization of the United Nations, FAO Corporate Document Repository. Meat and meat products in human nutrition in developing countries. Page 98
2. Aymerich, T. Picouet, P.A. and Monfort, J.M. (2008). Decontamination technologies for meat products. Meat Science, 78(1–2): 114-129.
3. USDA (2008). Additives in meat and poultry products. Food Safety Information. Available in: http://www.fsis.usda.gov/PDF/Additives in Meat and Poultry Products
4. Hourant, P. (2004). General properties of the alkaline phosphates: Major food and technical applications. Phosph. Res. Buln.15: 85-94.
5. Knipe, L. (2003) .Phosphates as meat emulsion stabilizers, In: C. Benjamin (Ed.), Encyclopedia of Food Sciences and Nutrition, Academic Press, Oxford. pp. 2077-2080.
6. 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
7. Molins, R.A. (1991): Phosphates in Food. CRC Press, Inc., Boca Raton
8. Feiner, G. (2006). Meat products handbook - Practical science and technology. Woodhead Publishing Limited, Cambridge, England
9. Offer, G. and Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci., 8: 245-281.
10. Shu Qin XU, Guang Hong Z., Zeng Qi P., Li Yan Z., Rui Y.A.O. (2009). The influence of polyphosphate marination on Simmental beef shear value and ultrastructure. J. Muscle Foods, 20: 101-116.

11. Siegel, D.G and Schmidt, G.R. (1979). Ionic, pH, and temperature effects on the binding ability of myosin. J. Food Sci. 44: 1686-1689.
12. Trout, G.R and Schmidt, G.R. (1986). Effect of phosphates on the functional properties of restructured beef rolls: the role of pH, ionic strength, and phosphate type. J. Food Safety, 51: 1416-1423.
13. Ritz, E., Hahn, K., Ketteler, M., Kuhlmann, M.K and Mann, J. (2012). Phosphate Additives in Food - a Health Risk. Dtsch Arztebl Int. 109(4): 49–55.
14. Lyasoglu, H., Nilüfer, D. and Boyacioglu, D. (2003). I 14- "Hydroxyproline determination methods in meat products" IFT Annual Meeting & Food Expo., Chicago, IL, USA.
15. Stanley, D.W. (1983). Relation of structure to physical properties of animal materials. In M. Peleg & E.B. Bagley (Eds.), Physical properties of foods. Westport, Connecticut: Avi Publish. Comp., Inc.
16. Messia, M.C. and Marconi, E. (2011). Innovative and Rapid Procedure for 4-Hydroxyproline Determination in Meat-Based Foods Amino Acid Analysis. Methods and Protocols, 828: 281-289.
17. Pearson, D. (1984). Chemical analysis of foods. 9th Ed., publishing Co. Churchilli Livingstone, Edinburgh, London, United Kingdom.
18. SPSS 14 (2006): Statistical package for social science, SPSS for window Release 14. 0. 0, standard version, copyright SPSS Inc., 1989-2006 All Rights Reserved, copyright ® SPSS Inc.
19. Pearson, A.M. and Gillette, T.A. (1996). Processed meats. 3 rd Ed. New York Albany, Bonn, Boston, London.
20. 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
21. Ruusunen, M. and Puolanne, E. (2005). Reducing sodium intake from meat products. Meat Sci., 70: 531-541.
22. 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: 292-297.
23. El-Zahaby- Dina, I.M. (2013). Chemical evaluation of some food additives in meat products. Ph.D. Thesis, (Meat Hygiene). Fac. Vet. Med., Benha Univ.
24. El- Sayed, A.E. (2006). Organoleptic and chemical evaluation of some locally manufactured beef burger. M.V.Sc. Thesis, (Meat Hygiene). Fac. Vet. Med., Benha Univ.
25. EOS (2005):EOS.No. (1688/2005) for beef burger. EOS.No. (1971/2005) for canned sausage.EOS.No.(1973/2005)for kofta. Egyptian Standard Specification or Egyptian Organization for Standardization and Quality Control for meat products.
26. FAO (2007). Food and Agriculture organization of the United Nations. Regional office for Asia and the Pacific Bangkok. Meat processing technology for small-to medium- scale producers (non-meat ingredients).
27. Trespalacios, P. and Pla, R. (2007). Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. Food Chemistry 100(1): 264-272.
28. Ranken, M. D. (2000). Handbook of meat product technology. Blackwell Science Ltd., Oxford
29. Code of Federal Regulations (2003). Tittle 21. Food and drugs. Parts182. Office of the Federal Register, National Archives, and Records Administration, Washington, D.C. 3:456-468.
30. Sullivan, C., Sayre, S.S., Leon, J.B, (2009). Effect of food additives on hyperphosphatemia among patients with

end-stage renal disease: a randomized controlled trial. JAMA. 301:629–635.
31. Uribarri, J. (2007). Phosphorus homeostasis in normal health and in chronic kidney disease patients with special emphasis on dietary phosphorus intake. Semin Dial. 20: 295–301
32. Lawrie, R.A. (1985). Meat Science. pp. 401-459, 4th Ed., Pergamum Press, Oxford, UK.
33. Jiang, S.T. (1998). Contribution of muscle proteinases to meat tenderization. Review: Proceedings of the National Science Council, ROC, Part Bi Life Sciences, 29(3): 97-107.
34. Marais, G.L. (2007). Evaluation of genetic and physiological parameters associated with meat tenderness in South African feedlot cattle. Partial fulfillment of the requirements for the degree M.Sc. (Agric.) production physiology, Pretoria Univ.
35. Beattie, V.E., Weatherup, R.N., Moss, B.W. and Walker, N. (1999). The effect of increasing carcass weight of finishing boars and gilts on joint composition and meat quality. Meat Science, 52: 205-211.
36. Nada- Shaimaa, M.M. (2012). Chemical indices as quality measures in some meat products. Ph.D. Thesis, (Meat Hygiene). Fac. Vet. Med., Benha Univ.
37. Kamkar, A., Bokaei, S., Behrozi, M. and Rokni, N. (2005). Determination of hydroxyproline as measure of collagen content in meat product by colorimetric method. J. Vet. Med. Univ. Tehran. 60 (1): 25-30.
38. KURĆUBIĆ1, V.S., VUKAŠINOVIĆ2, M.P., MAŠKOVIĆ1, P.Z. and PETROVIĆ1, M.D. (2012). Examination of the certain chemical characteristics of different types of boiled sausages produced in Serbia. Journal of Central European Agriculture, 13(4):.643-653.
39. Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D.A., Li, P, Li, X., McKnight, J.R., Satterfield, M.C. and Spencer, T.E. (2011). Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino Acids. 40(4):1053-1063.
40. Drabik-Markiewicz, G.B., Dejaegher, B., De Mey, E., Impens, S., T. Kowalska, T., H. Paelinck, H. and Heyden, V.Y. (2010). Evaluation of the influence of proline, hydroxyproline or pyrrolidine in the presence of sodium nitrite on *N*-nitrosamine formation when heating cured meat. Analytica Chimica Acta, 657(2): 123–130

الكشف عن الفوسفات والهيدروكسي برولين فى بعض منتجات اللحوم

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الملخص العربي

تمت هذه الدراسة على 60 عينة من منتجات اللحوم شملت البيف برجر و الكفتة والسجق المعلب بواقع 20 عينة من كل نوع وذلك لتحديد نسبة املاح الفوسفات والهيدروكسي برولين فى هذه المنتجات وكان متوسط املاح الفوسفات فى هذه المنتجات 0,40± 0,019، 0,41±0,025, 0 و53 ,0± 0,020 على التوالي. وكان عدد العينات التي تخطت الحد المسموح به طبقا للمنظمة القياسية المصرية 16 (80%) و20 (100 %) لعينات البيف برجر والسجق المعلب على التوالي بينما كان عدد العينات التي تخطت الحد المسموح به طبقا للمنظمة العالمية (الفاو) 4 (20 %) و5 (25 %) و14 (70 %) لعينات البيف برجر و الكفتة و السجق المعلب على التوالي. كما كان متوسط الهيدروكسي برولين 1,16±0,153، 1,57±0,192و 603 ,0±0,092 0للبيف برجر الكفتة و السجق المعلب على التوالي. كما تمت مناقشة الخطورة الصحية لزيادة املاح الفوسفات عن الحد المسموح به. كما تمت مناقشة دلالات تواجد نسبة عالية من الهيدروكسي برولين فى هذه المنتجات.

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