SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Print: 1304-7582, Online: 2146-9377
http://www.academicfoodjournal.com

Akademik Gıda 11(1) (2013) 13-19

Research Paper / Araştırma Makalesi

Effects of Storage Time and Temperature on Detection of Irradiated Hamburgers with DNA Comet and Gas Chromatography/Mass Spectrometry Analyses

Ayça Aylangan[1, ✉], Halil Vural[2]

[1]Turkish Atomic Energy Authority, Saraykoy Nuclear Research and Training Center, Ankara, Turkey,
[2]Hacettepe University, Department of Food Engineering, Ankara, Turkey

Received (Geliş Tarihi): 09.01.2013, Accepted (Kabul Tarihi): 10.03.2013
✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): ayca_yarali@yahoo.com (A. Aylangan)*
✆ 0 312 810 17 36 ☎ 0 312 815 43 07

ABSTRACT

This study investigated the formation 2-dodecycylobutanone (2-DCB) with gas chromatography/mass spectrometry (GC/MS) and DNA Comet Assay in irradiated hamburgers. Hamburgers were irradiated using a ^{60}Co source at three targeted absorbed doses of 0.4, 0.8, and 1.2 kGy. The samples were analyzed immediately after irradiation and post irradiation on days 2, 5 and 8 during storage at +4°C, and on days 2, 5, 8, 15 and 30 during storage at -12°C. Fragmented DNA stretched towards the anode and the damaged cells appeared as a comet in irradiated samples. DNA fragmentation in non-irradiated hamburger occurred during storage at two different temperature (+4° and -12°C). The results indicated that the presence of 2-DCB may be used as an irradiation indicator in 1.2 kGy irradiated hamburgers since it does not occur in non-irradiated samples. The effect of storage temperatures on formation and diagnosis of 2-DCB of 1.2 kGy irradiated hamburgers was insignificant.

Key Words: 2-Dodecycyclobutanone; DNA comet assay, Gamma irradiation

DNA Komet ve Gaz Kromatografisi/Kütle Spektrometrisi Analizleri ile Işınlanmış Hamburger Köftelerin Tespitinde Depolama Süresi ve Sıcaklığın Etkisi

ÖZET

Bu çalışmada ışınlanmış hamburgerler gaz kromatografisi / kütle spektrometresi ile 2-dodesilsiklobütanon (2-DSB) oluşumu açısından ve DNA Komet analizi ile incelenmişlerdir. Hamburgerler ^{60}Co kaynağı kullanılarak 0.4, 0.8 ve 1.2 kGy olmak üzere hedeflenen 3 farklı dozda ışınlanmışlardır. Örnekler ışınlamadan hemen sonra ve ışınlama sonrası +4°C'de 2, 5 ve 8 gün, -12°C'de 2, 5, 8, 15 ve 30 gün depolanarak analiz edilmişlerdir. Parçalanmış DNA anoda doğru yayılma eğilimindedir ve hasar görmüş hücreler ışınlanmış örneklerde kuyruklu yıldız görüntüsü vermektedirler. Işınlanmamış hamburgerlerde ise DNA parçalanması iki farklı sıcaklıkta (+4° ve -12°C) depolama sırasında görülmektedir. Bu çalışmadan elde edilen sonuçlar 1.2 kGy'lik dozda ışınlanmış hamburgerlerde 2-DSB varlığının ışınlanmamış örneklerde oluşmaması nedeniyle, ışınlanmanın bir indikatörü olarak kullanabileceğini göstermektedir. 2-DSB'nin oluşumu ve tespitine 1.2 kGy'lik dozda ışınlanan hamburgerlerde depolama sıcaklığının etkisi önemli bulunmamıştır.

Anahtar Kelimeler: 2-Dodesilsiklobütanon, DNA komet analizi, Gama ışınlama

INTRODUCTION

Food irradiation is the process of exposing food to a controlled source of ionizing radiation for the purposes of reduction of microbial load, destruction of pathogens, extension of product shelf life, and/or disinfestations of produce [1]. Although properly irradiated food is safe and wholesome, consumers should be able to make their own free choice between irradiated and non-irradiated [2]. Processing of food with ionizing radiation does not change its identity. Nonirradiated and irradiated foods are visually and sensorially identical [3]. For this purpose labelling is indispensable. Labelling of irradiated foods will ensure the consumer's freedom of choice. In order to check compliance with existing regulations, detection of radiation treatment by analysing the food itself is highly desirable [2, 4]. Ten international standards regarding different detection procedures for irradiated food have been adopted by the European Committee for Standardization (CEN) and are now available to food control agencies [5, 6].

The European Union has officially adopted the DNA "comet assay" as EN 13784 for detection of irradiated foods. Since the large molecule of DNA is an easy target for ionizing radiation, changes in DNA offer potential as a detection method [7, 8]. DNA damage [strand breaks] in the cells is determined in the comet assay by agarose gel electrophoresis. A drawback of this method is the non-specificity of radiation to fragment DNA. DNA fragmentation also occurs by heat treatment, and repeated freezing/thawing cycles as well as long storage periods may also interfere. The DNA "comet assay", therefore, seems suitable as a pre-screening test to detect whether food has been radiation processed [9].

For the detection of irradiated food containing fat such as meat, chicken etc., the other officially adopted method by EU is the analysis of 2-alkylcyclobutanones (2-ACB). 2-ACB's are considered to be unique radiolytic products of irradiated fatty foods [8, 10, 11]. These are cyclic compounds formed by rearrangement of free fatty acids when exposed to irradiation. The resulting compounds have the same number of carbon atoms as the precursor fatty acids, with an alkyl group attached at ring position two [12]. Therefore, when four of the major fatty acids present in most lipid-containing foods, namely, palmitic, stearic; oleic and linoleic acid, are exposed to ionizing radiation, the respective 2-dodecycl- (2-DCB); 2-tetradecyl- (2-TCB); 2-tetradecenyl- and 2-tetradecadienyl-cyclobutanones are formed [13]. Since, the cyclobutanones present are considered to be radiation specific, no background levels have been found in nonirradiated samples, this method has a great potential [2]. The main goal of the research was to investigate the application of the two detection methods, EN 13784 and EN 1785 in irradiated hamburgers. As for GC/MS analysis, it was preliminary carried out on the 2-DCB (induced by irradiation of palmitic acid, the most abundant saturated fatty acids in beef hamburgers) standard to evaluate its retention timeIn particular we have studied if the irradiation treatment was detectable as a function of the storage time and different storage temperature.

MATERIALS and METHODS

Food Samples

Hamburger samples with 0.8% NaCl content produced from beef obtained from a local butcher's shop in Ankara, Turkey. Meat was ground using a 3 mm plate. The hamburgers were put into plates with a diameter of 8 cm and a height of 1 cm. Then, the samples were packed in polyethylene bags and irradiated at room temperature.

Chemicals

Among the 2-alkylcyclobutanones produced in irradiated meat, we have chosen the 2-dodecyclobutanone (2-DCB) which is formed from palmitic acid during irradiation, to make analysis. 2-DCB and 2-cyclohexylcyclohexanone which is the internal standard were supplied by Fluka, Sigma-Aldrich, Germany. Florisil-mesh 60-100 (Sigma-Aldrich, Germany) was maintained at 550°C for 5 hours. After this process, deactivated Florisil was prepared by adding 20 mL of distilled water to 100 g of absorbent. All other chemicals and solvents used were of the highest purity available.

Irradiation

Three irradiation doses (0.4, 0.8 and 1.2 kGy) were applied to samples in ^{60}Co Gamma-cell (Tenex, Issledovatel, Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center). Dose values (dose rate 0,79 kGy/h in July 2010) were calculated by using Harwell Amber 3042 dosimeters and the dose rate was measured with Fricke dosimetry. After irradiation, packed samples were immediately stored in two different temperature (4±2°C and -12±2°C). All analyses were applied to samples on immediately after irradiation and storage for 2, 5 and 8 days at +4°C; for 2, 5, 8, 15 and 30 days at -12°C.

DNA Comet Assay

Once ready prepared for analysis, the samples were tempered at room temperature for 10 to 15 min or until they got soft enough (for especially at -12°C storaged samples) to be homogenization. About 1.0 g of very thin slices of samples were transferred to small beaker with 5 mL ice-cold phosphate buffer saline (PBS) and stirred for 5 min with a rate of 500 min^{-1}. The suspension was filtered first through 500 µm and then through 200 µm cloth sieves, and left to be settled on ice for about 5 min. The supernatant was used as a cell suspension. The cell suspension (100 µL) was mixed with 1 mL of low-melting agarose (0.8 % in PBS). A 100 µL of this mixture was spreaded on precoated slides. Then, the cells were lysed for disruption of membranes using 2.5 % SDS for about 10 min, and electrophoresed at a potential of 2 V /cm using 0.045 M Tris-borate, 1 mM EDTA buffer with pH 8.4 for 2.0 min. Silver staining was employed to

visualise DNA [8,14]. Slides were examined using a microscope (Olympus BX 51 model) at magnification rate of 20 x 10 by digital color video camera (Pixera).

Gas Chromatography-Mass Spectrometry Analyses of 2-DCB

Once prepared ready for analysis, the samples were tempered at room temperature for 10 to 15 min or until soft enough (for especially at -12°C storaged samples) to be homogenization. Samples were placed in semi-automatic fat extraction unit (Soxtec Avanti – 2055 Soxtec, Foss Tecator) and fat extraction was performed on samples with n-hexane. The analysis of the 2-DCB was perfomed by following EN 1785 standard method in Sarayköy Nuclear Research and Training Center Laboratories [8,15].

2-DCB were separated using a Varian Factor Four, VF 5MS column, 30 m x 0.25 mm internal diameter with a 0.25 µm stationary phase (95% dimethyl, 5% polysiloxane) in a Varian CP 3800 gas chromatograph directly linked to a Varian 1200 L quadrupole mass selective detector. Conditions used were as follows: injector temperature: 250°C; column temperature program: 55°C (1 min), first ramp, 15°C/min to 300°C, first temperature hold, 300°C (5 min); injection volume: 1 µl; - injection mode: splitless; carrier gas: He, 1 mL/min; MSD: selected ion monitoring of ions m/z 98 and m/z 112.

RESULTS and DISCUSSION

Results of DNA Comet Assay

Figure 1 illustrates the results obtained by Comet Assay on irradiated/non-irradiated hamburgers. The results show that the distance of DNA migration, "comet length", increases with radiation dose at the first day of storage. Similar results were obtained in studies already published by different authors with different foodstuffs. With increasing radiation dose, more DNA fragmentation occurs and these fragments migrate further during the electrophoresis [16,17,18] Villavicencio et al. [19] reported that the size of the dose may be indicated by shape of the comet. This is not, however, an obligatory requirement of methods for detection of irradiated food. It is of most importance to be able to distinguish between non-irradiated and irradiated samples. The non-irradiated hamburgers were in form of round or conical stains of DNA, whereas irradiated samples showed nice comets. The comets for 0.4; 0.8 and 1.2 kGy could be easily distinguished on the basis of DNA material the tails. The tails of the comets were densely loaded with distinct thinly stained narrow neck for 1.2 kGy; however, in 0.4 kGy, the necks were continued from head to tail. Khan et al. [20] found similar results for detection of radiation treatment of several kinds of leguminous beans.

Figure 2 illustrates the results obtained by Commet Assay on hamburgers after 2 days of storage at +4 °C. The irradiation, freezing, thawing, refreezing [18] and longer storage time [16] were responsible factors of the DNA degradation. Non-irradiated samples show different types of comets probably due to natural DNA degradation in dead cells. In this study, after irradiation at 2 days of storage at +4°C and -12°C, non-irradiated hamburger samples showed comet shapes. Cerda [21] concluded that the comet assay can be used for detection of fresh irradiated chicken, pork and fish. Because, one week after irradiation there was a general increase of DNA damage caused by storage at +2 °C. Cells from all irradiated samples (2.5 and 5.0 kGy) thus showed comets with larger tails than on day one. Our results are in common with similar studies shown in literature. Several authors used DNA Comet Assay to detect irradiated food [16,17,19,22,23]. By employing the DNA comet assay it is possible to detect the irradiation treatment of commercial poultry samples using the various radiation doses applied (1.5; 3.0; 4.5 kGy) to chicken meat. Several authors reported that the DNA "comet assay" offers high potential as a rapid screening test for qualitative detection of irradiation treatment foods [19,20,24]. Micro electrophoresis of single cells is a simple and rapid test for DNA damage. It is worth to say that as most studies realized, we also did not use an image analyzer to quantify comets, due to the facility and simplicity of this method. Delincée [22] concluded that positive results need to be confirmed by a validated method to specially prove an irradiation treatment for hamburgers.

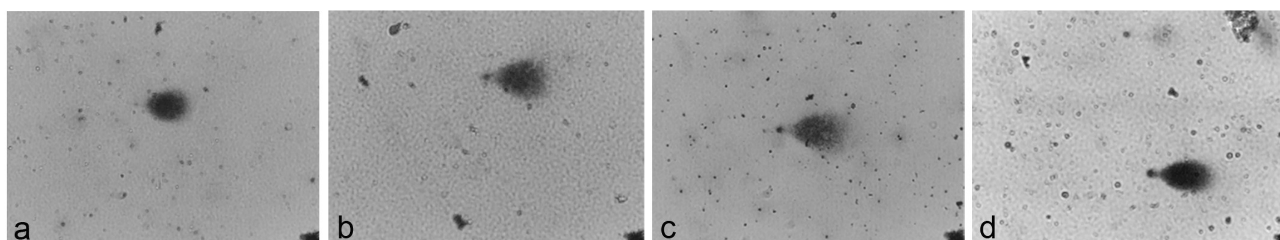

Figure 1. Photomicrographs of DNA comets from nonirradiated and irradiated hamburgers on the first day of storage (microscope objective X20); (a) non-irradiated; (b) dose of 0.4 kGy; (c) dose of 0.8 kGy; (d) dose of 1.2 kGy (Silver staining; anode to the right).

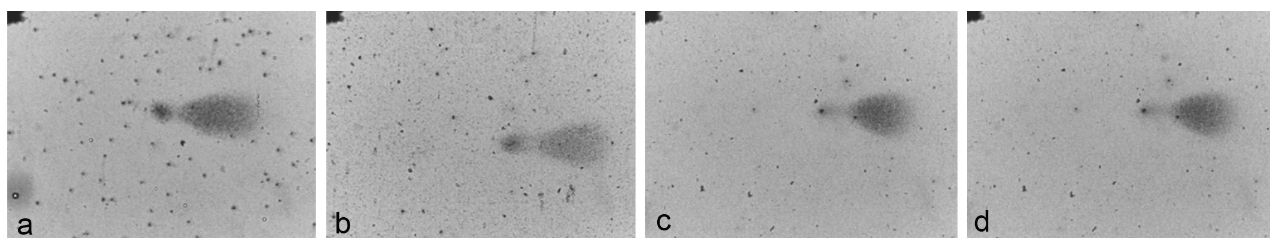

Figure 2. Photomicrographs of DNA comets from nonirradiated and irradiated hamburgers on the 2th day of storage at +4°C (microscope objective X20); (a) non-irradiated; (b) dose of 0.4 kGy; (c) dose of 0.8 kGy; (d) dose of 1.2 kGy (Silver staining; anode to the right).

Results of GC/MS

The occurrence of 2-DCB can be used as a marker in irradiated hamburgers. According to the EN 1785, the identification of the irradiation treatment was considered positive when peaks of ions m/z 98 and m/z 112 in a ratio approximately 4:1 were present in the mass spectrum of lipid extracts [14]. 2-DCB standard retention time was found to be between 13.0 – 13.25 min. The extract ions chromatogram of nonirradiated sample of hamburgers did not show any specific signal at the retention time attended for 2-DCB immediately after irradiation (Figure 3b). On the other hand, the 2-DCB, expected from hamburger lipids after irradiation, was clearly detected at expected retention time between 13.0 – 13.25 min (Figure 3a). D'Oca et al. [25] founded similar results in irradiated pork samples. Blanch et al. [26] concluded that 2-DCB may be used an irradiation indicator in sliced dry-cured ham since it was not occur in non-irradiated samples. Marchioni et al. [27] concluded that using gas chromatography-mass spectrometry, down to 0.5% [m:m] of irradiated mechanically deboned turkey meat included in non-irradiated chicken quenelles could be detected.

The chemical stability of 2-alkylcyclobutanones in food is quite good. It was observed that the moderate losses during storage do not reduce the validity of the standard method [28]. The 2-DCB in irradiated hamburgers was relatively stable during 8 and 30 days in two different storage temperature (+4°C and -12°C). After 8 days of storage at +4°C (Figure 4) and -12°C (Figure 5), the 2-DCB was clearly detected at retention time of 13.062 min and 13.103 min. Similarly, after 30 days of storage at -12°C, while non-irradiated sample of hamburgers did not show any specific signal at the retention time, 2-DCB was detected at 13.116 min retention time in 1.2 kGy irradiated sample. The alkylcyclobutanones in irradiated seasoned filefish were relatively stable during 6 months of low-temperature storage [29]. Parlato et al. [30] concluded that the European Standard 1785 can bu successfully applied on chicken irradiated at 3 kGy, soon after irradiation (skin or muscle). After storage for 30 days, the identification is still achievable provided that investigation is carried out on the skin, whereas on samples without skin technique is applicable only at higher doses.

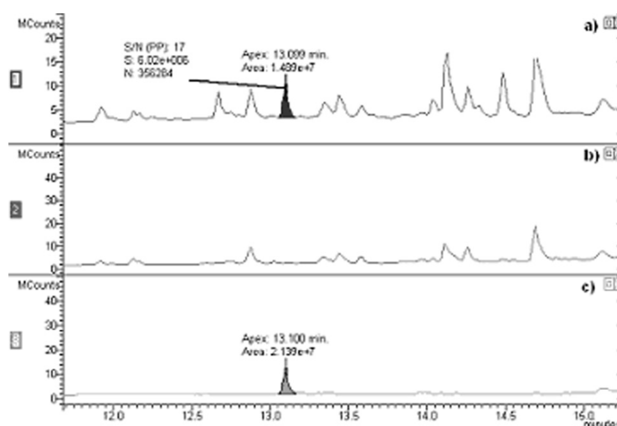

Figure 3. Immediately after irradiation 1.2 kGy irradiated and control sample chromatograms
a) 1.2 kGy irradiated sample; b) control; c) 0.5 ppm 2-DCB Standard

The yields of 2-alkylcyclobutanones formed by irradiation of foodstuffs do not seem to depend on the nature of the food matrix. But the amount of these products increases with irradiation dose [29, 31]. The analyses of 2-ACB's formed by radiolysis of triglycerides have in fact been validated on foodstuffs irradiated at doses usually higher than 0.5 kGy [31].

In this study, 2-DCB was not detectable for 0.4 kGy and 0.8 kGy irradiated hamburgers. This method has only been validated for the detection of irradiation treatment of eggs, chicken and pork meats, at doses higher or equal to 0.5 kGy [32]. Also, 2-DCB could be detected in exotic fruits [avocado, mango and papaya] irradiated at 0.1 kGy [33]. Ndiaye et al. [29] concluded that such an

objective will nonetheless require an increase in the sensitivity of this method by a factor of 10 to 20, for example by achieving a more specific extraction of the 2-ACB's from the food sample by performing a precoloumn purification of the extract to be analysed and/or by using a more sensitive detection device than the mass spectrometer.

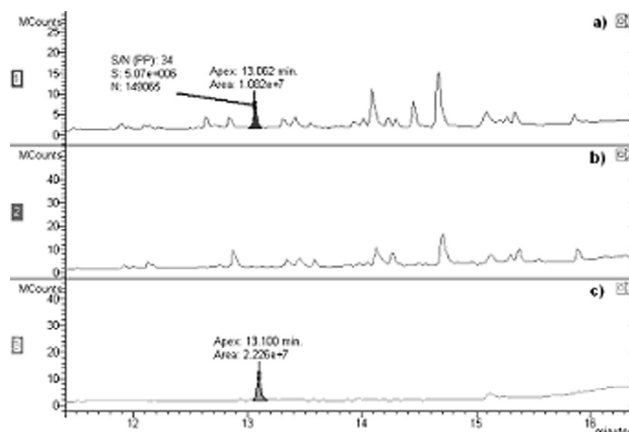

Figure 4. After 8 days of storage at +4°C 1.2 kGy irradiated and control sample chromatograms
a) 1.2 kGy irradiated sample; b) control; c) 0.5 ppm 2-DCB standard

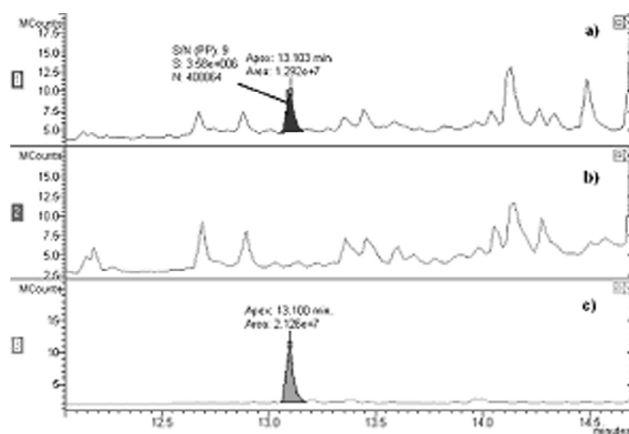

Figure 5. After 8 days of storage at -12°C 1.2 kGy irradiated and control sample chromatograms,
a) 1.2 kGy irradiated sample; b) control; c) 0.5 ppm 2-DCB standard

CONCLUSIONS

The DNA comet assay is a relatively simple, rapid screening and inexpensive method for a qualitative detection of irradiated foods. However, the test is restricted to foods not subjected heat or the other treatments, which also induce DNA fragmentation [19]. This technique may be used for a fressness indicator. It was possible to avoid the use of a costly image analyser as proposed by other authors [18, 34]. The method is not radiation specific and positive or suspected results may need to be confirmed by an officially validated method. As seen from the results of this study, the GC/MS technique can be successfully applied on irradiated hamburger samples (1.2 kGy) for detection of 2-ACB's. 2-ACB's are formed only during irradiation of hamburgers, these compounds provide a good way to confirm if hamburgers are irradiated or not. Unfortunately, most sample preparation, and isolation methods for these markers from their food matrices involve a long and very tedious clean-up regimen prior to analysis [13] such as Florisil coloumn choromotograpy. To reduce the extraction time and encourage selective extraction for 2-ACB's, supercritical fluid extraction [13,35], solid phase microextraction [26,36], silver ion chromatography [29] and accelarated solvent extraction [37] has been adopted analysis of 2-ACB's to detect irradiated foods.

ACKNOWLEDGMENTS

This work was supported by Turkish Atomic Energy Authority (TAEK). The authors gratefully thank the TUBITAK (Scientific and Technological Research Council of Turkey), National Scholarship Programme for PhD students.

REFERENCES

[1] Arvanitoyannis, I. S., 2010. Irradiation of Food Commodities, 1st Edition, Techniques, Applications, Detection, Legislation, Safety and Consumer Opinion, Academic Press.

[2] Delincée, H., 1998. Detection of food treated with ionizing radiation. *Trends in Food Science and Technology* 9: 73-82.

[3] Arvanitoyannis, I. S., Stratakos, A., Mente, E., 2009a. Impact of irradiation on fish and seafood shelf life: A comprehensive review of applications and irradiation detection. *Critical Reviews in Food Science and Nutrition* 49(1): 68-112.

[4] Arvanitoyannis, I. S., Stratakos, A., Tsarouhas, P., 2009b. Irradiation applications in vegetables and fruits. *Critical Reviews in Food Science and Nutrition* 49(5):427-462.

[5] Erel, Y., Yazici, N., Özvatan, S., Ercin, D., Cetinkaya. N., 2009. Detection of irradiated quail meat by using DNA comet assay and evaluation of comets by image analysis. *Radiation Physics and Chemistry* 78: 776-781.

[6] Marin-Huachaca, N., Delincee, H., Mancini-Filho, J., Villavicencio, A. L. C. H., 2005. Use of DNA comet assay to detect beef meat treated by ionizing radiation. *Meat Science* 71: 446-450.

[7] Barros, A. C., Freund, M. T. L., Villavicencio, A. L. C. H., Delincée, H., Arthur, V., 2002. Identification of irradiated wheat by germination test, DNA comet assay and electron spin resonance. *Radiation Physics and Chemistry* 63: 423-426.

[8] Aylangan, A., 2010. Effect of ionizing radiation on hamburgers' quality criteria, shelf life and detection of irradiated hamburgers, PhD thesis, Hacettepe University, Ankara.

[9] Delincée, H., 1996. Application of the DNA "comet assay" to detect irradiation treatment of foods. Edited by C. H. McMurray, E. M. Stewart, R. Gray, & J. Pearce, Detection methods for irradiated foods: Current status UK: The Royal Society and Chemistry, 349-352 p.

[10] Obana, H., Furuta, M., Tanaka, Y., 2006. Detection of 2-alkylcyclobutanones in irradiated meat, poultry and egg after cooking. *Journal of Health Science* 52(4): 375-382.

[11] Obana, H., Furuta, M., Tanaka, Y., 2007. Effects of temperature during irradiation on the production of 2-alkylcyclobutanones in beef. *Journal of Health Science* 53(2): 215-219.

[12] Gadgil, P., Smith, J. S., Hachmeister, K. A., Kropf, D. H., 2005. Evaluation of 2-dodecylcyclobutanone as an irradiation dose indicator in fresh irradiated ground beef. *Journal of Agricultural and Food Chemistry* 53: 1890-1893.

[13] Tewfik, I. H., Ismail, H. M., Sumar, S., 1998. A rapid supercritical fluid extraction method for the detection of 2-alkylcyclobutanones in gamma-irradiated beef and chicken. *Lebensmittel-Wissenschaft und-Technologie* 31: 366-370.

[14] EN 13784, 2001. Foodstuffs-DNA comet assay for the detection of irradiated foodstuffs–screening method. European Standard EN 13784. Brussels: European Committee for Standardization.

[15] EN 1785, 2003. Foodstuffs-Detection of irradiated food containing fat–Gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones. European Standard EN 1785. Brussels: European Committee for Standardization.

[16] Araújo, M. M., Marin-Huachaca, N. S., Mancini-Filho, J., Delincée, H., Villavicencio, A. L. C. H., 2004. Identification of irradiated refrigerated pork with the DNA comet assay. *Radiation Physics and Chemistry* 71: 183-185.

[17] Villavicencio, A. L. C. H., Araujo, M. M., Marin-Huachaca, N. S., Mancini-Filho, J., Delincee, H., 2004. Identification of irradiated refrigerated poultry with the DNA comet assay. *Radiation Physics and Chemistry* 71: 187-189.

[18] Duarte, R. C., Araújo, M. M., Salum, D. C., Marchioni, E., Villavicencio, A. L. C. H., 2009. Effects of the ionizing radiations, freezing and thawing duration on chicken liver cells quality. *Radiation Physics and Chemistry* 78: 631-634.

[19] Villavicencio, A. L. C. H., Mancini-Filho, J., Delincee, H., 2000. Application of a rapid screening method to detect irradiated meat in Brazil. *Radiation Physics and Chemistry* 57: 295-298.

[20] Khan, A. A., Khan, H. M., Delincee, H., 2002. Detection of radiation treatment of beans using DNA comet assay. *Radiation Physics and Chemistry* 63:407-410.

[21] Cerda, H., 1998. Detection of irradiated fresh chicken, pork and fish using the DNA comet assay. *Lebensmittel- Wissenschaft und-Technologie* 31: 89-92.

[22] Cerda, H., Delincée, H., Haine, H., Rupp, H., 1997. The DNA "comet assay" as a rapid screening technique to control irradiated food. *Mutation Research* 375: 167-181.

[23] Delincée, H., 2002. Rapid detection of irradiated frozen hamburgers. *Radiation Physics and Chemistry* 63: 443-446.

[24] Khan, H. M., Delincée, H., 1998. Detection of irradiation treatment of foods using DNA "comet assay". *Radiation Physics and Chemistry* 52(1): 141–144.

[25] D'Oca, M. C., Bartolotta, A., Cammilleri, M. C., Giuffrida, S. A., Parlato, A., Di Noto, A. M., Caracappa, S., 2009]. The gas chromatography/mass spectrometry can be used for dose estimation in irradiated pork. *Radiation Physics and Chemistry* 78: 687-689.

[26] Blanch, G. P., Caja, M. D. M., Flores, G., Castillo, M. L. R. D., 2009. Identification of 2-dodecylcyclobutanone and linear-alkanes as irradiation markers in sliced dry-cured ham. *Food Chemistry* 113: 616-620.

[27] Marchioni, E., Horvatovich, P., Ndiaye, B., Miesch, M., Hasselmann, C., 2002. Detection of low amount of irradiated ingredients in non-irradiated precooked meals. *Radiation Physics and Chemistry* 63: 447-450.

[28] Marchioni, E., 2006. Detection of irradiated foods. Edited by C. H. Sommers, & X. Fan, Food Irradiation Research and Technology, 1st edition, Blackwell Publishing and the Institute of Food Technologists, 85-103p.

[29] Kwon, J., Kausar, T., Noh, J., Kim, D., Byun, M., Kim, K., Kim, K., 2007. The identification of irradiated seasoned filefish [*Thamnaconus modestus*] by different analytical methods. *Radiation Physics and Chemistry* 79: 1833-1836.

[30] Parlato, A., Calderaro, E., Bartolotta, A., D'Oca, M. C., Giuffrida, S. A., Brai, M., Tranchina, L., Agozzino, P., Avellone, G., Ferrugia, M., Di Noto, A. M., Caracappa, S., 2007. Gas chromatographic/mass spectrometric and microbiological analyses on irradiated chicken. *Radiation Physics and Chemistry* 76: 1463-1465.

[31] Ndiaye, B., Jamet, G., Miesch, M., Hasselmann, C., Marchioni, E., 1999. 2-Alkycyclobutanones as markers for irradiated foodstuffs II. The CEN [European Committee for Standardization] method: Field of application and limit of utilization. *Radiation Physics and Chemistry* 55: 437-445.

[32] Stevenson, M. H., 1996. Validation of the cyclobutanone protocol for detection of irradiated lipid containing foods by interlaboratory trials. In: Detection Methods for Irradiated Foods. Current Status. The Royal Society of Chemistry, Cambridge

[33] Stewart, E. M., Moore, S., McRoberts, W. C., Graham, W. D., Hamilton, J. T. G., 1998. 2-Alkylcyclobutanones as markers for exotic fruits. *Food Science and Technology Today* 12, 103.

[34] Faullimel, C., Ennahar, S., Aoude-Werner, D., Guterl, P., Marchioni, E., 2005. DNA comet assay for the detection of time-temperature abuse during the storage of poultry. *Journal of Food Protection* 68(7): 1414-1420.

[35] Gadgil, P., 2006. Evaluation of toxicity, mutagenicity, metabolism and formation of 2-dodecylcyclobutanone in irradiated ground beef, PhD thesis, Kansas State University, Food Science Gradiate Program College of Agriculture, Manhattan, Kansas.

[36] Caja, M. M., Ruiz, M. L. D. C., Blanch, G. P., 2008. Solid phase microextraction as a methodology in the detection of irradiated markers in ground beef. *Food Chemistry* 110:531-537.

[37] Obana, H., Furuta, M., Tanaka, Y., 2005. Analysis of 2-alkylcyclobutanones with accelerated solvent extraction to detect irradiated meat and fish. *Journal of Agricultural and Food Che*mistry 53: 6603-6608.