J. Agric. Sci. Mansoura Univ., 34 (4): 3065 - 3079, 2009

ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY AS AN ANALYTICAL TOOL FOR DETECTING FREE RADICALS OF GAMMA-IRRADIATED MILK POWDERS

Abd El-Ghany, I.H.I. * and A. I. Atia**

*** Dairy Science and Technology Dept., Fac. of Agric. Cairo Univ. Giza, Egypt.**

**** Natural Products Dept., National Center for Radiation Res. and Technology; (NCCRT) Atomic Energy Authority, Nasr City, Cairo, Egypt.**

ABSTRACT

Electron spin resonance (ESR) spectroscopy has been used to assess the free radicals signal in different milk powders, buffalo's, cow's goat, camel, commercial full cream cow's milk powder, buffalo's butter milk and colostrum before and after γ-ray irradiation at does 1 and 2 KGy. Non-irradiated milk samples exhibited weak signal intensity, depending on the type of milk. However, ESR signal intensity of all irradiated milk samples gradually increased as the radiation dose increases from 1 to 2 KGy. The results showed that the radio-stability of milk powder depended on the original free radicals and radiation dose applied. Buffalo's butter milk and camel milk powders had the highest radio-stability, while buffalo's milk powder was the lowest. The results of this study recommended to use ESR as a new technique in evaluation quality control dairy products during processing and storage.

Keywords: Electron spin resonance (ESR) spectroscopy, free radicals , gamma-irradiation, milk powders

INTRODUCTION

Milk and dairy products are important in human nutrition. Milk contains compounds such as lipids (fatty acid, milk fat globule membrane, MFGM), proteins (whey proteins, lactoforrin), vitamins (tocopherols, ascorbic acid, β-caroten, riboflavin and endogenous enzyme as scavengers free radical such as superoxidase dismutase, catalasc, glutathione peroxidase,and xanthine oxidase. The concentration of these compounds are effected by species of animal, feeding rations and milk processing conditions (Aurand *et al.*, 1966, Fridovich, 1970, Lindmark-Mansson and Akesson, 2000, Levieux and Ollier, 1999, Ajems, 2007, Morin *et al.*, 2007, Al-Rowaily, 2008, Haddadin *et al.*, 2008 Kamal and Salama, 2008).

Free radicals can react with unsaturated fatty acids, protein, DNA and other compounds, and caused different types of damage related to several diseases like heat disease, cancer and critical illness (Shoji *et al.*, 2004, and Singh *et al.*, 2006). Oxidative reactions in milk can decrease the nutritional value, lead to formation of storage off-flavours and decreased the shelf-life of milk products. As is the case with lipids and proteins oxidation can be initiated by factors including light, heat treatments, storage, metal ions and gamma irradiation. King, 1963, Aurand *et al.*, 1977, Al-Rowaily 2008, Smet *et al.*, 2008).

Irradiation, physical treatment of exposing food to ionizing energy is considered an effective way of inactivation of food borne pathogens and changes the functional of foods, especially, milk and milk products. However, application of ionizing radiation up to a dose level of 10 KGy can be used to eliminate or greatly reduce the number of spoilage and pathogenic organisms without causing any toxicological hazard and with compromising the nutritional and sensory quality (Khorshid *et al.*, 1976, Ibrahim, 1984, Byun and Kang, 1995, WHO, 1999, Letendre *et al.*, 2002, Grolichova *et al.*, 2004, Ciesla *et al.*, 2004, 2006, Jo *et al.*, 2007, Uear, 2007, Osattli, 2007 and Kaddouri *et al.*, 2008, La Hoz and Nettro, 2008). Electron spin resonance (ESR) is a spectroscopic technique that directly detects chemical species with unpaired electron(s). These species include, but are not limited to, free radicals and transition metalions. ESR measurements based on the interactions between the unpaired electron and an applied magnetic field. ESR is widely used in many research field such as physics, chemistry, biology, life science, materials science, medicine, nutrition and nutraceutical food science. (Yu and Cheng, 2008).Recently electron spin resonance spectroscopy (ESR) technique has been used to study the physical and chemical changes occurring in compounds of milk and milk products. It has been used to investigate oxidation of milk and dairy products (Stapelfeldt *et al.*, a,b,c 1997, 1999, Kristensen and Skibsted, 1999, Friel *et al.*, 2002, Bradely *et al.*, 2003, Kristensen *et al.*,1999, 2004 Kondyli *et al.*, 2005), stuied the physical changes in ice cream during freezing and melting, intermolecular interactions between fatty acid bound to bovine, human serum albumin (Gurachvesky *et al.*, 2007, De simone *et al.*, 2007) also, action of milk and whey on the quality of powdery mildew (Crisp *et al.*, 2006).

Studies have demonstrated that ESR is a relatively fast, simple, and sensitive technique for detection certain foods, and the shapes of the detected ESR signals vary according the type of free radicals produced by the irradiation (Delicee, 1998, 2002, Kwon *et al.*, 2000, Shimoyama *et al.*, 2006, 2007, Yu and Cheng, 2008). However, there were little studies related to ESR spectroscopic features of radiated-inducedradicals species in milk powder (Hansen *et al.*, 1970 and Kispeter *et al.*, 2007). Therefore, the aim of this study was, to distinguish gamma irradiation at different doses(1 and 2KGY), induced radicals signal of different milk powders using ESR spectroscopy by comparison with unirradiated milk.

MATERIALS AND METHODS

Fresh whole buffalo's and cow's milk were obtained from the herd Faculty of Agriculture, Cairo University. Buffalo colostrum milk was taken during the first week of parturition. Sweet buffalo milk butter was prepared as described by El-Dieb *et al.*, (1995). Camel and goat milk samples were collected from Marsa Matrouh farm (Animal production research institute, Dokki, Giza, Egypt). Fresh commercial full cream cow's milk powder (Nido) was obtained from a local supermarket. All milk samples were freeze dried using centrifugal freeze-dryer (W.Edward and Co London). The freeze-dried

milk samples were stored at -20ºC in closed test tubes, conditions under which the free radical was unaffected.

Freeze-dried of different milk samples were exposed at two level of gamma radiation 1 and 2 KGy at ambient conditions. The treatment was performed in Cobalt-60 gamma cell irradiation unit 220 located at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr city, Cairo Egypt with a dose rate of 1.03 rad/sec.

The unirradiated and irradiated freeze-dried milk samples were transferred to cylindrical quartz ESR tube (inner diam 4mm; Wilmad glassco. NJ, U.S.A) and tapped a few times against the tube, ESR experiments were carried out using a Bruker EMX spectrometer (X-band, Germany), using the standard Bruker ER 4102 reclgular cavity. The operating condition for ESR spectaometer were as follow: microwave power 0.201 mv, modulation amplitude = 4.00 Gauss, moldulation frequency=100 KHz, sweep width = 200 Gauss, microwave frequency = 9.775 KHz, time constant = 81.92 ms and conversion time 20.48 ms. Stability of the ESR spectrometer sensitive was cheeked before and after each series of measurements using reference alanine dosimeters irradiated to know dose. The intensity of the ESR spectra (arbitray units) was measured as the peak-to-peak high of the broad ESR single divided by the peak-to-peak height of reference signal from a strong pitch samples, divided by the density of the milk powder in the tube (g/length), also the spectroscoptic splitting factor (g) values were estimated according to Brudiving, 1995 as a function of irradiation dose in KGy. All ESR measurements were carried out at room temperature 25± 2ºC with respect to 0 and 90 degree orientation. Each results was obtained as the average of more than two independent measurements

RESULTS AND DISCUSSION

It is the benefit of food irradiation that changes (oxidation) in the major nutrients (lipid, protein, carbohydrates, vitamins) of the food are so minute, whereas undesirable organisms inactivated. In addition, many changes induced by irradiation very closely resemble those resulting from other kinds of food processing (Delincee, 1998). However, many factors for degradation of lipid due to oxidation, and one of the major cause of this defect has been attributed to oxidation of unsaturated lipid. The rate of the oxidation is dependent upon the unsaturated lipids, the amount of oxygen presents, presence of prooxidats especially iron or copper and lipoxidase (Rawls and Santen, 1970, and Whang and Peng,1988). lipid oxidation occurs by two major mechanisms; (1) free radical autooxidation of unsaturated fatty acids with tripliet oxygen generated by irradiation (2) the oxidation of milk lipids with singlet oxygen. The autoxidation reaction proceeds by a three stage radical process: imitation, propagation and termination. Also, oxidation of riboflavin by irradiation caused lipid oxidation through singlet oxygen inserted at either end of the double bound of the unsaturated fatty acids to yield an allylic hydroperoxidic that are very unstable and are broken down further aldhydes and ketones that are responsible for off-flavours. Beside,

methionine reacts with singlet oxygen produced through oxidation of riboflavine under irradiation condition to yield free radicals (hydroxyl radicals or superoxide anions) (dimethyl disulfide, methyl sulfide). The reactions between lactoperoxidase and H_2O_2 in the presence of bovine serum albumin, β-Lg and casein are sources for the formation of protein radicals (Morello *et al.*, 2002, Grolichova *et al.*, 2004 ,Matak, 2004 and Hedegaard et al,2006) . Thefore, the presence of free radicals as rediolytic products is common for all physico-chemical changes induced by ionizing radiation in milk and milk products.

Representative ESR spectra of unirradiated and irradiated lyophilized different milk with gamma radiation at does (1 and 2 KGy) are shown in Figs. (1,2,3). The ESR singles of non-irradiated and irradiated milk samples can be reasonable of free radicals species of different origin as a results of its chemical compounds and oxidation induced by irradiation (Singh *et al.*, 2006, Yu and Cheng, 2008). Fig. (1). Shows the ESR spectra of raw freeze-dried milk powder (buffalo, cow, goat and camel) after irradiation (1 and 2 KGy) compared to those unirradiated milk samples. The ESR spectra of milk samples shows a weak signal intensities centered at g = 2.01126, g = 2.01129 , g = 2.01046 and g = 2.01090 for unirradiated buffalo,cow, goat, and camel milk, respectively. Irradiation resulted in increase in ESR signal intensities with changes in values of g factor at the same magnetic field (3400-3500 G). However, the ESR signal intensity of irradiated milk samples gradually increased as the radiation dose increases from 1 up to 2 KGy (Fig.1). At 1 KGy, the values of g factor were 2.01118, 2.01130, 2.01082 and 2.01106 for buffalo, cow, goat and camel milk corresponding values 2.01115, 2.01141, 2.01087 and 2.01089 at 2 KGy. Similar ESR spectra were observed for g value of different food in literature (Hargreaves *et al.*, 1994, Gardner, 1998, Kwon *et al.*, 2000, Nissen *et al.*, 2002, Nakamura *et al.*, 2006,Shimoyama *et al.*, 2006, Zhou *et al.*, 2006 Polat and Korkmaz, 2008, Prasuma *et al.*, 2008 Ukai *et al.*, 2008and Espinoza *et al.*,2009) . Kristensen and Skibsted (1999) showed that the free radical of freeze-dried processed cheese detection by ESR at 3480-3500G and g value = 2.0046 is carbon-centered radical. On the other hand, Stapelefeldt, (1997 a,b,c, 1999) showed that ESR measurements on unheated freeze-dried milk quantitates lipid-based radicals rather than protein-based or topheryl radicals. β-Lg irradiated showed no significant changes compared to non-irradiated sample (La Hoz and Nettro, 2008). Therefore, Ibrahim (1984) indicated that the exposure of buffalo, cow and goat milk to gamma rays caused increase of total saturated fatty acid while unsaturated fatty acids decrease. While Hansen *et al.*, (1970) examined the free radicals of grinded milk protein detected with ESR signals. The signals had g values closed to that free electron 2.0000. The cysteine-containing β-Lg and K-casein yield more intense free radical signals than α_s and β-casein, and the signal intensity increased with grinding time.

Irradiation also caused singlet oxygen formation and detected by ESR. The singlet oxygen which is very reactive with double-bound containing compound (lipids) can easily react with vitamins and aromatic and sulfur amino acids. The reactions could lower nutritional quality and produce undesirable flavour compound.

T1

Therefore, to minimize the singlet oxygen reactions in milk products, the products should be protected from light during processing and storage (Aurand *et al.*, 1977, Bradley *et al.*, 2003 and Gillies and Greenley, 2006). β-caroten's reactivity with singlet oxygen may account for its ability to protect the lipids from autooxidation (Rawls and Santen, 1970). Ascorbate free radical a maker oxidative stress can be detected by ESR (Buetter and Jurkiewicz,1993)

Data presented in Fig. (1) show that the ESR signal intensities of goat milk were lower that those of other milk samples. This may attribute to higher oxidative stability of goat milk. Kondyli *et al.*, (2005) stated that the oxidative stability of goat milk by ESR technique, and the level of free radicals generated in milk have been correlated to the natural antioxidants of goat milk i.e. α-tocopherol and ascorbic acid (Lindmark-Mamsson and Akesson, 2000).

Fig. (2) shows the ESR spectraum of commercial whole milk powder before and after gamma irradiation at 1 and 2 KGy. The spectrum exhibited shoulder signal centered at g = 2.00147, which corresponds to free radicals of non-irradiation milk sample. Upon radiation at dose 1 and 2 KGy the sharp signals centered at g = 2.00161, and 2.00153, respectively. The increase in the signal intensities was found to originate free from generation of new radicals during radiation. This might be attributed to the Maillard reaction.

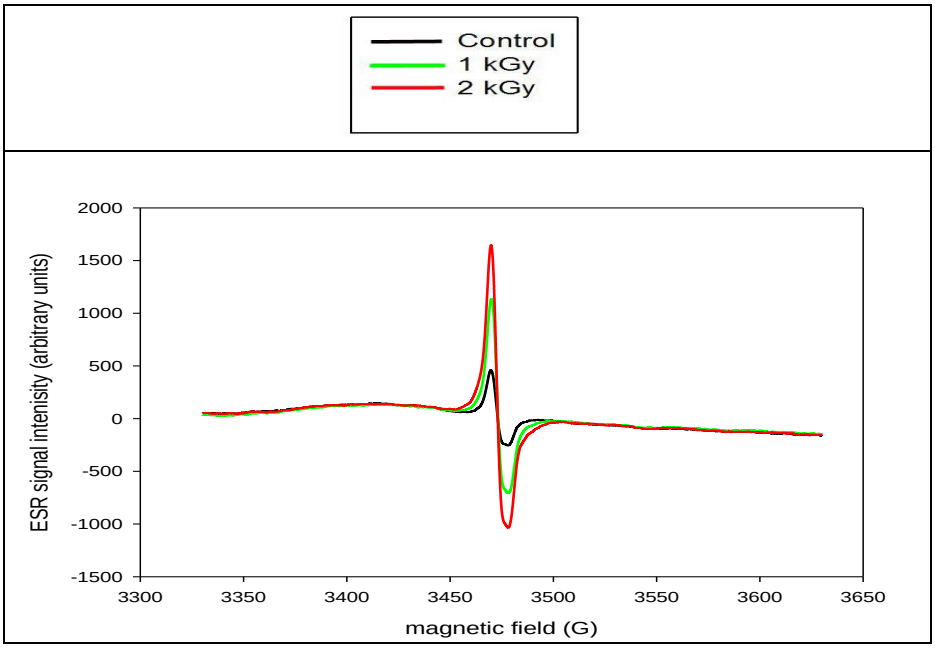

Fig (2). The ESR spectrum of irradiated commercial full cream cow's milk powder .

There are series of free radicals generated in the reaction between amino and glycoladehyde or glyceraldeyde (Yen and Hsieh, 1995 and Roberts and Lioyd, 1997). However, the characteristic features of these new radicals were found to be similar to those of the original free radical present in the raw milk samples. The ESR signals intensities were higher than those of raw milk samples. This attributed to spray-dried milk powder is much more subjected to oxidation than raw milk. Al-Rowaily (2008) showed that heat treatment of milk increased oxidation products (hydroperoxide or peroxide) compared to that of raw milk. The oxidation level in UHT milk produced from milk powder (reconstituted milk) was significantly higher than those of UHT produced from fresh milk. Therefore, it is unsuitable to use milk powder in the production of UHT milk. Smet *et al.*, (2008) suggested that electron spin resonance spectroscopy can be used to direct detection of early free radicals formation to predict generation of off-flavours during storage of pasteurized milk. On the other hand, the content of free radicals observed by ESR spectraoscopy was higher during storage of milk powders at 45ºC than in powders stored at 25ºC (Stapelfedt *et al.*, 1997b).

As regards, buffalo's milk butter and colostrum, Fig. (3) shows the ESR spectra of milk samples before and after irradiation at different dose (1 and 2 KGy). In non-irradiated milk samples the amplitudes of the central peaks of ESR spectra were characterized by g values of 2.01115 and 2.01123 for butter milk and colostrums, respectively. Both the signals are essentially the same magnetic field (3450-3500 G), shape and structure. However, the ESR signal intensity of colostrum was higher than that of butter milk. This attributed to the the higher free radicals scavengers in the colostrum than in butter milk. The free radical scavergers of colostrum include whey protein fraction lactoferrin ,enzymes (superoxide dismutase, gluthathione peroxidase, catalase, latoperoxidase-hydrogen peroxide-thiocyanate system) vitamins (β-carotene, C,E) and iron as a free radical generator (Kappeler *et al.*, 1999, Levieuk and Ollier, 1999, Lindmark-Mansson and Akession, 2000, Friel *et al.*, 2000, Marek *et al.*, 2003 , Nawar, 2006 , and Alamed *et al.*,2009). Whereas butter milk containes milk fat globule membrane (MFGM) as initiators for free radical formation owing to its relatively high concentration of phosplipids, xanthine oxidase and catalase. Stapelefeldt *et al.*, (1999) reported that the relationship between α-tocopherol content in milk and MFGM and the ESR signal is important since lipid oxidation in milk is believed to be initiated in the unsaturated phospholipids present in MFGM. Therefore, Kristensen *et al.*, (2004) showed that butter milk from the more unsaturated milk fat was less oxidatively stable during storage (11 days/4ºC) than butter milk from the more saturated milk fat.

Milk xanthine oxidase (X.O) containing redox centers serves as electron transfer processes. The radiolysis of X.O generated inorganic and organic free radicals as well as metal ions (Andesont *et al.*, 1986). Also, X.O can produce reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide (Fridovich, 1970, Kuppusamy and Zweier, 1989 and Granelli *et al.*, 1995).

T3

On the other hand, the ability of xanthine oxidase, catalyzing the oxidation of hydroxanthine to promate peroxidation of milk lipids (Aurand *et al.*, 1977 and Kankare, 1982). Therefore, food containing antioxidants against superoxide anion will be the first line of defence against oxidative stress (Singh *et al.*, 2006 and Zhang *et al.* 2009).

Fig. (3) also showed that gamma irradiatiation at dose of 1 and 2 KGy produced increase in signal intensity of both samples. There is positive correlation between signal intensity and irradiation dose, whereas the g value were not affected with the increase of the applied dose (2.01111 and 2.01115) for irradiated butter milk samples at dose of 1 and 2 KGy respectively corresponding values 2.01097 and 2.01098 for colostrum samples. Irradiation may cause decomposition of fat globule membrane and protein that relase copper and certain amino acid which act as prooxidants. On the other hand,the signal intensities of irradiated colostrum were higher than those of butter milk (Fig.3), although colostrum has the strongest free radical scaverging activity against generated free radical by irradiation. This might be due to irradiation caused degradation of free radical scavenging of colostrum thus reduced or inactivated antioxidant activity. Singh *et al.*, (2006) showed that antioxidative enzyme are one line of defense against free radicals. Hydrogen peroxide can become by lactoperoxidase catalase and gluthione peroxidase. Also, fatty acid hydroperoxides and phospholipids hydroproxides can be reduced by different gluthione peroxidases. The conversion of hydrogen peroxide into hydroxyl radical can be controlled by the a viability of iron ions influenced by lactoferrin and transferrin (Alamed *et al.*, 2009). Peng *et al.*, (2009) showed that the ESR signal intensities of superoxide anion and hydroxyl radical can be reduced by hydrolysed whey protein isolate (WPI). However, the lower signal intensities of irradiated butter milk may be attributed to inactivation of xanthine oxidase by radiation process and stable lipophilic antioxidant such as α-tocopherol and β-carotene. Ubaldi *et al.*, (2005) reported that α-tocopherol more stable and less oxidatable. Therefore Kristensen *et al.*, (2004) indicated that the oxidative stability of butter milk detected with ESR method depends on the content of α-tocopherol and β-carotene.

Table (1): The relative ESR signal intensities of non-irradiated and irradiated milk powders

Type of milk powder	Non-irradiated	Irradiation dose			
		1 KGy	Change %	2 KGy	Change %
Buffalo	60	360	5.00	525	7.75
Cow	160	310	0.93	540	2.38
Goat	50	125	1.5	163	2.26
Camel	150	240	0.56	292	0.95
CFCMP	480	1120	1.3	1650	2.44
Butter milk	100	148	0.48	168	0.68
Colostrums	160	305	0.91	608	3.25

CFCMP = Commercial full cream milk powder

Table (1) shows the relative ESR spectra signal intensities of non-irradiated and irradiated milk powders. The results indicated that the signal intstenensities of ESR spectra are dependent on the free-radicals origin of milk and irradiatation dose applied. However, the magnitude of radio stability based on change of ESR signal intensity. Butter milk and camel milk had the highest radio stability, while buffalo's milk was the lowest.

In conclusions, these previous results suggest the potential of ESR technique as sensitive, direct and accurate tool to monitor reactive species (free radicals) generated by radiation, oxidative stability and radio stability of dairy products, and more detailed studies of reactions mechanisms are warranted. Future studies are planned to include development of new application of ESR to provide additional information to identify the free radicals to advance our understanding of dairy products and interactions among individual components during formulation, processing and storage (Hedegaard *et al.*, 2006, Kopani *et al.*, 2006 and Yu and Cheng, 2008).

REFERENCES

Ajems, F. (2007). Fatty acid profile of camel. Food Agriculture Nutrition and development. 1: 5-8.

Al-Rowaily, M.A. (2008). Effect of heating treatments, processing methods and refrigerated storage milk and some dairy products on lipids oxidation. Pakistan J. of Nutrition, 1: 118-125.

Alamed, J., Chaiyasit, W. McClements, D.J. and Decker, E.A. (2009). Relationships between free radical scavenging and antioxidant activity n foods. J. of Agriculture and Food Chemistry: Under publishing.

Anderson, R.H, Hilles, K and Plassey, V. (1986). The radical chemistry of milk xanthine oxidase as studied by radiation chemistry techniques. J. of Biological Chemistry. 261: 15870-15876.

Aurand, L.W, Singleton, J.A. and Noble, B.W. (1966). Photooxidation reaction in milk. J. Dairy Sci.,49 138-143.

Aurand, L.W, Boone, N.H. and Giddings G.G. (1977). Superoxide and singlet oxygen in milk lipid peroxidation. J. Dairy Sci., 69: 363-369.

Bradely, D.G, Lee, H.O. and Min, D.B. (2003). Singlet oxygen detection in skim milk by electron spin resonance spectroscopy. J. Food Sci. 68: 491-494.

Brudving, G.W. (1995). Electron paramagnetic resonance spectroscopy. Methods in Enzymology 246-536-556.

Bueller, G. R., and Jurkiewiez, B.A. (1993). Ascorbate free radical a maker of oxidative stress: an ESR study: Free Radic. Biol. Med. 14: 49-55.

Byun, M. W. and Kang, I. J. (1995). Properties of soy milk and tofu prepared with γ-irradiated soy beans. J. Sci., Food Agric. 67: 477-483.

Ciesla, K, Salmieri, S. Lacroix, M and Tien, C, Le. (2004). Gamma irradiation influence on physical properties of milk proteins. Radiation physics and chemistry 71: 93-97.

Ciesla K, Salmieri, S. and Lacroix, M. (2006). Gamma-irradiation influence on the structure and properties of calcium caseinate-whey protein isolate based films. Part-Radiation effect on the structure of proteins of gels and films. J. Agric. Food Chem. 54: 63674-6384.

Crisp, P., Wicks, T.J, Troup, G, Scott, E.S. (2006). Mode of action of milk and whey in the control of grapevine powder mildew. Australasian Plant Pathology. 35: 487-493.

De Simone, F., Guzzi, R, Sportelli, L, Marsh, D and Batucci, R. (2007). Electron sin-echo studies of spin-labelled lipids interacting with human serum albumin. Biochemical Biophysical Acta. 1768: 1541-1549.

Delincee, H. (1998). Detection of food treated with ionizing radiation. Trends in food science and technology 9: 73-82.

Delicee, H, (2002). Analytical methods to identify irradiated food- a review. Radiation physics chemistry. 63: 455-458.

El-Dieb, S.M, Ramadan, F.A. M. and Abd El-Ghany, I.H.I. (1995). The use of butter milk and milk fat globule membrane to improve the body and texture of ice-milk. Egyptian J. Dairy Science: 249-257.

Espinoza, M, Olea-Azr, C, Spesiky, H, and Rodriguez, J. (2009). Determination of reactions between free radicals and selected Chilean wines and transition metals by ESR and UV. Vis technique. Spectrochimica Acta part A. 71: 1638-1643.

Fridovich, I. (1970). Quantitive aspects of the production of superoxide anion redical by milk xanthine oxidase. J. of Biological Chemistry. 245-4053-4057.

Friel, J. K, Martin, S.M, Langdon, M, Herzberg and Buttner, G.R. (2002). Milk from mothers of both premature and full-term infants provides better antioxidant protection than does infant formula. Pediatr, 51: 612-618.

Gardener, P.T., Mcphail, D, B, Duthie, G.G. (1998). Electron spin resonance spectroscopic assessment of the antioxidant potential of teas in aqeous and organic media. J. Sci., Food Agric. 76: 257-262.

Gillies, D.G and Greenley, K.R. (2006). ESR/Spin prob study of ice cream. Journal of Agriculture and food chemistry 54: 4943-4947.

Granelli, K, Bjorck, L and Appelqvist, L-A. (1995). The variation of superoxidse dismutase (SOD) and xanthine oxidase (xo) Activities in milk using improved method to quantitate SOD activity. J. Sci Food Agric. 67: 85-91.

Grolichova, M, Dvorak, P and Musilova, H. (2004). Employing ionizing radiaton to enhance food safety. A review. Acta vent. Brno. 73: 143-149.

Gurachevsky, A, Shimanovitch, E, Gurachevskaya, T. and Muravsky, V. (2007). Intra-albumin migration of bound fatty acid probed by spin label ESR Biochemical and Biophysical research communications. 3.60: 852-856.

Haddadin, M, Sy, Gammah, S.I. and Robinson, R.K. (2008). Seasonal variations in the chemical composition of camel milk in Jordan. J. of Dairy Research. 75: 8-12.

Hansen, P.M.T., Harper, W.J. and Sharma, K.K. (1970). Formation of free radicals in diary milk proteins. J. Food Sci., 35:598-600.

Hargreaves, J, Meste, M, L, Cornec, M and Popineau, Y. (1994). Electron spin resonance studies of wheat protein fractions. J. Agric. Food Chem. 42: 2698-2702.

Hedegaard, R.V., Kristensen, D., Nielson, J.H, Frost, M.B, Ostdal, H., and Hermansen, J.E. (2006). Composition of descriptive sensory analysis and chemical analysis for oxidative changes in milk. J. Dairy Sci., 89:495-504.

Ibrahim, M. Kh (184). Effect of gamma radiation on properties of milk and milk products. Ph.D. Thesis. Fac. of Agric. Cairo Univ.

Jo, C, Kim, H-J, Kim, D-H, Lee, W-K, Ham, J-S, and Byum, M-W. (2007). Radiation sensitivity of selected pathogens in ice cream. Radiation physica and chemistry. 74: 101-109.

Kaddouri; H, Mimoun, S, El-Mecherfi, K.E., Chekroun, A, Keroua, O, and Saidi, D. (2008). Impact of gamma-radiation on antigenic properties of cow's milk beta-lactoglobulin. J. Food Production. 71:1270-1772.

Kamal, A.M and Salama, O.A. (2008). Lipid fractions and fatty acid composition of colostrums, transitional and mature she-camel milk during the first month of lactation. Asian J. of Clinical Nutrition. 1: 23-30.

Kankare, V, and Antila, V (1982). The effect of xanthine oxidase and superoxide dismutase as well as cell counts on the oxidation of fat in bovine milk. Finnish J. Dairy Sci., 2: 32-40.

Kappeler, S.R, Ackermann, M, Farah, Z and Puhan, Z.C. (1999). Sequence analysis of camel (*Camelus dromedaries*) lactoferrin Inter. Dairy J. 9: 481-486.

Khorshid, M.A., Ahmed, N.S. and Ismail, A. A. (1976). Effect of gamma ray irradiation on some chemical and physical properties of buffaloes milk. Milshwissenschaft; 31: 409-413.

King, R.L. (1963). Oxidation of milk fat globule membrane material. I. Thiobarbituric acid reaction as a measure of oxidized flavour in milk and model systems. J. Dairy Sci., 46: 1163-1170.

Kispeter, J. Kiss, L.I and Horvath, L.I. (2007). Effect of ionizing gamma radiation on thermo luminescence and spin resonance intensities in milk protein concentrate powders. Food Structure. 11: 165-170.

Kondyli, E., Andersen, M.L. and Skibsted, L.H. (2005). Evaluation of the oxidative stability of goat milkby electron spin resonance spectroscopy (ESR). Milchwissenchaft. 60: 271-274.

Kopani, M, Ceiec, P. Danisovic, L. Michalka, P. And Biro, C. (2006.Oxidaive stress and electron spin resonance. Clinica Chimica Acta 364:61-66.

Kristensen, D and Skibsted, L.H. (1999). Comparison of three methods based on electron spin resonance spectrometery for evaluation of oxidative stability of processed cheese. J. Agriculture and Food Chemistry. 47: 3099-3104.

Kristensen, D, Hedegad, R.V, Nielsden, J.H. and Skibsted, L.H. (2004). Oxidative stability of butter milk as influenced by the fatty acid composition of cow's milk manipulated by diet. Journal of Dairy Resarch . 71: 46-50.

Kuppusamy, P. and Zweier, J. L. (1989). Characterization of free radical generation by xanthine oxidase. J. Biol Chem. 264: 9880-9884.

Kwon, J.H, Chung, H-W, and Byun, M-W (2000). ESR spectroscopy for detecting gamma-irradiated dried vegetables and estimating absorbed does. Radiations. Physics and Chemistry. 57: 319-324.

La Hoz, L, de and Nettro, T.M. (2008). Structural modification of β-lactoglobulin subjected to gamma radiation. International Dairy J. 18: 1126-1132.

Letendre, M, D'Aprans, G, Delmas-patterson, G, Laroix, M. (2002). Isothermal calcorimetry study of calcium caseinate and whey protein isolate edible films cross-linked by heating and gamma-irradiation. J. Agric. Food Chemistry. 50: 6053-6057.

Levieux, D, and Ollier, A. (1999). Bovine immunoglobulin G, β-lactoglobulin, α-lactoalbumin and serum albumin in colostrums and milk during the early post partum period. J. of Dairy Research. 66: 421-430.

Lindmark-Mansson, H and Askesson, B. (2000). Antioxidative factors in milk. British J. of Nutrition. 84 suppl 1 : S103-S110.

Marek, A, Sziagatya, A, Liberek, A, Goa-Gebk, M, Sikorsk-Wisniwsaka, G, Marek, K, Raezkowska-Kazak, J. and Bako, W. (2003). Human milk multipotent immune system fortifier Signature Med Sci., Monit. 9: 75-81.

Matak, K.E. (2004). Effect of UV irradiation on the reduction of bacterial pathogens and chemical indicators of milk. Ph.D. Thesis. Food Science and Technology. Blacksburg. Virginia.

Morells, M.J., Shahidi, F. an Ho, Ch-T. (2002). Free radicals in foods: Chemistry. Nitration, and health. ACS Symposium Series 807. Washington, DC; Amerian Chmical Society.

Morin, P, Britten, M, Jimenez-Flores, R, and Pouiiot, Y. (2007). Microfiltration of buttermilk and washed cream buttermilk for concentration of milk fat globule membrane components. J. Dairy Sci. 90: 2132-2140.

Nakamura, H, Ukai, M, Shimoyama, Y. (2006). An electron spin resonance study of γ-ray irradiated ginseng. Spectrochimica Act Part A 63: 883-887.

Nawar, M.A. (2006). Antimicrobial factors in buffalo milk: Changes during the first month lactation. Egyptian J. Dairy Sci. 34: 133-138.

Nissen, L.R., Hung-Ba, T, Petersen, M.A, Bertelsen, G. and Skibsted, L.H. (2002). Potential use of electron spin resonance spectroscopy for evaluating the oxidative status of potato flakes. Food Chemistry. 79:387- 394.

Osattli, T-M, Shaker, R.R., Abu-El-Hasan, Ayyash, M.M, Martin, E.M. (2007). Inactivation of *Enterobacter sakazakii* in infant milk formula by gamma irradiation: determination of D_{16}-value. J. Food Sci., 72:M85-88.

Peng, X, Xiong, Y.L. and Kong, B. (2009). Antioxidant activity of peptide fraction from whey protein hydrolysates as measured by electron spin resonance. J. Agric. Food Chemistry: Under publishing.

Polat, M, and Korkmaz, M, (2008). Detection of irradiated black tea (*Camellia siensis*) roobios tea (*Aspalathus linearis*) by ESR spectroscopy. Food Chemistry: 107: 956-961.

Prasuma, C,P. L. Chakradhar, R.P.S. Pao, J.L. and Gopal, N.O. (2008). EPR as an analytical tool in assessing the mineral nutrients and irradiated food products. Vegetables. Spectrochimica Acta Part A . 71: 809-813.

Rawls, H.R., and Snaten, P.J. (1970). A possible role for singlet oxygen in the initiation of fatty acid outoxidation. J. of the American of Chemical Society. 47:121-125.

Roberts, R.L, Lioyd, R.VV. (1997). Free radical formation from secondary amines in the Maillard reaction. J. Agric. Food Chem. 45: 2413-2418.

Shimoyamma, Y, Ukai, M and Nakamura, H, (2006). ESR dection of wheat flour before and after irradiation. Specrochimica Acta part A : 63:888-890.

Shimoama, Y, Ukai, M and Nakamura, H. (2007). Advanced protocol for the detection of irradiated food by electron spin resonance spectroscopy . Radiation physics and Chemisty.1837-1839

Shoji, H, Shimizu, T., Shinohara, , Oguch, S, Shiga, S, and Yamashiro, Y. (2004). Suppressive effect of breast milk on oxidative DNA damage in very low birth weight infant. Arch Dis Child Fetol Neonatal Ed. 89: F 136-F 138.

Singh, R.P., Sharad, S, and Kapui, S. (2006) Free radicals and oxidative stress in neurodegenerative disease relevance of dietary antioxidants. J. Indian Academy of Clinical Medicine. 5: 218-225.

Smet, K, Raes, K., De Block, J, Herman, L, Dewthinck, K., Coudizer, K. (2008). A change in antioxidative capacity as measure of onset to oxidation in pasteurized milk. International Dairy J. 18: 520-530.

Stapelfeldt, H, Mortensen, G. and Skibsted, L.H. (1997a). Early events in oxidation of whole milk powder detected by electron spin resonance spectrometry. Carry-over effect from butter oil used for instantization. Milchwisseschaft. 52: 266-269.

Stapeifeldt, H; Nieisen, B. R, and Skibsted, L.H. (1997b). Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. Int. Dairy Journal. 7: 331-339.

Stapeifeldt, H; Nieisen, B.R, and Skibsted, L.H. (1997c). Towards use of electron spin resonance spectrometry in quality control of milk powder. Correlation between sensory score of instant whole milk powder and concentration free radicals and 2-thiobarbituric acid reactive substance. Milchwissenchaft. 52: 682-685.

Stapeifeldt, H, Nieisen, K.N, Jensen, S.K, and Skibsted, L.H. (1999). Free radical formation in freeze-dried raw milk relation to its α-tocopherol level. J. Dairy Research 66: 461-466.

Ubald, A, Debono, GM Fusari, and Serventi, P. (2005). Quick HPLC method to determine vitamin E concentration in cow's milk. Am. Faac. Medie Vet. Di Pama. Xxx: 101-110.

Uear, A. (2007). The effects of foods irradiation on nutrition and health. Paskistan J. of Social Sciences. 4: 662-666.

Ukai, M, Kameya, H, Nakamura, H, Shimoyama, Y. (2008). An electron spin resonance study of dry vegetables before and after irradiation. Spectrochemia Acta part A 69: 1417-1422.

Whang, K. and Peng, I.C. (1988). Electron paramagnetic resonance studies of effectiveness of myogloblin and its derivatives as photosensitizers in singlet oxygen generation. J. Food Sci., 53. 1863-1866.

WHO (World Health Organization. 1999). High-dose irradiation: wholesomeness of food irradiated with dose above 10 KGy. WHO technical series 890. Geneva: World Health Organization.

Yen, G-C and Hsieh, P-P. (1995). Antioxidative activity and scavenging effect on active oxygen of xylose-lysine Maillard reaction products. J. Sci. Food Agric. 67: 415-420.

Yu, L.L. and Cheng, Z. (2008). Review: Application of electron spin resonance (ESR) spectrometry in nutraceutical and food research Mot. Nut. Food Res. 52: 62-78.

Zhang, L, Huang, D, Konda, M, Fan, E, Ji, H, Kou, Y and Ou, B. (2009). Novel high-throught assay for antioxidant capacity against superoxide anion. J. Agric. Food Chem. Under publication.

Zhou, K, Yin, j-j, AND Yu, L.L. (2006). ESR Determination of the reaction between selected phenolic acids and free radicals or ransition metals. Food Chemistry. 95: 446-457.

استخدام تقنية جهاز الرنين الالكتروني الدورانى (ESR) في كشف الشقوق الحرة للألبان المجففة والمشعة بأشعة جاما

إسماعيل حسين إسماعيل عبد الغنى* و أحمد إبراهيم عطية**

*** قسم علوم وتكنولوجيا الألبان كلية الزراعة – جامعة القاهرة – الجيزة – مصر**

**** قسم المنتجات الطبيعية – المركز القومي لبحوث وتكنولوجيا الإشعاع – هيئة الطاقة الذرية – مدينة نصر – القاهرة - مصر**

يهدف هذا البحث إلى تقييم استخدام جهاز الرنين الالكتروني (ESR) في الكشف عن الشقوق الحرة للألبان المجففة المختلفة (جاموسى ، بقرى ، غنم ، ابل ، لبن بقرى تجارى ، لبن خض وسرسوب جاموسى) والمشعة بجرعات مختلفة (١ ، ٢ كيلو جراى KGy) لأشعة جاما واستدل على وجود الشقوق الحرة ظهور الإشارات (Signals) الناتجة من جهاز ESR وأوضحت النتائج إلى ما يلي

١- أظهرت عينات اللبن المختلفة الغير مشعة بأشعة جاما إشارات ضعيفة تعتمد على نوعية اللبن المستخدم
٢- شدة إشارات (ESR) الناتجة من تشعيع الألبان المختلفة تزداد بزيادة الجرعة الإشعاعية المستخدمة من ١ إلى ٢ كيلو جراى
٣- درجة ثبات اللبن المجفف لأشعة جاما تعتمد أساسا على وجود الشقوق الحرة في اللبن قبل التشعيع وشدة الجرعة الإشعاعية المستخدمة
٤- كان اللبن الخض (الجاموسى) ولبن الإبل أكثر ثباتاً لأشعة جاما بينما كان اللبن الجاموس اقل ثباتاً

ونظرا لكون جهاز الرنين الالكتروني الدورانى (ESR) من الأجهزة الحديثة وما يمتاز بة من سرعة ودقة النتائج المتحصل عليها لذلك توصى هذة الدراسة بإمكانية استخدام هذا الجهاز في تقيم والتنبأ بجودة المنتجات اللبنية المختلفة مما يساهم في تجنب التغيرات الغير مرغوب فيها أثناء الصناعة والتخزين.

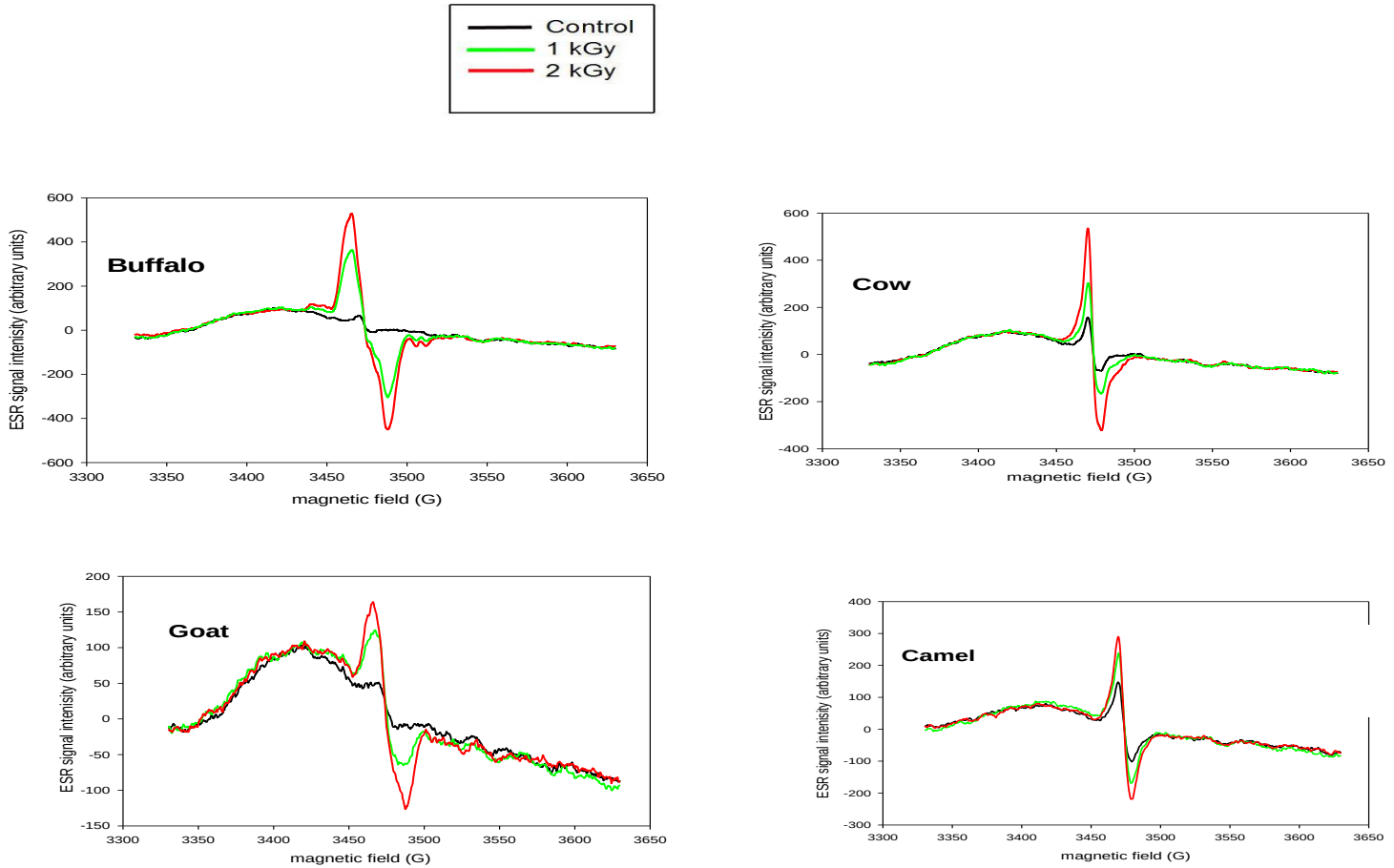

Fig (1). The ESR spectra of irradiated different milk powders (buffalo, cow, goat and camel).

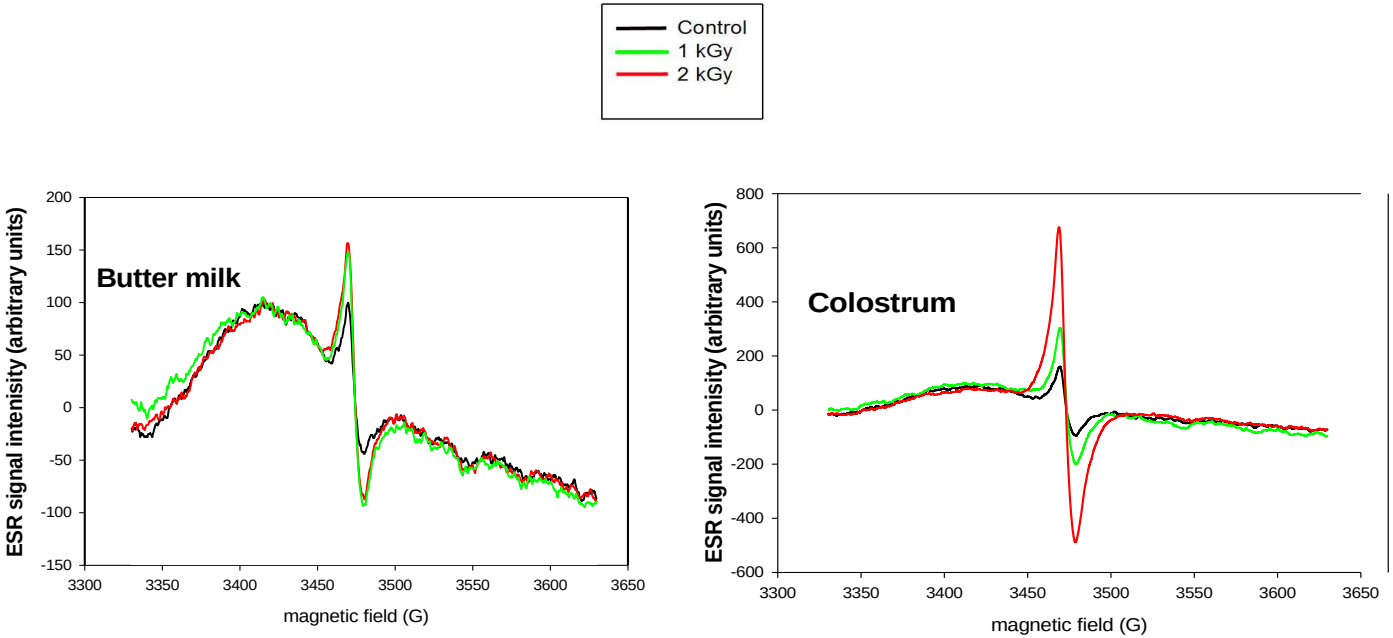

Fig (3). The ESR spectra of irradiated buffalobutter milk and colostrum powders