# Development and validation of an analytical testing method to evaluate the irradiation history of dried chicken meat

by

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B.S., Urmia University, 2008
M.S., University of Agriculture and Natural Resources of Gorgan, 2012

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Food Science

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# Abstract

There have been health concerns regarding the consumption of irradiated chicken jerky treats (CJT), and there is a lack of an established method to quantify the irradiation dose within the legal limits. Radiolytic lipid products can be used to back-trace the irradiation history of foods. The formation kinetics of 2-dodecylcyclobutanone (2-DCB) and hydrocarbons were studied in gamma-irradiated (0 to 50 kGy) tripalmitin, chicken fat, and chicken jerky treats (CJT) to assist in method development for dose prediction.

Among different methods to extract the food irradiation markers, solid phase micro extraction (SPME) could provide a faster and simpler method to estimate the irradiation history of fat containing food products. The SPME technique was optimized to extract 2-DCB and hydrocarbons from irradiated CJTs. The analytes were identified and quantified using Gas Chromatography–Mass Spectrometry (GC-MS) in scan and selected ion modes (SIM).

Thirty-nine different hydrocarbons, aldehydes, and ketones were identified, among which 33 increased with irradiation dose. Formation of such radiolytic products occurred along with reduction of all major fatty acids of chicken fat up to 20 kGy. Pentadecane, 1-pentadecene, 1-tetradecene, and 8-heptadecene, which are likely derived from palmitic and oleic acid, were shown the highest concentration and linear regression (Slope of 658531, $R2 = 0.998$).

Water dilution (1:5) was needed to mobilize 2-DCB and allow partition to the headspace form the CJT matrix. Increasing the incubation temperature up to 80 °C resulted in higher response. Spiking control jerky samples with 2-DCB from 10 to 150 ppb compared with spiking water revealed a significant effect that the food matrix has in lowering the signal obtained from 2-DCB. The concentration of 2-DCB increased linearly (10.8 ppb/kGy, $R2 = 0.999$) from 0 to 543 ng/g CJT with irradiation from 0 to 50 kGy. Addition of glycerol to the formulation of

chicken jerkies did not significantly affect the formation or measurement of 2-DCB. Pentadecane is the major hydrocarbon in irradiated fat and could be used for dose measurement of fat containing irradiated samples showing the highest concentration among hydrocarbons and a linear regression of 0.95.

SPME-coupled GC-MS analysis can serve as a suitable technique to quantify 2-DCB and hydrocarbons concentrations in CJT as irradiation markers. A limit of detection of 50 ppt, and a limit of quantitation of 4 ppb was determined for 2-DCB using the optimized parameters of the method. This method provides a fast, simple, and environmentally friendly alternative for the existing solvent extraction methods for analyzing irradiation markers.

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# Abstract

There have been health concerns regarding the consumption of irradiated chicken jerky treats (CJT), and there is a lack of an established method to quantify the irradiation dose to ensure the legal limits. Radiolytic lipid products can be used to back-trace the irradiation history of foods. The formation kinetics of 2-dodecylcyclobutanone (2-DCB) and hydrocarbons were studied in gamma-irradiated (0 to 50 kGy) tripalmitin, chicken fat, and chicken jerky treats (CJT) to assist in method development for dose prediction.

Among different methods to extract lipid-based irradiation markers, solid phase micro extraction (SPME) could provide a faster and simpler method to estimate the irradiation history of fat containing food products. The SPME technique was optimized in terms of fiber stationary phase, extraction solvent, time, and temperature to extract 2-DCB and hydrocarbons from irradiated CJTs. The analytes were identified and quantified using Gas Chromatography–Mass Spectrometry (GC-MS) in scan and selected ion modes (SIM).

Thirty-nine different hydrocarbons, aldehydes, and ketones were identified, among which 33 increased with irradiation dose. Formation of such radiolytic products occurred along with reduction of all major fatty acids of chicken fat up to 20 kGy. Pentadecane, 1-pentadecene, 1-tetradecene, and 8-heptadecene, which are likely derived from palmitic and oleic acid, were shown the highest concentration and linear regression (formation kinetic of 658531 peak area/kGy, $R^2 = 0.998$).

Water dilution (1:5) was needed to mobilize 2-DCB and allow partition to the headspace form the CJT matrix. Increasing the incubation temperature up to 80 °C resulted in higher response. Spiking control jerky samples with 2-DCB from 10 to 150 ppb compared with spiking water revealed a significant effect that the food matrix has in lowering the signal obtained from

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SPME-coupled GC-MS analysis can serve as a suitable technique to quantify 2-DCB and hydrocarbons concentrations in CJT as irradiation markers. A limit of detection of 50 ppt, and a limit of quantitation of 4 ppb was determined for 2-DCB using the optimized parameters of the method. This method provides a fast, simple, and environmentally friendly alternative for the existing solvent extraction methods for analyzing irradiation markers.

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# Dedication

I dedicate this dissertation to my parents Nasrollah and Hava Taghvaei who always supported me and made my journey to here possible.

# Chapter 1 : Introduction

## Food irradiation history

The idea of using ionizing radiation to extend the shelf life of food was initially introduced in late 1800s, but it was not until 1890s that systems to apply irradiation to food was developed (Molins, 2001). In 1940s to 1970s an extensive research around the development of this new technology and the wholesomeness of irradiated food was done by researches (Molins, 2001). Since then, the majority of efforts regarding irradiation technology revolved around regulating its application. Opposing environmental groups in one hand, and toxicological concerns about irradiated foods on the other hand, required monitoring agencies to regulate the use of irradiation in food products. Perhaps the most important regulatory aspects of food irradiation was established by joint FAO/IAEA/WHO Expert Committees (JECFI) in 1980 when it was declared "irradiation of any food commodity up to an overall average dose of 10 kGy causes no toxicological hazard; hence, toxicological testing of food so treated is no longer required" (Molins, 2001). After that, a variety of food products (mostly meet and poultry) were approved by FDA for irradiation up to certain levels (Table 1.1). The formation rate and fate of free radicals and compounds produced during irradiation could vary in foods with different composition and moisture content; hence, different legal limits were imposed to irradiation of different food products (table 1.1)

**Table 1.1 The current FDA limitations regarding irradiation in the production, processing and handling of food for various purposes and products (Code of Federal Regulations, 2018).**

| Use | Limitations |
|---|---|
| For control of Trichinella spiralis in pork carcasses or fresh, non-heat-processed cuts of pork carcasses | Minimum dose 0.3 kiloGray (kGy) (30 kilorad (krad)); maximum dose not to exceed 1 kGy (100 krad) |
| For growth and maturation inhibition of fresh foods | Not to exceed 1 kGy (100 krad) |
| For disinfestation of arthropod pests in food | Do |
| For microbial disinfection of dry or dehydrated enzyme preparations (including immobilized enzymes) | Do not to exceed 10 kGy (1 megarad (Mrad)) |
| For microbial disinfection of the following dry or dehydrated aromatic vegetable substances when used as ingredients in small amounts solely for flavoring or aroma: culinary herbs, seeds, spices, vegetable seasonings that are used to impart flavor but that are not either represented as, or appear to be, a vegetable that is eaten for its own sake, and blends of these aromatic vegetable substances. Turmeric and paprika may also be irradiated when they are to be used as color additives. The blends may contain sodium chloride and minor amounts of dry food ingredients ordinarily used in such blends | Not to exceed 30 kGy (3 Mrad) |
| For control of food-borne pathogens in fresh (refrigerated or unrefrigerated) or frozen, uncooked poultry products that are: (1) Whole carcasses or disjointed portions (or other parts) of such carcasses that are "ready-to-cook poultry" within the meaning of 9 CFR 381.l(b) (with or without nonfluid seasoning; includes, e.g., ground poultry), or (2) mechanically separated poultry product (a finely comminuted ingredient produced by the mechanical deboning of poultry carcasses or parts of carcasses) | Not to exceed 4.5 kGy for non-frozen products; not to exceed 7.0 kGy for frozen products |
| For the sterilization of frozen, packaged meats used solely in the National Aeronautics and Space Administration space flight programs | Minimum dose 44 kGy (4.4 Mrad). Packaging materials used need not comply with 179.25(c) provided that their use is otherwise permitted by applicable |

| | |
|---|---|
| | regulations in parts 174 through 186 of this chapter |
| For control of foodborne pathogens in, and extension of the shelf-life of, refrigerated or frozen, uncooked products that are meat within the meaning of 9 CFR 301.2(rr), meat byproducts within the meaning of 9 CFR 301.2(tt), or meat food products within the meaning of 9 CFR 301.2(uu), with or without nonfluid seasoning, that are otherwise composed solely of intact or ground meat, meat byproducts, or both meat and meat byproducts | Not to exceed 4.5 kGy maximum for refrigerated products; not to exceed 7.0 kGy maximum for frozen products |
| For control of Salmonella in fresh shell eggs | Not to exceed 3.0 kGy |
| For control of microbial pathogens on seeds for sprouting | Not to exceed 8.0 kGy |
| For the control of Vibrio bacteria and other foodborne microorganisms in or on fresh or frozen molluscan shellfish | Not to exceed 5.5 kGy |
| For control of food-borne pathogens and extension of shelf-life in fresh iceberg lettuce and fresh spinach | Not to exceed 4.0 kGy |
| For control of foodborne pathogens, and extension of shelf-life, in unrefrigerated (as well as refrigerated) uncooked meat, meat byproducts, and certain meat food products | Not to exceed 4.5 kGy |
| For control of food-borne pathogens in, and extension of the shelf-life of, chilled or frozen raw, cooked, or partially cooked crustaceans or dried crustaceans (water activity less than 0.85), with or without spices, minerals, inorganic salts, citrates, citric acid, and/or calcium disodium EDTA | Not to exceed 6.0 kGy |

## Irradiation source

Gamma and X-rays, as well as machine generated electron beams are able to knock the electrons in food components out of their orbits resulting a compound that is not electrically balanced. This is the reason this category of radiation is called ionizing radiation (irradiation). The most common source of irradiation is cobalt 60, which is synthesized from natural cobalt 59.

The cobalt 60 will produce a controlled emission of γ-rays during transformation down to the stable state of nickel 60. The γ-irradiation could also be achieved from cesium 137. This is an isotope of cesium produced from reprocessing of used nuclear reactor rods. The use of nuclear reactor waste for food irradiation brought many negative attention which limited the use of cesium 137 as an irradiation source. A newer source of γ-irradiation is electron accelerators that could produce a high voltage electron beams. This will ionize compounds by taking away electrons from their valence shell. The advantage of this technology is that it could easily be switched on and off without the need for nuclear energy byproducts. This leads to higher public acceptability compared with other irradiation sources. One big disadvantage of electron beams is that their penetration depth into food is not as much as gamma and X-rays (Stain, 1996).

Currently, four irradiation sources are approved by FDA to be used for food: 1) Gamma rays from sealed units of the radionuclides cobalt-60 or cesium-137. 2) Electrons generated from machine sources at energies not to exceed 10 million electron volts. 3) X rays generated from machine sources at energies not to exceed 5 million electron volts MeV. 4) X rays generated from machine sources using tantalum or gold as the target material and using energies not to exceed 7.5 MeV (Code of Federal Regulations, 2018).

## Irradiation chemistry

The structure of fatty acids or triglycerides breaks down to a number of different free radical fragments under irradiation depending on the position of the cleavage (figure 1.1). Such fragments could accept hydrogen radicals, which results in formation of a stable hydrocarbon, aldehyde, ketone, mono and di-glyceride (LeTellier & Nawar, 1972). The cleavage of the C-O bond (position a) results in formation of a ketone, which could later change to a more stable

aldehyde. The cleavage of the C-C bond α to the carbonyl group (position b) will result in formation of a hydrocarbon containing one carbon less than the original fatty acid. The cleavage of the C-C bond β to the carbonyl group (position c) will result in formation of a hydrocarbon containing two carbons less than the original fatty acid (Marchioni, 2013).

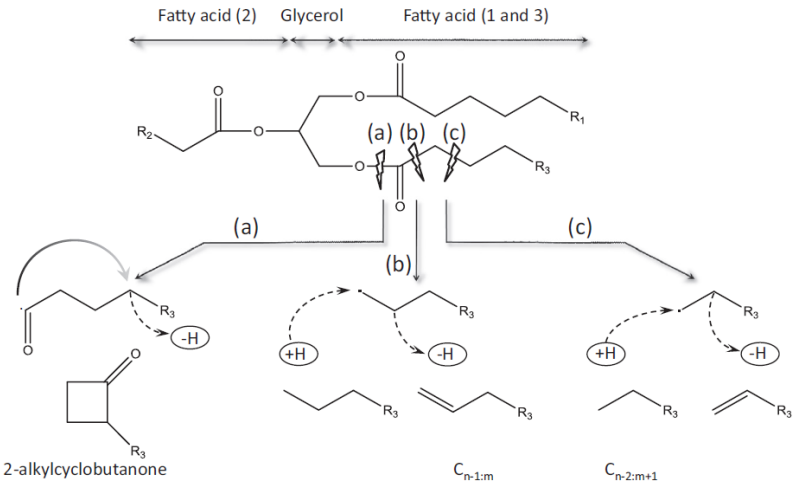


**Figure 1.1 Radiolysis of a triglyceride (taken from Marchioni, 2013)**

Among all possible compounds, two major irradiation products of fatty acids are 2-acylcyclobutanones (2-ACBs) and hydrocarbons. Ionizing waves could remove an electron from the fatty acid and the positive charge produced in this way will transfer toward the head of molecule (ester link). This could result in different pathways. If the electron removed from oxygen bonding carbon is not compensated by oxygen, carbon will lose the electron shared with neighboring carbon and c-c bond will break. The result of this cleavage is a free radical of the hydrocarbon chain having one carbon less (oxygen bonding carbon) than the original fatty acid (figure 1.2). This radical is stabilized by gaining a hydrogen radical (resulting an alkane), or

losing a hydrogen radical (resulting an alkene). The second pathway occurs when the electron removed from oxygen bonding carbon is compensated by oxygen. The oxygen that lacks an electron could gain a hydrogen from carbon number 4 to form an stable ring. This is an unstable intermediate, that is stabilized by formation of oxygen-carbon double bond with transferring hydrogen to the oxygen attached to the glycerol backbone. This results in formation of 2-ACBs (Fan, 2013a; Stewart, 2001).

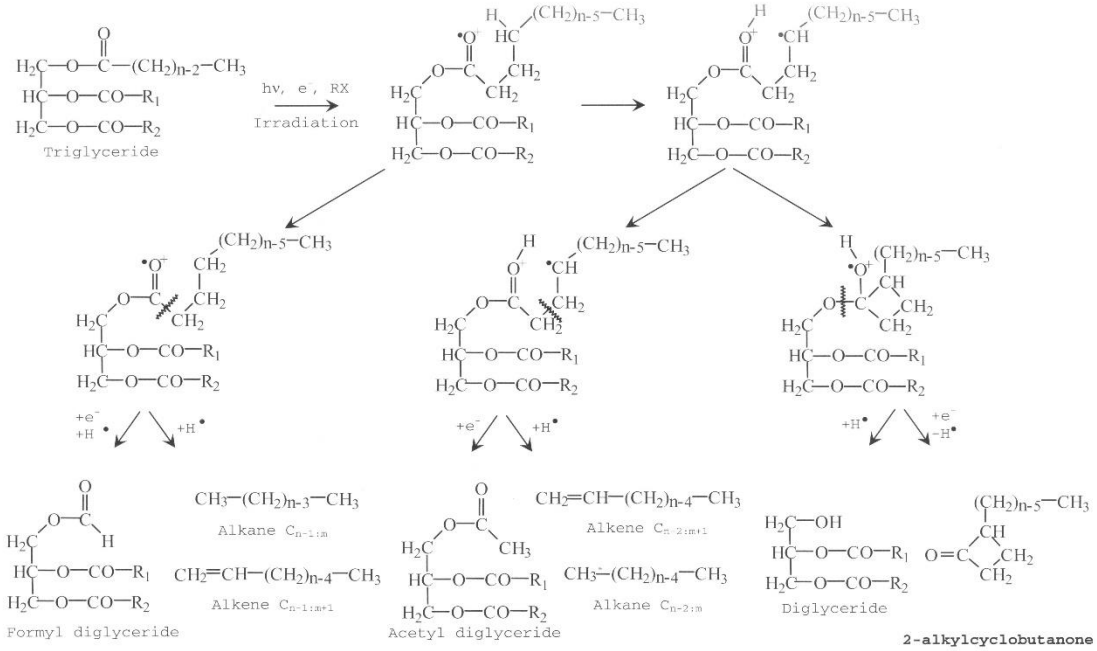


**Figure 1.2 Possible reaction pathways of triglycerides under gamma irradiation (taken from Fan, X., 2013a)**

2-dodecylcyclobutanone (2-DCB), is one of the 2-ACBs that is suggested for the detection of irradiated foods containing fat (European committee for standardization, 2003). This compound is formed through cyclisation of palmitic acid and formation of a cyclobutanone ring that is attached to a long hydrocarbon chain (Fan, 2013a; Stewart, 2001). Since this reaction is only induced by ionizing irradiation, 2-DCB is only formed in irradiated food with a

concentration directly related to irradiation dosage that is used during the process (Gadgil, Hachmeister, Smith, & Kropf, 2002; Hijaz, & Smith, 2010). No other food processing technique, including cooking, drying, pasteurization and sterilization, slicing, smoking, and curing, will lead to formation of 2-DCB (Fernades, Pereira, Antonio, & Ferreira, 2018). Although Variyar, Chatterjee, Sajilata, Singhal, and Sharma (2008) showed the natural occurrence of 2-DCB in non-irradiated cashew nut and nutmeg samples, their study has not been confirmed by another independent study and further investigation of the Joint Research Centre (JRC) of the European Commission did not approve the natural existence of 2-DCB in cashew nut and nutmeg (Breidbach, & Ulberth, 2015).

Another group of compounds that are produced during irradiation are hydrocarbons derived from fatty acids. Nawar and Balboni (1970) showed that irradiation of pork produces mainly six hydrocarbons of pentadecane, 1-tetradecene, heptadecane, 1-hexadecene, 8-heptadecene, and 1,8-hexadecadiene. These hydrocarbons are derived from palmitic, stearic, and oleic acid, which are the main fatty acids found in pork fat. The concentration of such hydrocarbons proved to increase at higher irradiation doses, while they are absent or found in very small quantity in non-irradiated foods (Nawar, & Balboni, 1970). Their occurrence is proved to be independent of the presence of air, moisture, and mild heating during irradiation; however, presence of a specific free fatty acid would highly increase the production of resulting hydrocarbons from that fatty acid (Dubravcic, & Nawar, 1976). Dubravcic and Nawar (1976) showed that the amount of tridecane produced from myristic acid (free fatty acid) was 34 times higher than that from trimyristin (triglyceride).

Many other similar compounds such as aldehydes and shorter chain saturated and unsaturated hydrocarbons could also be produced in lower quantity during irradiation of animal

fat (Champagne, & Nawar, 1969). An aldehyde with the same carbon number as the original fatty acid was shown to be the most predominant in this category of compounds (Dubravcic, & Nawar, 1976).

Another common reaction in lipids is oxidation, which could happen automatically in presence of oxygen, or be catalyzed by light and oxidants such as heavy metals. Oxidation and irradiation both induce the formation of free radicals but differ some of the final products. The autooxidation reaction, which is the most common in lipid containing foods, starts with formation of free radicals of the carbon near the double bond of fatty acids resulting an unstable proxy intermediate. This intermediate proxy accepts a hydrogen to form a more stable hydroperoxide (autooxidation primary product). The hydroperoxide formed on the carbon near the double bond changes into various aldehydes, ketones, alcohols,… (autooxidation secondary product). Hence, the products of autooxidation from fatty acids are shorter chain aldehydes, ketones, and alcohols, rather than long chain hydrocarbons. Irradiation involves formation of free radicals and breaking the carbon-carbon single bond near the ester group and liberating a long hydrocarbon chain without oxygen, or the rearrangement of the ester bond and formation of cyclobutanone ring, which is unique to irradiation (Stewart, 2001). As both reactions involve formation of free radicals, irradiation in presence of oxygen facilitate the oxidation reaction. In presence of oxygen, radiolytic and autoxidation reactions happens in parallel; however, autoxidation does not occur in saturated fatty acids. This could be one of the reasons that irradiation products from saturated fatty acids are unique to irradiation and could be used as a marker.

## Toxicological concerns

Toxicological studies are performed in four main steps. The first step is the hazard identification. This is the process of defining the scope and potential of hazard at different doses, and whether or not the hazard leads to an adverse effect. The second step is dose-response assessment, which is the process of measuring the lowest concentration at which a compound shows an adverse effect and determining the acceptable daily intake for human. The third step is exposure assessment, which is the process of assessing the ways in the environment that we could be exposed to a chemical, as well as the ways that a chemical could transferred into our body. The exposure dose, which is the concentration of the chemical entered the body or ingested, should be differentiated with the internal dose, which is the concentration of the same chemical in the target site to have a more realistic risk assessment. And the final step is risk characterization, which is the final evaluation of the hazard with considering the hazard list, dose response, and actual and expected exposure, followed by calculation of probability of adverse effect (EPA, 2019). Based on the toxicological studies that are done on irradiated foods, concerns were categorized as follow (Sommers, Delincee, Smith, & Marchioni, 2013):

a) Benzene: benzene and toluene are known to be the decomposition products of phenyl alanine, that are also found in cooked foods; however, irradiated foods show somewhat higher concentration of such compounds. The amount of such compounds in irradiated foods are still low enough that does not raise any significant health concern.

b) Formaldehyde: Formaldehydes also formed from irradiation of carbohydrates. Unless the food has low protein or high amount of water, these compounds are not found, due to their high affinity to bond with proteins.

c) Amines: Irradiation is suggested to lower the amount of nitrosamine in food, although the dosage required for significant impact (10 kGy or higher) is higher than the dosage used in food industry.

d) 2-ACBs: 2-ACBs are the product of irradiation of lipids, which does not occur in non-irradiated foods. If all the meat and poultry consumed by human were to be irradiated, an intake value of 40 µg 2-ACBs/person/day was suggested. This low amount of intake is comparable with the current consumption of acrylamide from fried foods; however, there has not been much toxicological studies for 2-ACBs as it was for acrylamide. At the current time, studies regarding the toxicity of 2-ACBs are controversial. Some studies failed to show any mutagenic effect in bacteria or cytotoxicity in human cells, the others show cytotoxicity of some 2-ACBs at concentrations higher than 100 µM and tumor promotion in rats.

The main concern about the safety of irradiated foods is targeted toward 2-ACBs, however, after the evaluation of the safety concerns regarding irradiated food, the FDA, CDC, and USDA approved and endorsed the safety of food irradiation (Code of Federal Regulations, 2018). The high dosage used and validity of experiments that showed the adverse effects of 2-ACBs are the reason for not considering 2-ACBs as a health concern by regulatory agencies at the current time.

The most extensive study on the safety of irradiated food was done by the World Health Organization in 1994, reviewing hundreds of short term and long term studies in more than 60 countries, that led to approval of one or more foods for irradiation. It was concluded that “when requirements for good manufacturing practice are implemented, food irradiation is safe and effective” The studies reviewed failed to show connection between any deleterious effect and the consumption of irradiated food (World Health Organization, 1994). However, it was not before

1990 that 2-ACBs are detected in a irradiated food sample, and the controversy over the safety of this group of compounds started at 1998 when a concentration of 0.3 to 1.25 mg/ml of 2-DCB showed genotoxicity using in-vitro Comet assay (Delincée, & Pool-Zobel, 1998).

A more recent comprehensive study on toxicity of 2-DCB and other 2-ACBs was done by Health-Canada at March 2003. The experiments reviewed in this study showing controversial results, but the evaluation committee voted for safety of irradiated foods by questioning the validity of methods used in studies showing 2-ACBs adverse effects. Also the dosage used in many studies are much higher than exposure dose for Canadian population based on consumption of irradiated beef and poultry. The committee concluded that "as the adverse effects noted refer almost entirely to in vitro studies, it is not appropriate, on the basis of these results, to make a risk assessment for human health associated with the consumption of 2-ACBs present in irradiated fat-containing foods" (Government of Canada, 2003)

Another study for the safety of irradiated food was carried out by a joint FAO/IAEA/WHO study group in 1999. This study concerned both health effects and nutritional aspects of irradiated food by reviewing also studies regarding the nutritional loss during irradiation. The group concluded that “food irradiated to any dose appropriate to achieve the intended technical objective was both safe to consume and nutritionally adequate” (World Health Organization, 1999).

## Nutritional concerns

The nutritional effects of irradiation could be studied in two separate areas of food macronutrients and food micronutrients. There has been no significant effect of irradiation on food macronutrients’ digestibility, bioavailability, metabolism, energy and nitrogen balance.

However, studies show different amounts of reduction in some micronutrients depending on the composition of food and the dosage used.

Irradiation induced oxidation changes ascorbic acid (vitamin C) to dehydroascorbic acid. However, it is important to note that dehydroascorbic acid could be converted back to vitamin C in presence of a reducing agent and it also has vitamin C activity. Reduction in lutein, violaxanthin, and beta-caroten was also observed. Vitamin E and thiamin (B1) are also among other vitamins sensitive to irradiation. However, it is important to note that the reduction of vitamins occurs in most thermal processes of foods. In this regard, irradiated foods are equivalent or superior to thermally sterilized foods (Fan, 2013b).

Irradiation maintained or increased (during storage after irradiation) the amount of total phenols, carotenoids, and flavonoids of fruits and vegetables. As fruits and vegetables are living tissues even after harvest, the oxidative stress caused by irradiation induces higher production of phenolics and antioxidants during the storage after irradiation (Fan, 2013b).

In conclusion, the only nutritional consideration for a diet of irradiated foods would be thiamin supplementation, if all the foods containing this vitamin are irradiated at high doses. In order to minimize the nutrition loss during the irradiation, it is recommended that foods are irradiated frozen and under vacuum. This minimizes the irradiation induced oxidation and effect of water's radiolytic products in decomposition of nutrients.

## Chicken jerky treats problem

Chicken jerky pet treats are one of the products that have been commonly treated with irradiation for disinfection and pathogen destruction. However, over the last decade or so there have been concerns for the safety of irradiated pet food products due to numerous cases of illness

and death in dogs and cats associated with the consumption of chicken jerky treats (CJT) in USA and dry cat food in Australia, respectively (FDA, 2019; Burke, 2009). Since 2007, FDA received about 3600 consumers' report about jerky treat related illnesses, while about 580 of those incidences resulted in pet's death (U. S. Food and Drug, 2013). Most illnesses involved decreased appetite, decreased activity, vomiting, diarrhea (sometimes with blood or mucus), increased water consumption, and/or increased urination. In severe cases, kidney failure, gastrointestinal bleeding, and a rare kidney disorder was observed (U. S. Food and Drug, 2013). Despite detailed biological and chemical analyses of the affected animals and related products by official and private institutions, the causation is still unclear. An ongoing investigation in the areas of microbiological testing, compositional testing, chemical toxicology testing, evaluation of jerky treat irradiation, formaldehyde testing, radioactivity testing, and viral testing is being carried out by FDA (U. S. Food and Drug Administration, 2018). The evaluation of irradiation history of the jerkies received by FDA from cases that caused pets ill or collected from the market is still pending on development and validation of a testing method. The current available standard method for detection of 2-DCB in foodstuff (European committee for standardization, 2003) only shows the presence/absence of irradiation treatment and does not measure the irradiation dose used for the product. This method also consists of timely Soxhlet extraction of fat with hexane and several steps of cleanup and drying. There is, therefore, a large need for a suitable method for quantification of the irradiation dose in pet food products, such as CJT, after treatment. Such method should be universal, precise, accurate, fast and easy. A suitable method should also be able to measure compounds that are specific to irradiation, are formed more at higher irradiation doses, and are stable throughout the shelf life of the product. Amongst these, the specificity, stability and formation range of the analyte being monitored is utmost importance

that determine the reliability of the analytical method. In low-moisture food matrixes, irradiation-specific lipid decomposition products such as hydrocarbons and alkylcyclobutanones (2-ACBs) are shown a linear dose response (Gadgil, Hachmeister, Smith, & Kropf, 2002; Hijaz, & Smith, 2010). The objective of this study was to develop a user-friendly, reliable and sensitive method based on irradiation-specific lipid decomposition products for quantification of irradiation dose in CJTs. The results of this study can serve as guidelines for regulatory agencies, such as Food and Drug Administration (FDA), for surveillance of the irradiated pet food products, including CJT.

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# Chapter 2 : Objectives

Among several compounds that are produced by irradiation, some are either do not exist in non-irradiated foods, or exist at very low level. Such compounds also show an increasing concentration at higher irradiation doses and could be used as irradiation chemical markers. Most of the compounds that are suggested to be used as an irradiation marker in fat containing food products, including CJT, are derived from lipids. Hence, the preliminary step of this study was to investigate the effect of irradiation in food lipid model systems. All the volatile compounds that are produced during the irradiation of lipids needed to be identified and their formation kinetic with the irradiation needed to be measured in ordered to select a suitable irradiation marker. Because our ultimate goal was to analyze the irradiation history of CJTs, we used chicken fat and tripalmitin as model lipid systems. Using tripalmitin also helped us to study the effect of fatty acid diversity on the formation kinetics. By identifying all the volatile compounds produced during irradiation of lipids and their dose response, we aimed to select the compounds with the highest formation rate, concentration, stability, and lowest variability.

After selection of the best candidates to be used as irradiation marker, the next step was to develop a method that could extract and detect such compounds in irradiated CJTs. Since the concentration of irradiation markers are directly used to predict the irradiation history of CJTs, such a method should be able to measure the concentration of markers accurately with lowest variability. Our objective in this step was to study the effect of different variables of the method, such as solvent, incubation temperature, SPME fiber type, etc, on the peak area, linear regression, and variability of analyte concentration. This helped us to develop the most accurate and precise method. Another purpose of this study was to study the effect of CJT additive,

glycerol, on the SPME of CJT to develop a method that could work for both CJT with and without glycerol the same.

The application of different internal standards was also studied to correct for various experimental errors during and after extraction. Our objective was to select an internal standard with physical-chemical characteristics closest to the analytes that could result in the lowest variability, and to develop an analytical approach using multiple points internal standard. In this study we also wanted to investigate the formation of irradiation markers in CJT treated with glycerol by irradiating CJT samples with and without glycerol separately. Our purpose was to ensure the accurate dose prediction in all CJT samples, including those treated with glycerol.

After selecting the best irradiation markers and developing an analytical testing method for those compounds, our final goal was to detect irradiation and predict the irradiation dose that was used to treat CJTs.

# Chapter 3 : Analysis of Radiolytic Lipid Products in Model Lipid Systems from Gamma Irradiation[1,2]


## Abstract

Radiolytic lipid products can be used to back-trace the irradiation history of foods. The formation kinetics of 2-dodecylcyclobutanone (2-DCB) and hydrocarbons were studied in gamma-irradiated (0 to 50 kGy) tripalmitin and chicken fat to assist in method development for dose prediction. A solid phase micro extraction (SPME) technique was used to extract volatile compounds. The analytes were identified and quantified using Gas Chromatography–Mass Spectrometry (GC-MS). The analysis of 2-DCB showed good correlation with increasing irradiation dose (R2 = 0.999). Thirty-nine different hydrocarbons, aldehydes, and ketones were identified, among which 33 increased with irradiation dose. Formation of such radiolytic products occurred along with reduction of all major fatty acids of chicken fat up to 20 kGy. Pentadecane, 1-pentadecene, 1-tetradecene, and 8-heptadecene, which are likely derived from palmitic and oleic acid, were shown the highest concentration and linear regression (Slope of 658531, R2 = 0.998). Pentadecane is the major hydrocarbon in irradiated fat and could be used for dose measurement of fat containing irradiated samples.


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1. Abstract presented in part at IFT Annual Meeting 2019
2. Taghvaei, M., Sommers, C., Ceric, O., Linghu, Z., Smith, J. S., Yucel, U. (2019) Analysis of Radiolytic Lipid Products in Model Lipid Systems from Gamma Irradiation. Manuscript Submitted.

# Introduction

Ionizing radiation, commonly known as irradiation, is a non-thermal process used for sterilization, disinfection, decontamination, shelf-life extension and pathogen destruction in human and pet food products (Smith & Pillai, 2004). The irradiation process is approved to be safe below the threshold doses determined by Food and Drug Administration (FDA) in U.S. (FDA, 2015). Over the last decade or so there have been concerns related to safety of irradiated pet food products (FDA, 2019). In Australia, illness and death in cats were suspected to be associated with the consumption of dry cat food which had been subject to gamma irradiation upon entry to Australia, at levels greater than or equal to 50 kilo-Grays (kGy), (Burke, 2009). Jerky pet treats fed to dogs in the United States are commonly irradiated by producers to reduce pathogen load. Improper irradiation of these products, with the creation of toxic byproducts, was one hypothesis put forward to explain some of the illnesses associated with jerky treats reported to the FDA. Needed still are methods that can identify whether products are irradiated within or above the legal limits. One of the ways to measure the irradiation history of samples is to measure compounds that are induced by irradiation and have a linear dose response with irradiation. For this reason, a better understanding of the changes occurring to food components and formation of irradiation markers during the irradiation process is needed. Up to this point, all the suggested compounds by the European Committee for Standardization (CEN) to be used as an irradiation marker are derived from food lipids (Fernades, Pereira, Antonio, & Ferreira, 2018). The triglycerides and fatty acids break down to a number of different free radical fragments under irradiation, which can be used as irradiation markers. Such fragments could accept hydrogen radicals, which results in formation of a stable hydrocarbon, aldehyde, ketone, mono and di-glyceride (LeTellier & Nawar, 1972).

CEN has suggested 2-dodecylcyclobutanone (2-DCB) as one of the compounds to detect irradiated foods containing fat (European committee for standardization, 2003). This compound is formed through cyclisation of fatty acid with cleavage of ester bond, followed by a formation of a cyclobutanone ring that is attached to the hydrocarbon chain (Fan, 2013; Stewart, 2001). Since this reaction is only induced by ionizing irradiation, 2-DCB is only formed in irradiated food with a concentration directly related to irradiation dosage that is used during the process (Gadgil, Hachmeister, Smith, & Kropf, 2002; Hijaz, & Smith, 2010).

Another group of compounds that are produced during irradiation are hydrocarbons which are derived from fatty acids. Nawar and Balboni (1970) showed that irradiation of pork produces mainly six hydrocarbons: pentadecane, 1-tetradecene, heptadecane, 1-hexadecene, 8-heptadecene, and 1,8-hexadecadiene. These hydrocarbons are derived from palmitic, stearic, and oleic acid, which are the main fatty acids found in pork fat. The concentration of such hydrocarbons proved to increase at higher irradiation doses, while they are absent or found in very small quantity in non-irradiated foods (Nawar, & Balboni, 1970).

Many other similar compounds such as aldehydes and shorter chain saturated and unsaturated hydrocarbons could also be produced in lower quantity during irradiation of animal fat (Champagne, & Nawar, 1969). An aldehyde with the same carbon number as the original fatty acid was shown to be the most predominant in this category of compounds (Dubravcic, & Nawar, 1976). Separation and detection of such compounds at low concentration in different food matrixes has been a challenging task for food scientists.

SPME coupled with GC-MS is an effective technique to detect irradiation markers at low concentration. The application of SPME technique to detect irradiation induced hydrocarbons was previously examined in ground beef (Hwang et al., 2014), chilled beef (Li, Ha, Wang, & Li,

2010), and beef patties (Panseri et al., 2015). Thomazini, Contreras, and Miyagusku (2006) also studied the effect of irradiation dose up to 7 kGy on raw chicken thigh with SPME and identified 6 volatile compounds (mostly aldehydes). The analysis of off flavor compounds (volatiles) from ready-to-eat chicken breast was also successfully performed by Yun et al. (2012) using SPME technique. About 21 hydrocarbons were identified and hexanal and toluene showed the highest abundance.

In the current study, SPME technique was used to extract hydrocarbons from tripalmitin and chicken fat for the first time. With further modifications, this extraction technique could be used to analyze the irradiation history of CJTs.

The objective of this study was to apply SPME-GC-MS to investigate the dose response of radiolytic products in chicken fat and tripalmitin as model systems to choose suitable irradiation markers for detection and dose quantitation. The results of these studies is intended to be applied to analyze the irradiation history of CJT and other products.

## Materials and Methods

### Materials

The SPME fiber (carboxen/polydimethylsiloxane (CAR/PDMS) with coating diameter of 75 µm) was purchased from Supelco, USA. The 14% methanol solution of boron trifluoride, fatty acids methyl ester standard mix (C8 to C24), and tripalmitin (glyceryl tripalmitate) was obtained from Sigma, USA. Chicken meat (Tyson Foods Inc, USA) was purchased from a local store.

## Sample preparation

Chicken fat was extracted from chicken tenders by blending with hexane (IKA model T18BS1, Germany). The slurry was filtered (Whatman #40), and the hexane removed using a rotary evaporator at 45 °C (Buchi model RE121, Switzerland). About 100 mL of Chicken fat and tripalmitin samples were irradiated by a Cesium-137 source, at doses of 5, 20, and 50 kGy in a research scale irradiator at the USDA Eastern Regional Research Center (Windmore, USA). Irradiated samples were shipped at room temperature and were stored at -80 °C upon arrival.

## Solid phase micro extraction

After thawing samples at room temperature, aliquots of chicken fat (0.5 g) and tripalmitin (0.1 g) were transferred to clear glass GC vials (2 mL) with screw cap. Different amounts of chicken fat and tripalmitin were used to have the same amount of palmitic acid in both samples (considering 20% palmitic acid in chicken fat. 2-DCB and hydrocarbons were captured from headspace of hermetically-sealed GC vials (PTFE/White Silicone PurePack Septa, ThermoScientific) using SPME. Vials were incubated for 30 minutes at 80 °C for thermal equilibrium (Pierce Heating/Stirring Module, USA), followed by exposing the SPME fiber to the vials' headspace for 15 minutes. The extraction conditions were determined by preliminary experiments.

## Analysis of radiolytic lipid products by GC-MS

Following SPME extraction, the SPME fiber was exposed to a GC-MS chromatograph (HP 5890, Agilent Technologies, USA) equipped with an HP-5MS (60 m × 0.25 mm × 0.25 µm) column. The analyte was desorbed from the SPME fiber at the injector port (for 1 minute at 250 °C) The GC-MS conditions were adapted from Hijaz and Smith (2010) with some modifications. The oven temperature initially was kept at 55 °C for 0.5 minutes and then brought up to 200 °C

at a rate of 20 °C per minute, follow by 15 °C per minute up to a final temperature of 270 °C. The fragment ions with m/z of 98, and 112 were monitored for the detection of 2-DCB. The analysis of hydrocarbons was done similar to 2-DCB with different oven temperature program. The initial temperature was 40 °C (kept for 0.5 minutes) and then brought to 200 °C at a rate of 5 °C per minute. Later, the temperature was increased to 270 °C at a rate of 20 °C per minute that kept for 4 minutes. The ions were quantified with a HP MS 5972 mass detector at 280 °C with voltage varying from 2,400 to 2,800 V. The ions with m/z of 30 to 400 were monitored in scan mode and compounds were identified by comparing their mass spectrum with those of the National Institute of Standards and Technology (NIST) database 2014.

**Fatty acid analysis**

The preparation of fatty acid methyl esters was achieved using the boron trifluoride method (Method Ce 2-66, AOCS, 1998; Ackman, 1998). Briefly, 100 mg of oil was mixed with 3 mL of 14% methanol solution of boron trifluoride and incubated for 40 minutes at 100 °C. Later, the methyl esters were extracted from the solution with hexane.

One µl of hexane extract was introduced to a GC-MS chromatograph (HP 5890, Agilent Technologies, USA) injector at 250 °C. The oven temperature initially was held at 60 °C for 4 minutes, and then brought up to 195 °C at a rate of 20 °C per minute. The final temperature was held for 5 minutes. An HP-23 (DB-23) fatty acid methyl ester (FAME) GC column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA) was used to separate the fatty acid methyl esters. Fragment ions with m/z of 30 to 400 were monitored in scan mode with a HP MS 5972 mass detector at 280 °C. The FAMEs were identified by comparing their retention time with fatty acids methyl ester standard mix (C8 to C24), as well as comparing their mass spectrum with those of the National Institute of Standards and Technology (NIST) database. Each fatty acid

was quantified using its corresponding peak with known concentration in the FAME standard mix.

### Statistical analysis

All data were collected in triplicates and the significance difference between means was analyzed using ANOVA. Individual treatments were compared using post-ANOVA Tukey's multiple comparison test using Minitab (v17, Minitab Inc).

## Results and discussion

### Hydrocarbons

Figure 3.1 provides sample chromatograms of tripalmitin (A) and chicken fat (B) irradiated at different doses. Some peaks show increasing area at higher irradiation doses for both tripalmitin and chicken fat samples. The identification of compounds and their structures are provided on the 50 kGy chromatogram. The compound identification was made by considering the parent ion and longer ion fragments. we accepted the NIST library identification if the probability was more than 90% and the parent ion matched with the molecular weight of identified compound. If NIST library gave a 90% or higher probability but the parent ion was absent, we considered the order of elution or the corresponding peak in the other sample (tripalmitin vs chicken fat) to ensure the correct identification. Thirty-nine different compounds were identified in tripalmitin and chicken fat samples, among which 33 show increasing trends at higher irradiation doses (Table 3.1).

All of the hydrocarbons derived from palmitic acid, which is a saturated fatty acid, have a saturated structure or are unsaturation at the first carbon position. This concurs with Stewart (2001) who stated two major hydrocarbons resulting from a fatty acid under irradiation are one

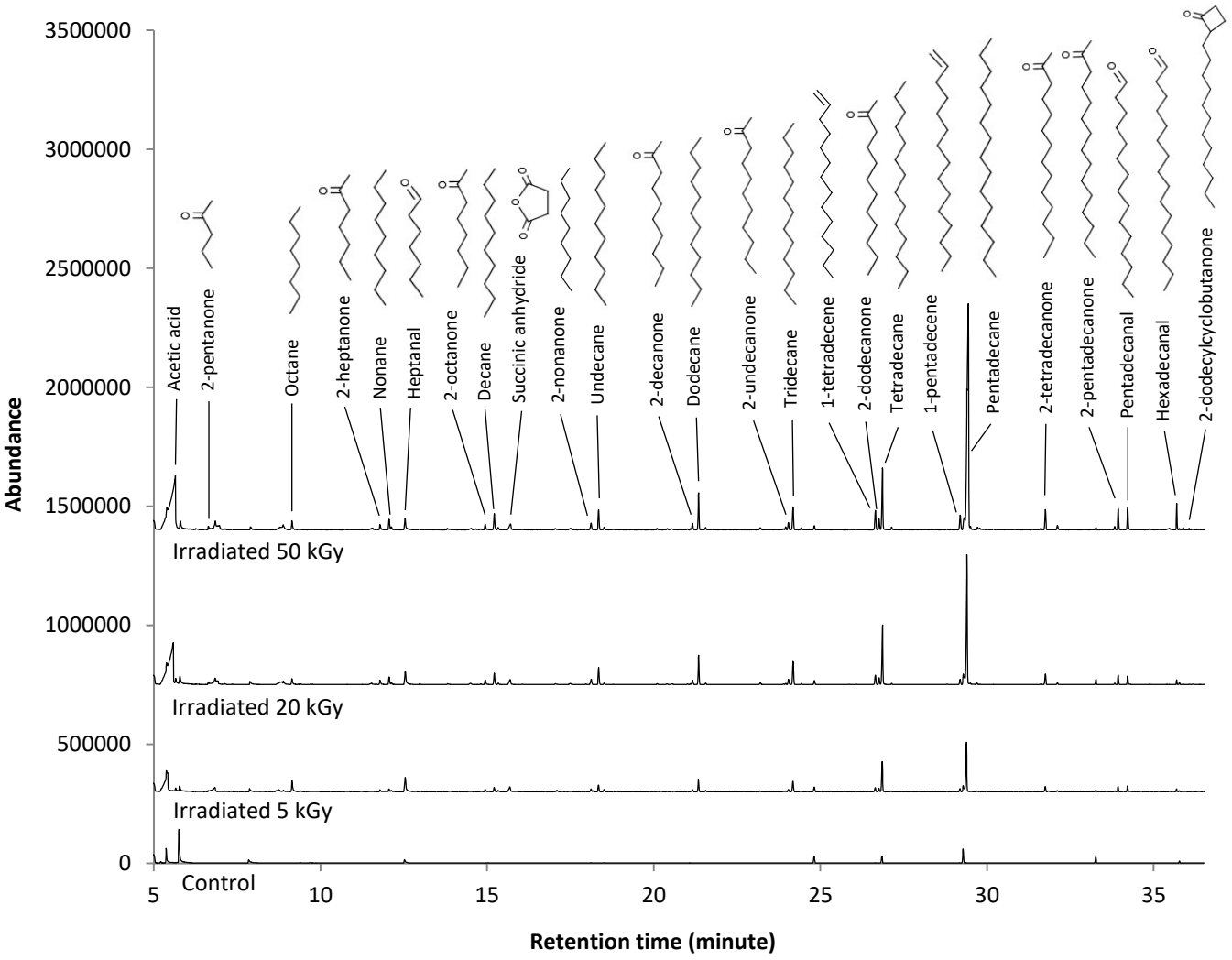
with a carbon less and the other with two carbons less and a double bond at the first carbon position. For example, pentadecane (one carbon less) and 1-tetradecene (two carbon less and double bond on first carbon) are two major radiolytic products of palmitic acid.

Many aldehydes and ketones (all ketones at the second carbon position) with different chain lengths were also be observed, suggesting these types of compounds as major irradiation products of fatty acids. Dubravcic and Nawar (1968) showed that irradiation of pure tripalmitin at 60 kGy promotes the production of pentadecane (88.4 mmol/100g) and tetradecane (19.4 mmol/100g) with all shorter chain hydrocarbons being less than 3 mmol/100g. The reason for less production of shorter chain hydrocarbons and the lack of aldehydes and ketones might be irradiation under vacuum in that study. In the present study, samples were irradiated in closed screw cap vials with little air in the headspace of vials. The presence of oxygen probably interfered with the formation of aldehydes and ketones, and therefore they are not suitable as irradiation markers.

Chicken fat samples showed similar results to tripalmitin (figure 3.1, B). Oleic and palmitic acids are the major fatty acids present in chicken fat. The radiolytic products of oleic acid (8-heptadecene, 1,9-tetradecadiene, and 1,9-dodecadiene) and compounds with unsaturation inside the chain (not just first position) are the main difference with the result of tripalmitin samples. The presence of aldehydes and ketones could also be observed similar to tripalmitin samples but mostly with unsaturation, probably due to the presence of oleic and linoleic acids. Three major compounds, 8-heptadecane, 1,9-tetradecadiene, and 1-tetradecene were only identified in irradiated chicken fat with increasing trend at higher irradiation doses. Among all fatty acids, palmitic acid was the only fatty acid found in both irradiated and non-irradiated chicken fat using SPME with increasing concentration at higher irradiation doses. This suggests

that either the cleavage of fatty acid steric bond occurs at a higher rate for palmitic acid or palmitic acid is more stable than other fatty acids under irradiation.

A-Tripalmitin



B-Chicken Fat

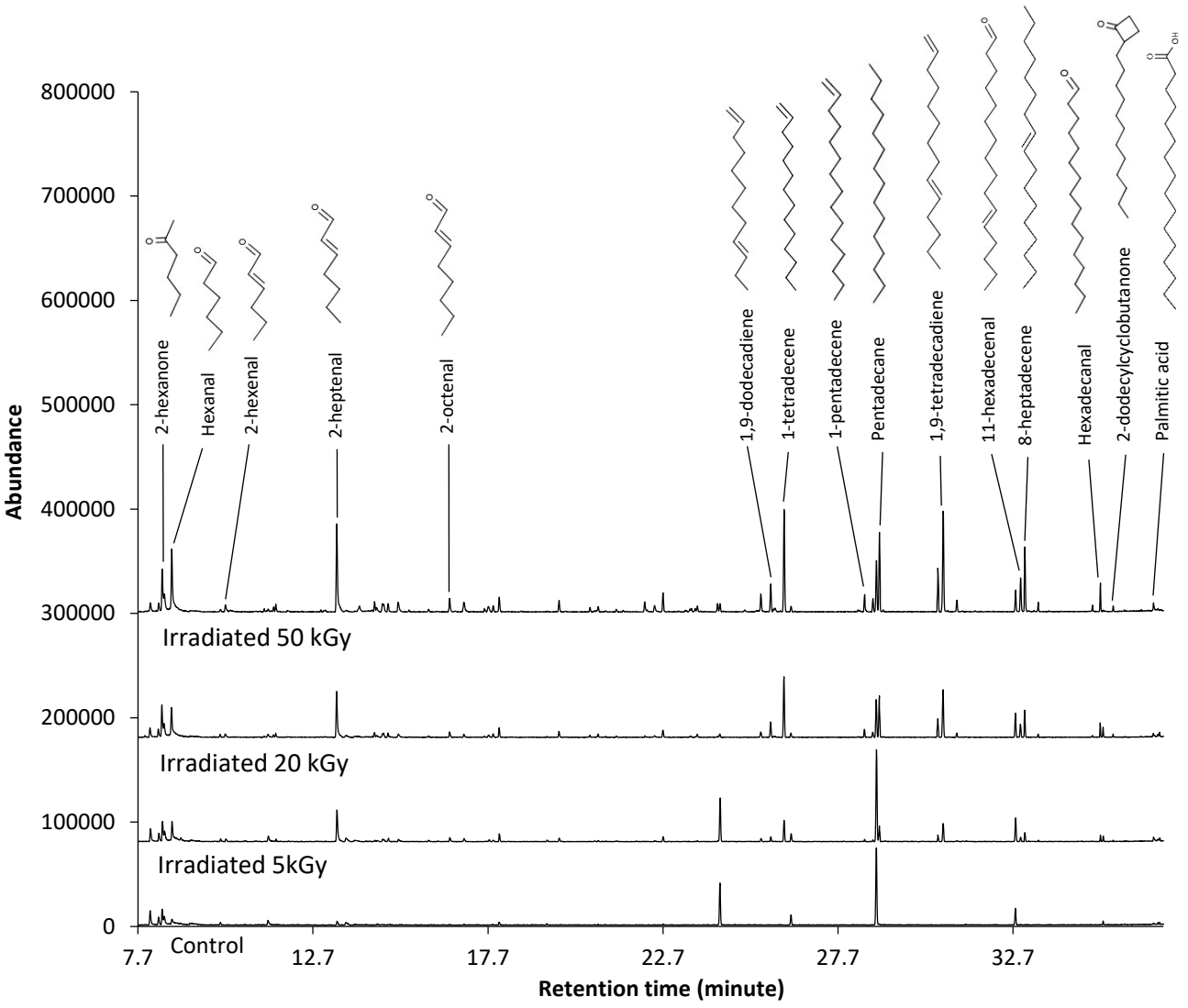
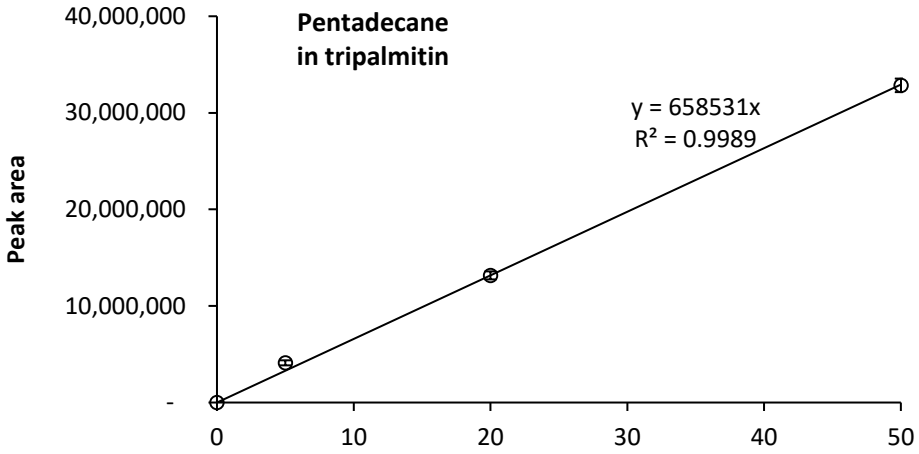



**Figure 3.1 The chromatogram with indication of identified compounds from irradiated A) tripalmitin and B) chicken fat at different doses.**

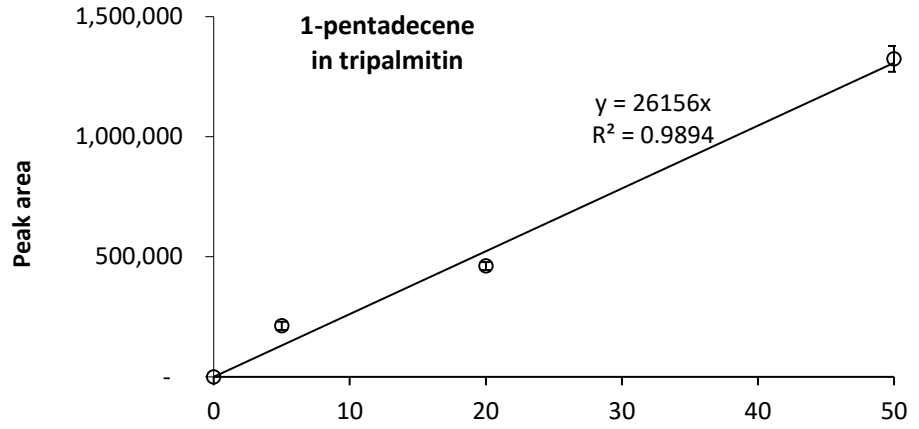
The amount of hydrocarbon products created is largely determined by the concentrations of the parent fatty acid molecule, but independent of the presence of air, moisture, and mild heating during irradiation (Dubravcic, & Nawar, 1976). Dubravcic and Nawar (1976) showed that the amount of tridecane produced from myristic acid (free fatty acid) was 34 times higher than that produced from trimyristin (triglyceride). For this reason, the amount of sample used in

this study was adjusted to represent the same amount of palmitic acid in both chicken fat and tripalmitin samples. This enabled us to compare the production of hydrocarbons derived from palmitic acid between these two samples. The pentadecane and 1-pentadecene peak area (both derived from palmitic acid) was still higher in pure tripalmitin compared with chicken fat (Figure 3.2 and 3.3). This might be due to the inhibitory effect of other fatty acids present in chicken fat samples.

A)




B)




C)

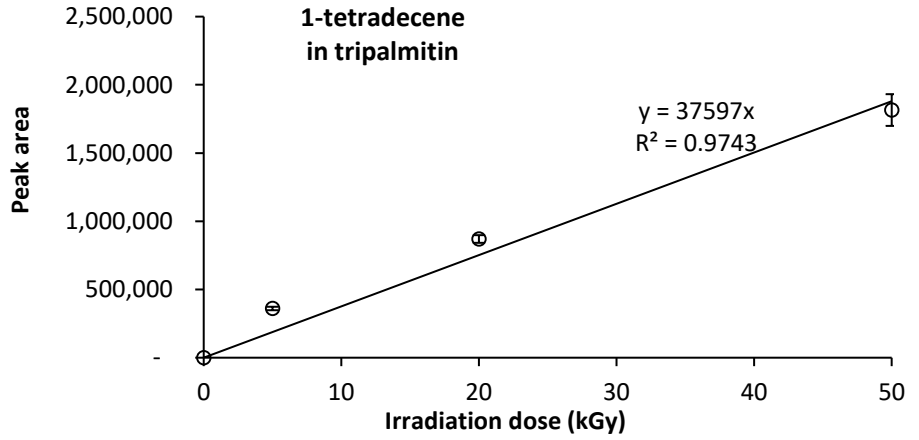
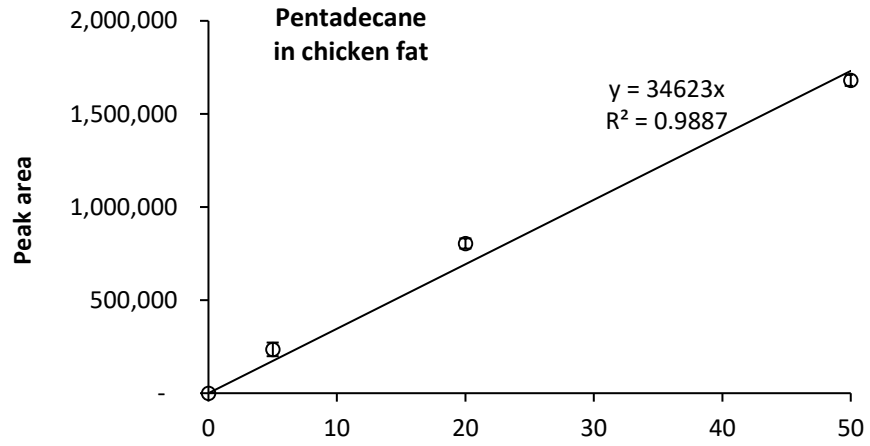
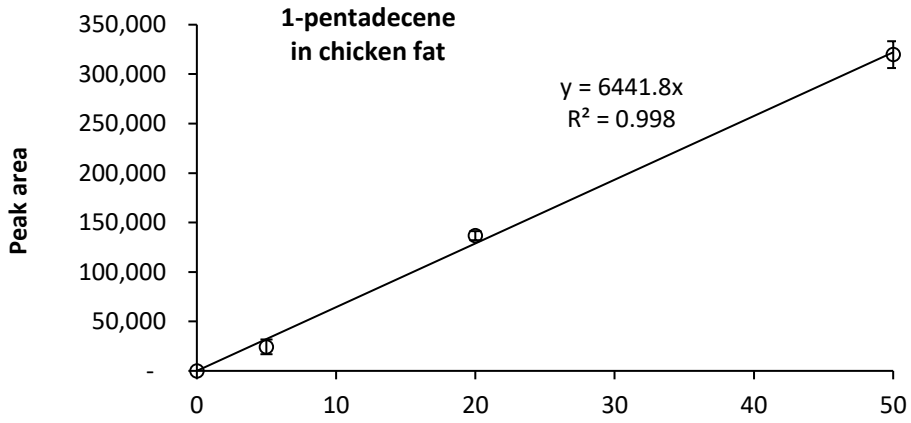



**Figure 3.2 Formation kinetics of pentadecane (A), 1-pentadecene (B), and 1-tetradecene (C) in tripalmitin to irradiation doses from 0 to 50 kGy. Data is the average of 3 replicates and bars represent standard deviation.**

A)




B)




C)

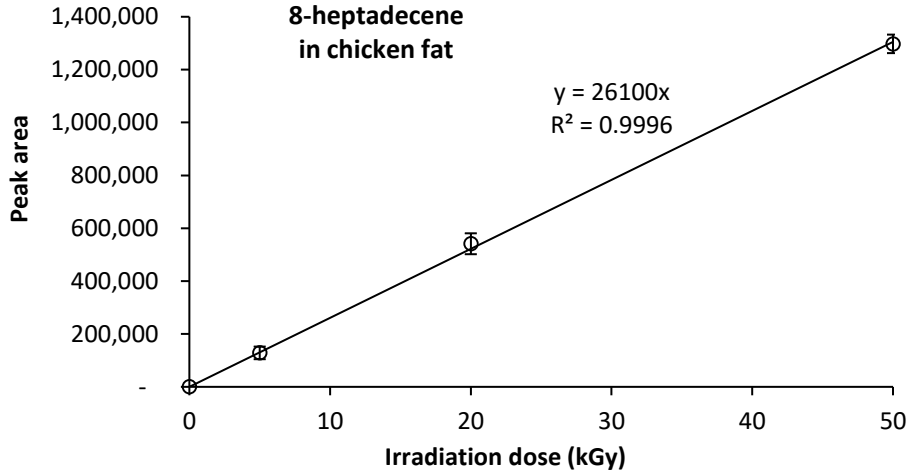



**Figure 3.3 Formation kinetics of pentadecane (A), 1-pentadecene (B), and 8-heptadecene (C) in chicken fat to irradiation doses from 0 to 50 kGy. Data is the average of 3 replicates and bars represent standard deviation.**

For all identified compounds a dose-response graph was made to evaluate the formation kinetics under irradiation. Some examples of such graphs are presented in figures 3.2 and 3.3 To choose an irradiation marker that would be able to predict better the irradiation history of product, a high formation kinetic (dose-response graph's slope), peak area, and linear regression (coefficient of determination close to 1), with low variability (low standard deviation) is needed. A suitable marker should also have a saturated structure (more stable) and not to be oxidized (not dependent on the presence of oxygen) so that it could be a good indicator of irradiation rate in different samples during shelf life. Table 3.1 shows the data obtained from the dose-response graphs including coefficient of determination ($R^2$) and the slope obtained from each compound separately. Among saturated hydrocarbons that does not contain oxygen, pentadecane showed the highest formation kinetic, peak area, and coefficient of determination. However, the formation kinetic of pentadecane in chicken fat was lower than tripalmitin (34,623 vs 658,531). This could be because unsaturated fatty acids in chicken fat are better accepters of electron and compete with palmitic acid, while irradiation of tripalmitin, where there is palmitic acid alone, could lead to higher breakdown of palmitic acid and formation of pentadecane. It could be concluded that pentadecane, which is a saturated hydrocarbon derived from palmitic acid, is the most suitable candidate to be used as an irradiation marker.

**Table 3.1 The identified compounds in control and irradiated tripalmitin and chicken fat. The $R^2$ value represents the linear trend regression of the data from 0 to 50 kGy and data is the average of three replicates ± the standard deviation.**

| | | Tripalmitin (total ions count × $10^{-4}$) | | | | | | Chicken fat (total ions count × $10^{-4}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RT (min)** | **Compound** | **0 kGy** | **5 kGy** | **20 kGy** | **50 kGy** | **$R^2$** | **Slope** | **0 kGy** | **5 kGy** | **20 kGy** | **50 kGy** | **$R^2$** | **Slope** |
| **5.11** | **Acetic acid** | - | 574.9 ± 19.4 | 1591.7 ± 132.7 | 2459.5 ± 109.7 | 0.8842 | 539093 | - | - | - | - | - | |
| **6.13** | **2-pentanone** | - | 4.5 ± 0.6 | 11.9 ± 2.2 | 18.5 ± 3.2 | 0.8813 | 4046 | - | - | - | - | - | |
| **7.39** | **Pyridine** | 47.0 | 33.0 ± 2.7 | 30.6 ± 2.7 | 29.6 ± 2.3 | 0.4664 | -2453 | - | - | - | - | - | |
| **8.38** | **2-hexanone** | - | 13.6 ± 1.7 | 35.5 ± 5.5 | 156.5 ± 7.3 | 0.9587 | 29407 | 29.2 | 39.8 ± 4.0 | 64.6 ± 1.9 | 90.8 ± 2.5 | 0.9665 | 11996 |
| **8.63** | **Octane** | - | 124.5 ± 5.5 | 50.1 ± 6.3 | 87.4 ± 4.7 | -0.5740 | 20502 | - | - | - | - | - | |
| **8.65** | **Hexanal** | - | - | - | - | - | | 9.4 | 34.7 ± 4.0 | 69.8 ± 3.5 | 154.3 ± 6.2 | 0.9946 | 28025 |
| **10.2** | **2-hexenal** | - | - | - | - | - | | - | 14.2 ± 14.5 | 8.9 ± 1.0 | 16.9 ± 0.4 | 0.0528 | 3745 |
| **11.27** | **2-heptanone** | - | 17.3 ± 1.0 | 39.3 ± 5.8 | 55.9 ± 3.6 | 0.7946 | 12538 | - | - | - | - | - | |
| **11.55** | **Nonane** | - | 24.6 ± 3.1 | 52.3 ± 7.2 | 89.5 ± 9.4 | 0.8963 | 19306 | - | - | - | - | - | |
| **11.61** | **Heptanal** | - | 11.6 ± 0.4 | 13.7 ± 1.0 | 27.1 ± 1.7 | 0.7759 | 5771 | - | - | - | - | - | |
| **12.02** | **Butyrolactone** | 49.1 | 205.7 ± 12.8 | 161.1 ± 17.3 | 135.2 ± 8.0 | 0.0231 | 4450 | - | - | - | - | - | |
| **13.37** | **2-heptenal** | - | - | - | - | - | | 7.8 | 65.4 ± 10.4 | 110.7 ± 3.1 | 224.1 ± 3.3 | 0.9120 | 46998 |
| **14.43** | **2-octanone** | - | 19.8 ± 1.1 | 35.7 ± 4.0 | 51.8 ± 5.4 | 0.7354 | 11642 | - | - | - | - | - | |
| **14.7** | **Decane** | - | 40.5 ± 3.1 | 82.0 ± 12.1 | 132.5 ± 14.4 | 0.8546 | 28949 | - | - | - | - | - | |
| **15.19** | **Succinic anhydride** | - | 65.5 ± 7.6 | 88.1 ± 4.1 | 87.9 ± 9.7 | -0.0400 | 22173 | - | - | - | - | - | |
| **16.59** | **2-octenal** | - | - | - | - | - | | - | 7.1 ± 1.6 | 12.2 ± 0.3 | 31.0 ± 0.1 | 0.9700 | 6257 |
| **17.61** | **2-nonanone** | - | 22.1 ± 6.9 | 47.6 ± 5.3 | 69.0 ± 3.6 | 0.7965 | 15426 | - | - | - | - | - | |
| **17.83** | **Undecane** | - | 65.3 ± 5.7 | 124.1 ± 18.0 | 176.3 ± 17.7 | 0.7362 | 39733 | - | - | - | - | - | |
| **20.65** | **2-decanone** | - | 17.0 ± 0.7 | 34.5 ± 2.5 | 55.1 ± 3.9 | 0.8486 | 12077 | - | - | - | - | - | |
| **20.83** | **Dodecane** | - | 117.1 ± 9.9 | 213.6 ± 29.4 | 324.2 ± 33.0 | 0.7780 | 72022 | - | - | - | - | - | |
| **23.53** | **2-undecanone** | - | 18.7 ± 1.1 | 42.8 ± 2.1 | 64.3 ± 2.9 | 0.8354 | 14239 | - | - | - | - | - | |
| **23.67** | **Tridecane** | - | 94.8 ± 6.0 | 175.6 ± 20.4 | 208.0 ± 23.1 | 0.5231 | 49177 | - | - | - | - | - | |
| **25.77** | **1,9-dodecadiene** | - | - | - | - | - | | - | 6.7 ± 1.1 | 26.2 ± 2.2 | 50.8 ± 1.6 | 0.9796 | 10598 |
| **26.15** | **1-tetradecene** | - | 36.1 ± 1.3 | 87.0 ± 2.8 | 181.5 ± 11.6 | 0.9743 | 37597 | - | 35.4 ± 4.0 | 113.1 ± 7.2 | 203.1 ± 2.9 | 0.9561 | 43054 |
| **26.25** | **2-dodecanone** | - | 25.3 ± 2.3 | 63.5 ± 2.5 | 92.5 ± 3.8 | 0.8345 | 20592 | - | - | - | - | - | |
| **26.35** | **Tetradecane** | 60.7 | 273.1 ± 10.0 | 505.9 ± 33.3 | 576.0 ± 41.6 | 0.3255 | 137719 | - | - | - | - | - | |
| **28.7** | **1-pentadecene** | - | 21.2 ± 1.7 | 46.2 ± 1.7 | 132.4 ± 5.4 | 0.9894 | 26156 | - | 2.4 ± 0.7 | 13.7 ± 0.5 | 32.0 ± 1.4 | 0.9980 | 6441 |
| **28.92** | **Pentadecane** | - | 410.9 ± 26.1 | 1316.5 ± 41.6 | 3284.7 ± 69.9 | 0.9989 | 658531 | - | 23.5 ± 3.7 | 80.4 ± 2.7 | 168.0 ± 3.0 | 0.9887 | 34623 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **30.7** | **1,9-tetradecadiene** | - | - | - | - | - | | - | 30.8 ± 4.5 | 104.4 ± 4.2 | 219.6 ± 2.6 | 0.9894 | 45202 |
| **31.24** | **2-tetradecanone** | - | 37.9 ± 5.9 | 108.0 ± 14.7 | 156.5 ± 22.8 | 0.8508 | 34786 | - | - | - | - | - | |
| **32.91** | **11-hexadecenal** | - | - | - | - | - | | - | 5.9 ± 1.2 | 28.0 ± 2.1 | 68.4 ± 1.6 | 0.9996 | 13701 |
| **33.03** | **8-heptadecene** | - | - | - | - | - | | - | 12.8 ± 2.4 | 54.1 ± 4.0 | 129.8 ± 3.5 | 0.9996 | 26100 |
| **33.43** | **2-pentadecanone** | - | 32.5 ± 6.6 | 87.7 ± 14.1 | 142.8 ± 25.5 | 0.9069 | 30959 | - | - | - | - | - | |
| **33.72** | **Pentadecanal** | - | 33.9 ± 6.3 | 81.2 ± 16.0 | 147.2 ± 26.2 | 0.9372 | 31288 | - | - | - | - | - | |
| **35.19** | **Hexadecanal** | - | 14.1 ± 4.4 | 38.7 ± 9.1 | 119.7 ± 29.0 | 0.9908 | 23347 | - | 6.3 ± 0.9 | 19.0 ± 1.1 | 39.9 ± 1.8 | 0.9861 | 8239 |
| **35.56** | **2-dodecylcyclobutanone** | - | 1.6 ± 0.3 | 3.5 ± 0.7 | 6.3 ± 1.4 | 0.9201 | 1338 | - | 1.3 ± 0.3 | 3.9 ± 0.8 | 8.5 ± 0.6 | 0.9916 | 1744 |
| **36.71** | **n-hexadecanoic acid** | 669.3 | 1535.4 ± 243.7 | 1745.7 ± 392.6 | 1748.0 ± 388.9 | 0.4463 | 444416 | 1.7 | 6.8 ± 1.5 | 7.6 ± 2.3 | 24.9 ± 8.6 | 0.9408 | 4365 |
| **37.02** | **Hexadecanoic acid, ethyl ester** | - | 5.4 ± 0.7 | 19.6 ± 5.8 | 64.2 ± 16.0 | 0.9873 | 12402 | - | - | - | - | - | |

## 2-DCB

2-DCB can only form with irradiation and it is not present in control (non-irradiated) tripalmitin and chicken fat samples (Table 3.1). Other researchers have reported similar results (Fernades, Pereira, Antonio, & Ferreira, 2018). Variyar, Chatterjee, Sajilata, Singhal, and Sharma (2008) reported the natural occurrence of 2-DCB in non-irradiated cashew nut and nutmeg samples. However, further investigation of the Joint Research Centre (JRC) of the European Commission did not verify the natural existence of 2-DCB in cashew nuts and nutmeg (Breidbach, & Ulberth, 2015). Therefore, it is considered , along with hydrocarbons, as a marker to detect the occurrence of irradiation in fat containing foods (The European Committee for Standardization, 2003).

The peak area obtained from 2-DCB linearly (slope of 1744, and R2 = 0.991) increased at higher irradiation doses for both tripalmitin and chicken fat samples (figure 3.4). The slope of the dose-response graph for 2-DCB in tripalmitin is 2956, whereas the slope for chicken fat sample is 1304. This shows, similar to the results of pentadecane, the formation kinetic of 2-DCB is

higher in tripalmitin where there is no competition for accepting electrons other than palmitic acid. Monitoring selected ion fragments (m/z of 98 and 112), improved the abundance and resolution of the resulting chromatogram, but didn't change the slope. The linear occurrence of 2-DCB in pure tripalmitin shows that the production of 2-DCB under irradiation does not depend on the presence of water or other food components, but is only a function of fatty acid composition.

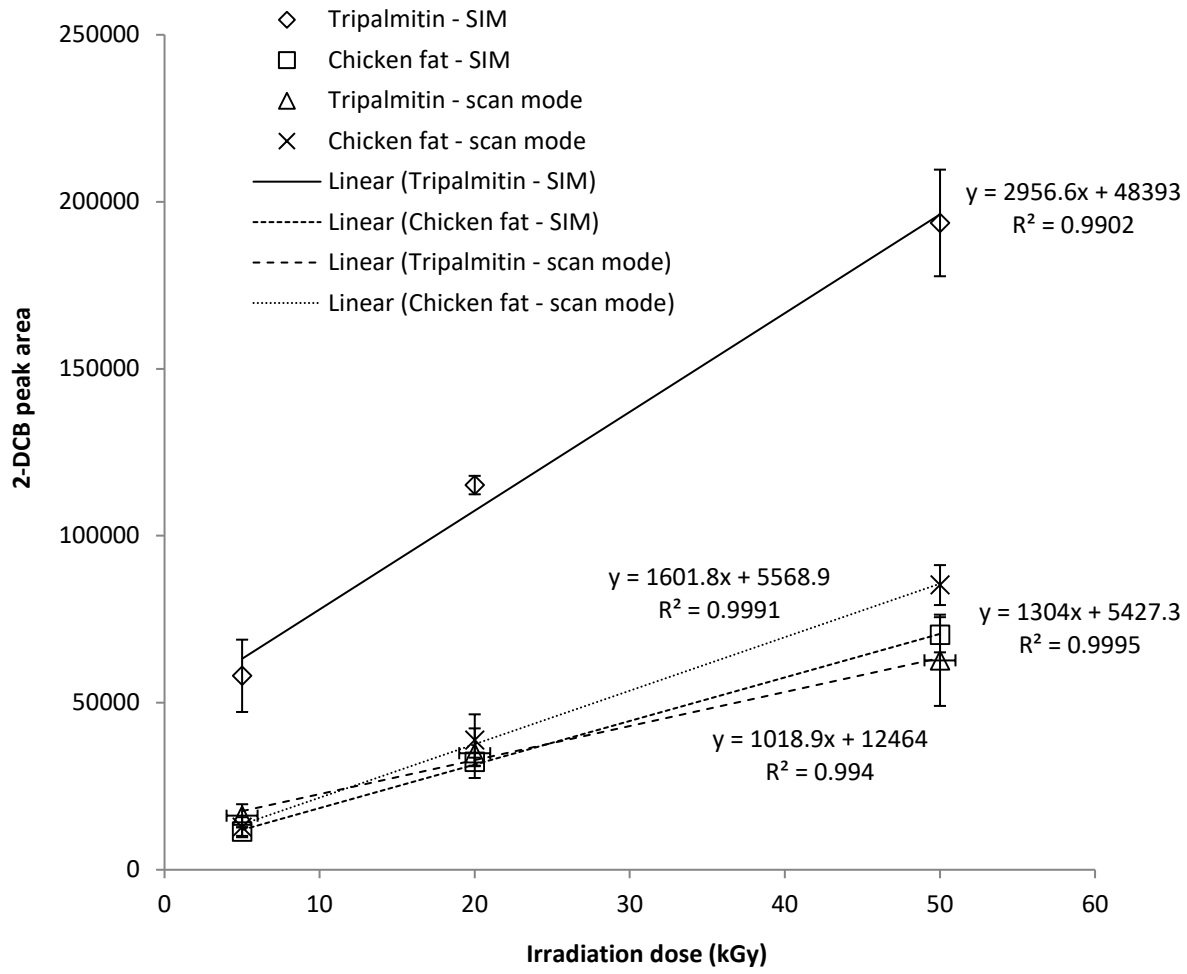



**Figure 3.4 The irradiation dose response of 2-DCB in tripalmitin and chicken fat using Scan mode (m/z of 30 to 400), and Selected Ion Mode (SIM) monitoring only m/z of 98 and 112.**

## Fatty Acids

Seven major fatty acids were identified in the chicken fat sample including oleic, palmitic, linoleic, linolenic, myristic, and stearic acid (in the order of highest to lowest concentration). The concentration of all fatty acids decreased up to 20 kGy of irradiation and remained essentially unchanged 50 kGy (Figure 3.5). The reduction rate up to 5 kGy is also

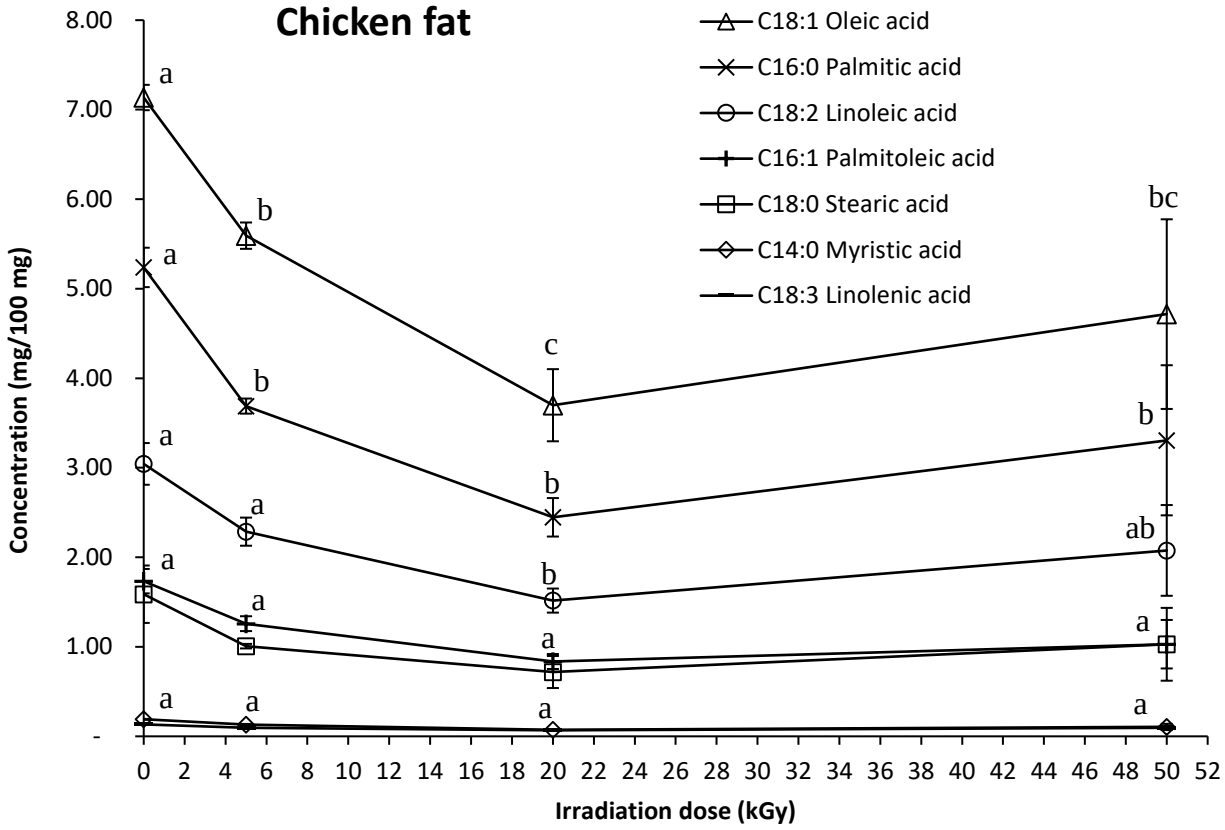
higher than the reduction rate between 5 to 20 kGy for all fatty acids. This shows a non-linear reduction of fatty acids from 0 to 20 kGy. A large variability in 50 kGy samples might be the reason for insignificance of the change after 20 kGy. The reduction of fatty acids could be parallel to formation of hydrocarbons and other radiolytic products of fatty acids.

Chen, Zhou, Zhu, Xu, Tang, and Gao (2007) showed irradiation up to 3.17 kGy has little to no effect on beef fatty acid composition. However, the percentage of fatty acids was calculated based on sum of peak areas for fatty acids. That calculation will result in values based on percent of each fatty acid in total fat (fatty acids) of sample and not independent concentration of each fatty acid in sample. In that case, if the concentration of all fatty acids change at a similar rate by irradiation, the percentage of each fatty acid compared with total remains unchanged. The same approach could also be seen in the study of Hong, Kim, Cho, and Park (2010) where the effect of irradiation on oleic acid was studied and the percentage of oleic acid was reported as the percentage of total fatty acids. Some studies reported a reduction of unsaturated fatty acids along with the increase of saturated fatty acids by irradiation. (Yalcin, Ozturk, Tulukcu, & Sagdic, 2011; Minami, Nakamura, Todoriki, & Murata, 2012; Olotu, Enujiugha, Obadina1, & Owolabi, 2014; Zhanga, Wanga, Wanga, & Ye, 2017). While the reduction of unsaturated fatty acids can be explained by oxidation or break down of such compounds, it is unclear what causes the increase in saturated fatty acids. The increase may simply be due to the reduction of unsaturated fatty acid and a change in the ratio of unsaturated to saturated fatty acids. When percent composition of each fatty acid is reported based on the total peak area of fatty acids, reduction of one fatty acid would simply result in increasing the remaining fatty acids share (%). For this reason, the concentration of each individual fatty acid should be measured by comparing with its

corresponding fatty acid standard (known concentration) in order to better understand the effect of irradiation on fatty acids.

The analysis of the concentration of palmitic acid in tripalmitin, however, did not show any significant change at higher irradiation doses. This could be because tripalmitin is made of only one fatty acid and slight changes in its concentration would be hard to observe.

A)




B)

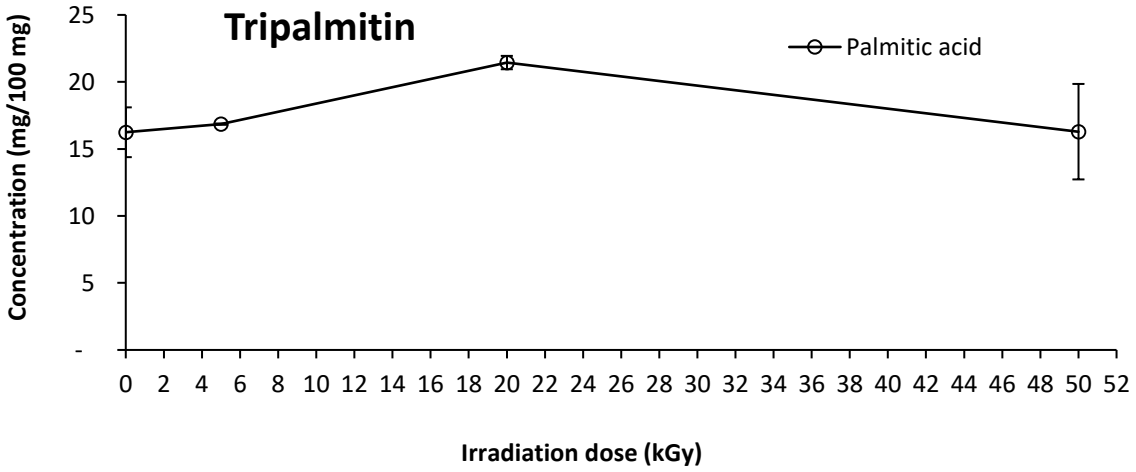



**Figure 3.5 The effect of irradiation at different dosage on fatty acid composition of chicken fat (A) and tripalmitin (B). Data is the average of three replicates and error bars represent standard deviation. Different letters for each fatty acid indicate the significant difference.**

## Conclusion

Irradiation of food lipids with either 5, 20, 50 kGy leads to a reduction of fatty acids (up to 20 kGy) along with formation of many different hydrocarbons, aldehydes, and ketones. The concentration of these compounds increases linearly at higher irradiation doses, which makes them suitable to be used to predict the irradiation history of samples. Among all, pentadecane, and 8-heptadecane, which likely derived from palmitic acid and oleic acid respectively, showed the highest concentration and linear regression.

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# Chapter 4 : Solid phase micro extraction of food irradiation marker 2-dodecylcyclobutanone (2-DCB) from chicken jerky treated with glycerol[1,2]


## Abstract:

Among different methods to extract the food irradiation marker, 2-dodecylcyclobutanone (2-DCB), solid phase micro extraction (SPME) could provide a faster and simpler method to estimate the irradiation history of fat containing food products. The SPME technique was optimized to extract 2-DCB from chicken jerky treats (CJT) irradiated at 0 to 50 kGy. This compound was detected using gas chromatography mass spectrometry (GC-MS) in single ion mode. Water dilution (1:5) was needed to mobilize 2-DCB and allow partition to the headspace form the CJT matrix. Increasing the incubation temperature up to 80 °C resulted in higher response. Spiking control jerky samples with 2-DCB from 10 to 150 ppb compared with spiking water revealed a significant effect that the food matrix has in lowering the signal obtained from 2-DCB. Irradiated samples showed a linear increasing trend for concentration of 2-DCB at higher irradiation doses. This method provides a fast, simple, and environmentally friendly alternative for the existing solvent extraction methods for analyzing 2-DCB.


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1. Abstract presented in part at IFT Annual Meeting 2018
2. Taghvaei, M., Sommers, C., Ceric, O., Hussain, F., Yucel, U., Smith, J. S. (2019) Solid phase micro extraction of food irradiation marker 2-dodecylcyclobutanone (2-DCB) from chicken jerky treated with glycerol. Manuscript Submitted.

# Introduction

The process of ionizing irradiation is commonly used in pet food manufacturing, primarily with the purpose of reducing the levels of parasites and pathogenic bacteria, as well as extending shelf-life. However, over the last decade or so there have been concerns for the safety of irradiated pet food products due to numerous cases of illness and death in dogs and cats associated with the consumption of chicken jerky treats (CJT) in USA and dry cat food in Australia, respectively (FDA, 2019; Burke, 2009). Despite detailed biological and chemical analyses of the affected animals and related products by official and private institutions, the causation is still unclear. Ensuring the products are irradiated within the legal limits remain as an important checkpoint. For regulatory agencies to monitor the use of food irradiation, a validated analytical method is needed to quantify the dose that was used during production. Among different compounds that are suggested as a food irradiation marker, 2-dodecylcyclobutanone (2-DCB) could be a suitable candidate due to its dose response relationship with irradiation (Soncin, Panseri, Rusconi, Mariani, & Chiesa, 2012). This compound is formed through cyclisation of palmitic acid and formation of a cyclobutanone ring that is attached to a long hydrocarbon chain (Fan, 2013; Stewart, 2001). Since this reaction is only induced by ionizing irradiation, 2-DCB is formed at a concentration directly related to irradiation dosage (Gadgil, Hachmeister, Smith, & Kropf, 2002; Hijaz, & Smith, 2010). Although Variyar, Chatterjee, Sajilata, Singhal, and Sharma (2008) showed the natural occurrence of 2-DCB in non-irradiated cashew nut and nutmeg samples, their study was not confirmed by further investigation of the Joint Research Centre (JRC) of the European Commission which did not accept the natural existence of 2-DCB in non-irradiated cashew nuts and nutmeg (Breidbach, & Ulberth, 2015).

The current available standard method for detection of 2-DCB in foods (European committee for standardization, 2003) only shows the presence of 2-DCB from irradiation (not quantitation of dosage) and consists of timely Soxhlet extraction of fat with hexane and several cleanup and drying steps. Presence of glycerol in the food formula could also interfere with the extraction. Alternatively, solid phase micro extraction (SPME) can serve as a fast and simple extraction method to efficiently analyze 2-DCB. With this technique, it is possible to concentrate organic compounds in the head space (even at very low concentrations) on the fiber coating and later analyze them by gas or liquid chromatography. The SPME technique could provide a faster and simpler extraction without using organic solvents compared with the current standard method.

Others used SPME technique to analyze 2-DCB in various food matrixes, such as irradiated ground beef (Caja, Ruiz del Castillo, & Blanch, 2008; Soncin, Panseri, Rusconi, Mariani, & Chiesa, 2012) and dry-cured ham (Blanch, Caja, Flores, & Ruiz del Castillo, 2009). The efficiency of the SPME technique is known to be affected by fiber type, incubation temperature, and the food matrix being analyzed (Caja, Ruiz del Castillo, and Blanch, 2008; Soncin, Panseri, Rusconi, Mariani, and Chiesa, 2012; Cho, Kong, and Oh, 2003). Additionally, the intrinsic factors, such as food composition and additives used in the food formula could affect SPME. Glycerol is an additive commonly used in the production of CJT. This sugar alcohol is added to CJT to improve taste, appearance, and the texture of the product by absorbing water (Ockerman, 1991). Three hydroxyl groups in glycerol provide a potential site for hydrogen-bonding with water resulting in lower water activity on the surface of jerky. The presence of glycerol also helps to maintain the moisture content inside the treats at the desired level. This ability to bond with water could affect the release of 2-DCB into the head space

during SPME. As irradiated CJTs in the market are treated with glycerol at different levels, it is important to understand how the presence of glycerol may affect the SPME of 2-DCB.

The objective of this study was to determine the SPME condition including fiber type, incubation temperature, solvent type and amount for hydration as well as sample composition (i.e., water, CJT with and without glycerol) to maximize 2-DCB isolation for subsequent detection with GC-MS.

## Materials and Methods

### Materials

2-DCB analytical standard and food grade glycerol (purity > 99%) were purchased from Sigma-Aldrich (USA). The SPME fibers (Polydimethylsiloxane (PDMS) with two coating diameters of 7 and 100 µm, carboxen/polydimethylsiloxane (CAR/PDMS) with coating diameter of 75 µm, and polydimethylsiloxane/divinylbenzene (PDMS/DVB) with coating diameter of 65 µm) were purchased from Supelco, USA. Chicken breast tenderloins (Tyson Foods Inc, USA) was purchased from a local store.

### Preparation of chicken jerky treats (CJT)

CJT were prepared by drying raw chicken breast tenderloins at 68 °C for 48 hours in a commercial food dehydrator (Sedona SD-P9000, Korea). Another batch of chicken tenderloin (4 kg) was mixed with 150 mL of food grade glycerol followed by 2 minutes of massaging the glycerol into the meat, before drying. Both chicken samples dried with and without glycerol were irradiated by a Cesium-137 source, at doses of 5, 10, 20, and 50 kGy at the USDA Eastern Regional Research Center (Windmore, USA). Irradiated samples were stored at -80 °C prior to analysis.

## Proximate analysis

Total protein was measured according to the Kjeldahl method (Official methods # Ac 4-91, 2011). Total fat was analyzed by acid hydrolysis (AOAC # 922.06 and 954.02, 2005a). The glycerol content of jerky samples was measured using high performance liquid chromatography according the method described by Sweeley, Bentley, Makita, and Wells (1963). The moisture content was measured by AOAC official method (AOAC # 925.09 and 926.08, 2005b), and the water activity was analyzed with an Aqualab series 3 meter calibrated with 6 mol/kg NaCl and 8.57 mol/kg LiCl standard solutions (Decagon Devices, Inc. USA).

## Standard solution

A 1000 ppm stock solution of standard 2-DCB (44197-5MG, Sigma-Aldrich, USA) was prepared by dissolving 5 mg of standard into 5 mL of methanol. This solution was kept in -80 °C freezer. A 1.00 ppm working standard solution was prepared by subsequent dilutions of the stock solution in water. This solution was prepared fresh each day of experiments.

## Solid phase micro extraction

Irradiated jerky strips (with glycerol) at 0, 5, 10, 20, and 50 kGy were ground for 1 minute, and 1.25 g of each sample was transferred into a 20 mL siliconized glass vial and mixed with 5 mL of water. The mixture was homogenized (Omni International TH01, USA) at the highest speed for 1 minute, and an 8 mm magnetic stirring bar was added to the mixture. Vials were sealed with silver crimp caps containing polytetrafluoroethylene/white silicon liners and incubated at 80 °C (Pierce Heating/Stirring Module, USA) with high speed magnetic stirring for 30 minutes, followed by exposing a solid phase micro extraction fiber (PDMS/DVB 65 µm fused silica, Supelco, USA) to the vial's headspace for 15 minutes.

The standard curve for measuring the concentration of 2-DCB in irradiated samples was made using a series of vials with 1.25 control jerky (with glycerol) and 5 mL water spiked with 2-DCB at concentrations of 0, 5, 10, 25, 50, 75, 200, and 400 ng/g CJT in triplicates. The rest of the experiment was performed similar to above.

### Extraction solvent and temperature

Two solvents of water and methanol (5 mL) were spiked with 2-DCB in three concentrations of 10, 50, and 100 ppb (one replicate for each concentration), and incubated at three temperatures of 25, 65, and 80 °C. The temperature of methanol extract were not analyzed at higher temperatures than 65 °C due to boiling of the solvent. Vials incubated with high speed magnetic stirring for 30 minutes, followed by exposing SPME fiber to the vial's headspace for 15 minutes.

### Temperature inside the vials

The temperature inside the vials during incubation was measured by adding 5 mL water to three vials and inserting a thermometer to each vial after capping. Vials were put in three different spots in the heating block and the temperature was recorded every two minutes during one hour of incubation.

### Matrix effect

Four different 2-DCB concentrations of 10, 50, 100, and 150 ppb were made in triplicates by spiking pure water (5 mL), water and control jerky without glycerol, and water and control jerky with glycerol. The dilution for the jerky and water mixtures was 1:5. Vials were incubated at 80 °C with high speed magnetic stirring for 30 minutes, followed by exposing SPME fiber to the vial's headspace for 15 minutes.

## Dilution factor

Control jerky without glycerol was mixed with water using three dilution rate of 1:4, 1:5, and 1:6. Three 2-DCB concentrations of 1, 5, and 10 ppb were made for each dilution rate in triplicates. Vials were incubated at 80 °C with high speed magnetic stirring for 30 minutes, followed by exposing SPME fiber to the vial's headspace for 15 minutes.

## Fiber stationary phase selection

A 2-DCB concentration of 5 ppb was made in 5 mL pure water in each vial and incubated at 80 °C with high speed magnetic stirring for 30 minutes. Four different SPME fiber stationary phases/coating diameters of PDMS with two coating diameters of 7 and 100 µm, CAR/PDMS with coating diameter of 75 µm, and PDMS/DVB with coating diameter of 65 µm were exposed to the vial's headspace for 15 minutes. This experiment was performed in duplicates.

Two separate standard curves were made using PDMS/DVB 65 µm and PDMS 100 µm fiber stationary phases. 1.25 g Jerky was mixed with 5 mL water and spiked with 2-DCB at concentrations of 1, 2, 5, 7.5, and 10 ppb. The experiment was done in triplicates and each vial incubated at 80 °C with high speed magnetic stirring for 30 minutes. SPME fibers were exposed to the vial's headspace for 15 minutes.

## Gas chromatography mass spectrometry (GC-MS)

Following extraction step, the SPME fiber was exposed to a GC-MS chromatograph's (HP 5890, Agilent Technologies, USA) equipped with an HP-5MS (60 m × 0.25 mm × 0.25 µm) column. The GC-MS conditions were adapted from Hijaz and Smith (2010) with some modifications. The analyte was desorbed from the SPME fiber at the injector port (for 1 minute at 250 °C). The oven temperature initially was kept at 55 °C for 0.5 minutes and then brought up to 200 °C at a rate of 20 °C per minute, follow by 15 °C per minute up to a final temperature of

270 °C. The fragment ions with m/z of 98, and 112 were monitored for the detection of 2-DCB in selected ion mode (SIM). The ions were quantified by HP MS 5972 mass detector at 280 °C with voltage varying from 2,400 to 2,800 V. The initial identification of the retention times and ion fragments were confirmed with monitoring m/z of 30 to 450 in scan mode, and compounds were identified by comparing their mass spectrum with those of the National Institute of Standards and Technology (NIST) database.

### Statistical analysis

Figures and tables were prepared in MS Excel (Microsoft Office, 2010). The significance of difference was analyzed using ANOVA, and individual treatments were compared using post-ANOVA Tukey's multiple compassion test using Minitab (v17, Minitab Inc).

## Results and discussion

### Extraction conditions

Initially, the effect of solvent and incubation temperature on the extraction efficiency of 2-DCB was evaluated. As the use of non-polar solvents is not recommended with polydimethylsiloxane (PDMS) fibers methanol and water were selected to test for their efficiency. Three different concentrations of 2-DCB (10, 50, and 100 ppb) were made by adding aliquots of 2-DCB standard to methanol, and water. Three incubation temperatures of 25, 65, and 80 °C was used. Using methanol solvent did not show an increasing trend from 10 to 100 ppb at any temperature (figure 4.1). The reason could be related to saturation of the fiber by methanol and leaving fewer adsorbing sites for 2-DCB. This makes water a better solvent for extraction of 2-DCB using a PDMS fiber. Incubation at 80 °C using water as the solvent resulted in the highest peak area for 2-DCB (Figure 4.1). This is similar to the work of Soncin, Panseri,

Rusconi, Mariani, and Chiesa (2012), who reported that incubation temperature of 80°C results in the highest extraction efficiency for SPME analysis of 2-DCB in beef.

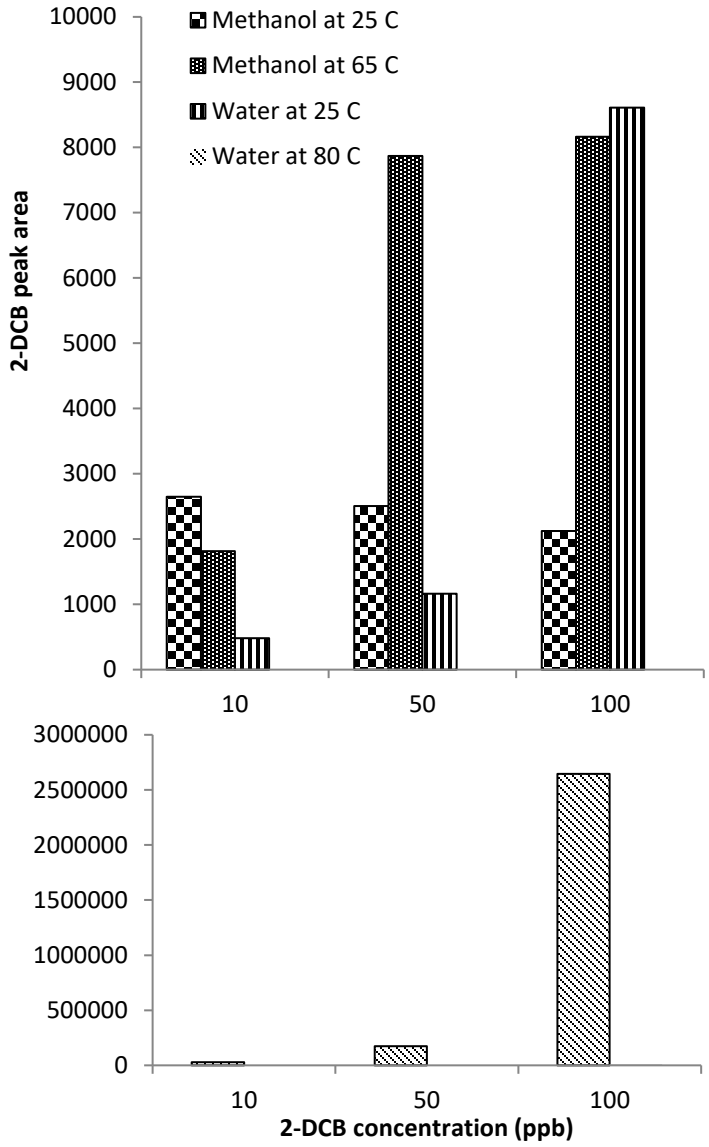


**Figure 4.1 The effect of solvent and incubation temperature on solid phase micro extraction of 2-DCB**

Increasing the incubation temperature from 25 to 80 °C resulted in an increase in the 2-DCB peak area from 8,600 to 2,600,000 (Figure 1, for the concentration of 100 ppb in water), which shows the profound effect of temperature on the extraction of 2-DCB. This could be due to increasing 2-DCB's volatility and release in the headspace at higher temperatures. For this

reason, a careful monitoring of the temperature inside the vials during incubation was performed to minimize possible error/variability caused by slight changes in temperature. The temperature equilibirium inside the CJT-water slurry was reached to 80 °C in 30 minutes (figure 4.2). Hence, for the analysis of samples, a 30-minute incubation step at 80 °C was done before introducing the SPME fiber into the headspace.

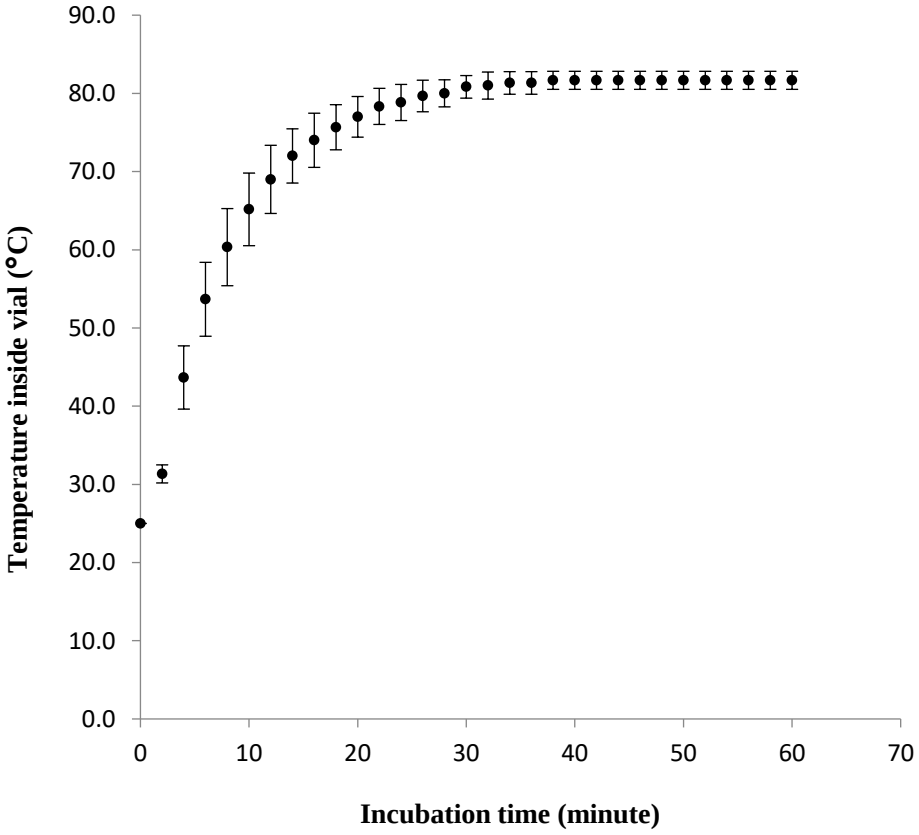


**Figure 4.2 The change in water temperature inside the vial over time in 85°C heating blocks. Data is the average of three replicates, and bars indicate the standard deviation.**

## Matrix effect

Extraction of an analyte from the head space mainly depends on the volatility and release of the analyte from the liquid or solid phase to the gas phase. Food matrix composition may bind

2-DCB at various degrees to modulate its volatility, and eventually affect the extraction efficiency. Cho, Kong, and Oh (2003) showed that an aqueous solution's ionic strength also affects the SPME by changing the vapor and partial pressure, and surface tension, which could lead to variation of the vapor-liquid equilibrium. With the same reason, Soncin, Panseri, Rusconi, Mariani, and Chiesa (2012) stated that the results obtained from adding standard 2-DCB to water will be different from those obtained in irradiated food samples due to the effect of food matrix on the equilibrium. Figure 4.3 (A) shows headspace SPME analysis of 2-DCB added to pure water. However, figure 4.3 (B) and (C) show results of experiments made by adding 2-DCB to a slurry of ground chicken jerky and water. As expected, the area obtained from pure water is much higher than the area obtained from the slurry (about 1.5 million vs 3,000 for 150 ppb). This could be explained by the effect of food matrix (chicken meat) on volatility and release of 2-DCB to the headspace. The interaction of 2-DCB with various food components (protein, fat, etc) may lower its volatility and its release to the headspace. As some manufacturers treat chicken meat with glycerol for the production of jerky, this could result in different extraction rate of 2-DCB in different products on the market. Hence, a comparison was made between chicken jerky without glycerol (figure 4.3, B) and chicken jerky that were made with adding glycerol (figure 4.3, C). The matrix effect for both samples were similar and no significant difference were found between the curves made for chicken jerky with and without glycerol. The chicken jerky treated with glycerol contained 5% higher moisture content, but due to the hygroscopic effect of glycerol, the water activity remained equal for both samples dried with and without addition of glycerol (Table 4.1).

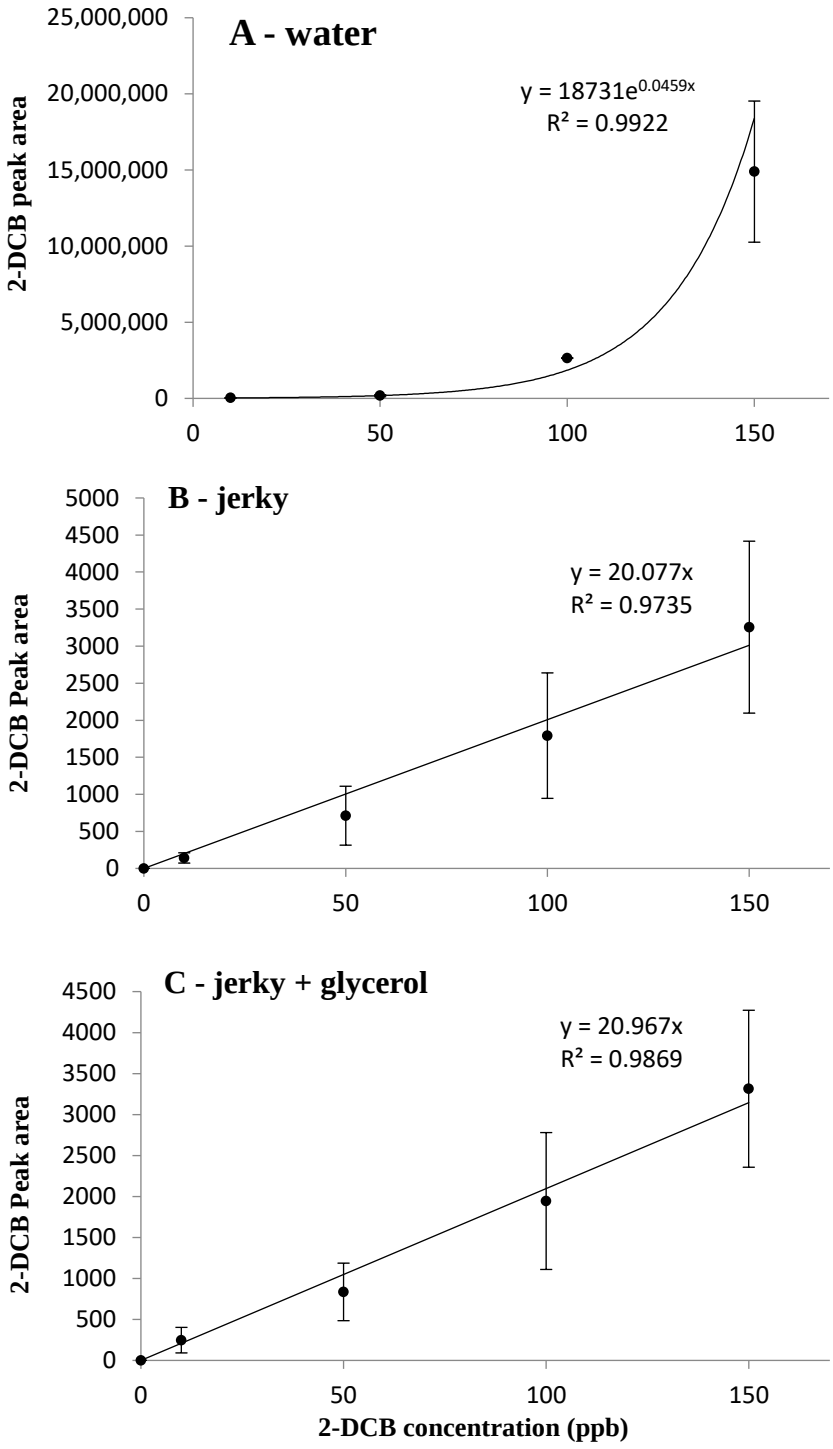


**Figure 4.3 Standard curves made with (A) spiking water, (B) chicken jerky that were dried without using glycerol and (C) with using glycerol from 10 to 150 ppb. Data is the average of four replicates and bars indicate standard deviation.**

**Table 4.1 The proximate analysis results of chicken jerky that are produced with glycerol and without using glycerol.**

| Component | Without Glycerol | With Glycerol |
|---|---|---|
| **Protein (g/100g)** | 85.3 | 69.2 |
| **Fat (g/100g)** | 2.6 | 2.1 |
| **Glycerol (mg/100g)** | <200 | 12700 |
| **Moisture (g/100g)** | 11.2 | 16.2 |
| **Water activity** | 0.625 | 0.601 |

The relationship in standard curves made using jerky without glycerol (Figure 4.3B) and jerky with glycerol (Figure 4.3C) was linear, while a logarithmic relationship was seen in standard curve made with spiking pure water (Figure 4.3A). Considering the amphiphilic structure of 2-DCB, it is possible that the attachment of 2-DCB to the fiber provides more bonding sites to adsorb more 2-DCB at higher concentrations of 2-DCB in the headspace (Figure 4.3A).

Overall, presence of glycerol has no matrix effect on the extraction of 2-DCB. However, the food matrix has a significant effect on the SPME of 2-DCB as compared to pure water. Hence, a matrix matched standard curve is needed for quantitation of 2-DCB.

## Effect of dilution factor

Dilution of jerky powder helps for better heat transfer, distribution of compounds, and magnetic stirring. The amount of dilution was one the factors that was changed to improve linearity and variability of data. Since generally at very low concentrations results show more sensitivity to various errors, we used concentrations as low as 1, 5, and 10 ppb of 2-DCB with three dilution factors of 4, 5, and 6. Figure 4.4 shows that dilution factor of 5 (one part jerky powder, four parts water) leads to the highest linear regression with lowest variability. The reason for low consistency in the results for the dilution factor of 4 could be coagulation of

proteins. When using low dilution, a coagulate was observed at the top of mixture, which could trap and lower the release of 2-DCB into the headspace.

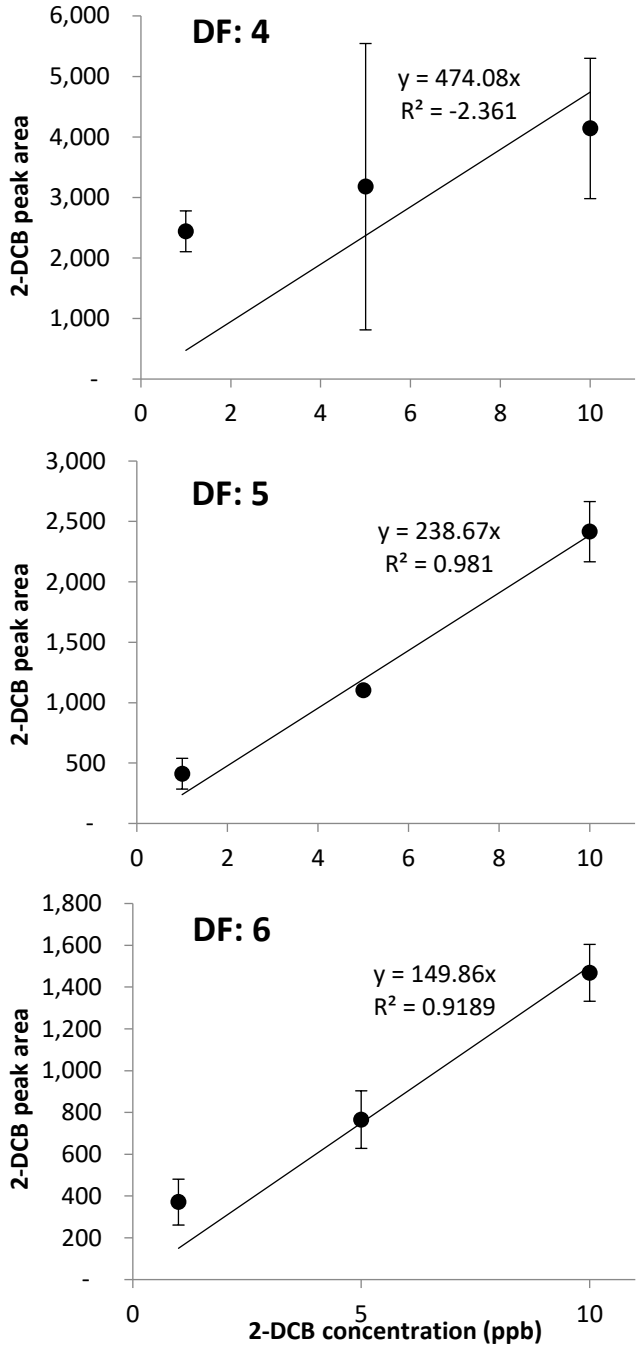


**Figure 4.4 The effect of three different dilution factors of 4 (DF:4), 5 (DF:5), and 6 (DF:6) when mixing water and jerky powder on extraction of 2-DCB at three concentration of 1, 5, and 10 ppb. The data is the average of three replicates, and bars indicate standard deviation.**

## Effect of SPME fiber stationary phase

One of the important factors that affect the SPME efficiency is the affinity of analyte to fiber's stationary phase. Caja, Ruiz del Castillo, and Blanch (2008) showed that among different fiber coating materials Poly Dimethyl Siloxane (PDMS) has the highest affinity for 2-DCB producing the highest peak area in GC-MS analysis. However, in that study 2-DCB could not be detected in jerky samples that were irradiated at 2 kGy doses or lower. Generally, the selection of fiber is based on polarity and molecular weight of analyte (Selection guide, 2018). In the current study, four different stationary phases with various coating diameters were selected. Polydimethylsiloxane (PDMS) with two coating diameters of 7 and 100 µm, carboxen/polydimethylsiloxane (CAR/PDMS) with coating diameter of 75 µm, and polydimethylsiloxane/divinylbenzene (PDMS/DVB) with coating diameter of 65 µm were initially tested for their affinity to 2-DCB (figure 4.5A). PDMS100 µm and PDMS/DVB 65 µm showed the highest affinity to 2-DCB and two separate standard curves were made using these two types of fiber (figure 4.5B). Comparing the standard curves shows that PDMS/DVB 65 µm result a lower variability and higher linear regression value.

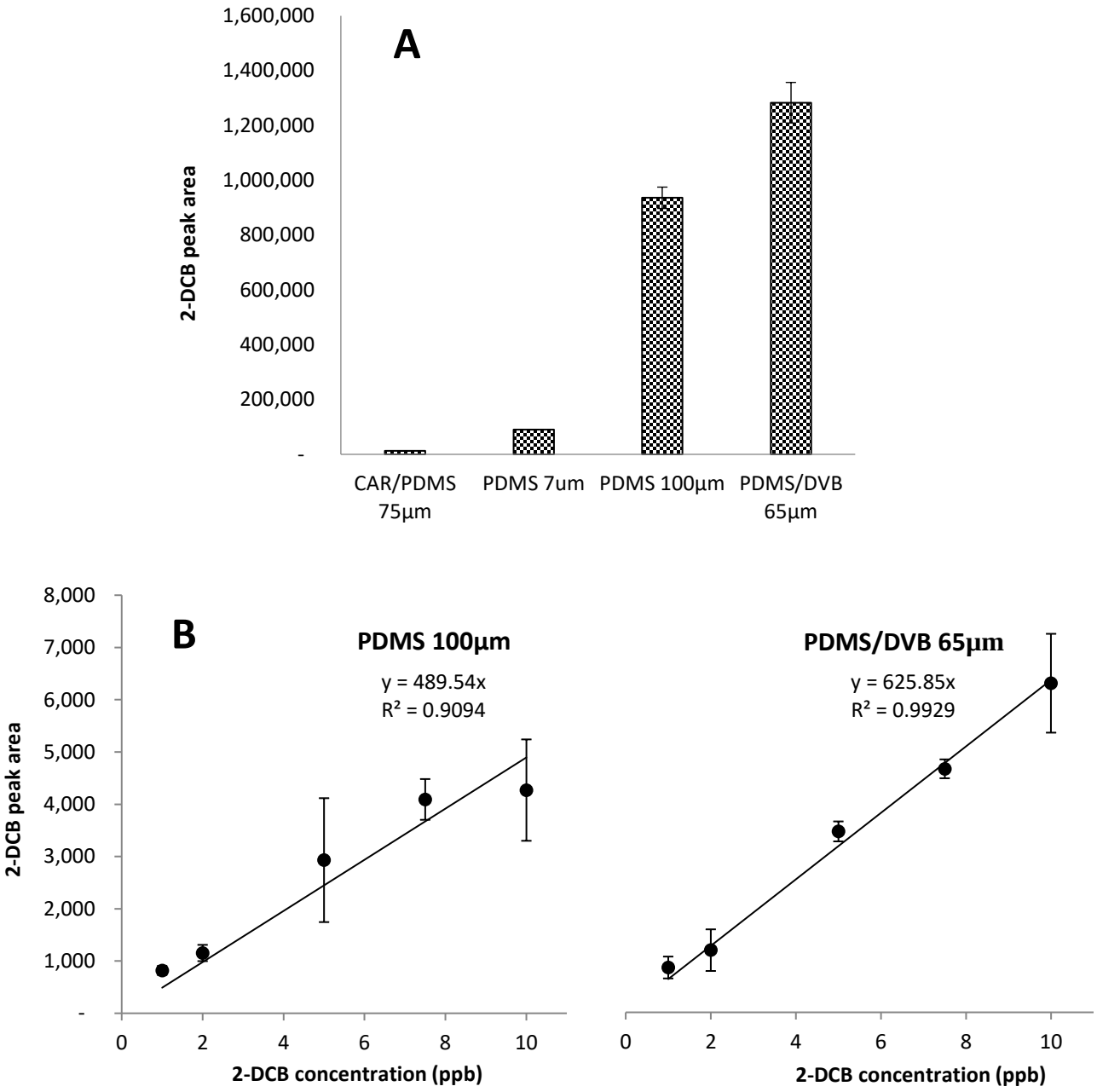


**Figure 4.5 A: The peak areas obtained from the analysis of 5 ppb 2-DCB standard in water with SPME fibers made of four different stationary phases/coating diameters. The data is the average of two replicates. B: Comparison of using PDMS/DVB 65 µm with PDMS 100 µm fiber stationary phase in analysis of 2-DCB mixed with water and jerky at 1 to 10 ppb. Data is the average of three replicates. Bars indicate standard deviation.**

## Analysis of 2-DCB in irradiated jerky

The 2-DCB concentration in CJT linearly increased from 0 to 369 ppb with irradiation from 0 to 50 kGy (Figure 4.6). Generally, the use of 2-alkylcuclobutanones as irradiation marker is suggested when the fat content of the food product is higher than 1% and an irradiation dose higher than 0.5 kGy is used (Barreira, Antonio, & Ferreira, 2018). Chicken breast meat is composed of 2.6 percent fat (table 4.1) and only 20 percent of its fat is palmitic acid, the substrate for formation of 2-DCB. However, we showed that SPEM technique can be used to quantify 2-DCB at such small concentrations. Additionally, the percentage of palmitic acid appears to be consistent among different commercial breeds of chicken (Boschetti, Bordoni, Meluzzi, Castellini, Dal Bosco, & Sirri, 2015). For this reason, variation coming from palmitic acid content was not a concern in this study. But food matrix has a significant effect on the SPME analysis of 2-DCB (as described in section 3.2). Hence, it is important to note that the obtained dose-response graph in this study was made using CJT with 69 to 85% protein, 2.1 to 2.6% fat, 0 to 13% glycerol, and 11 to 16% moisture content (table 4.1) prepared under the conditions described in this study.

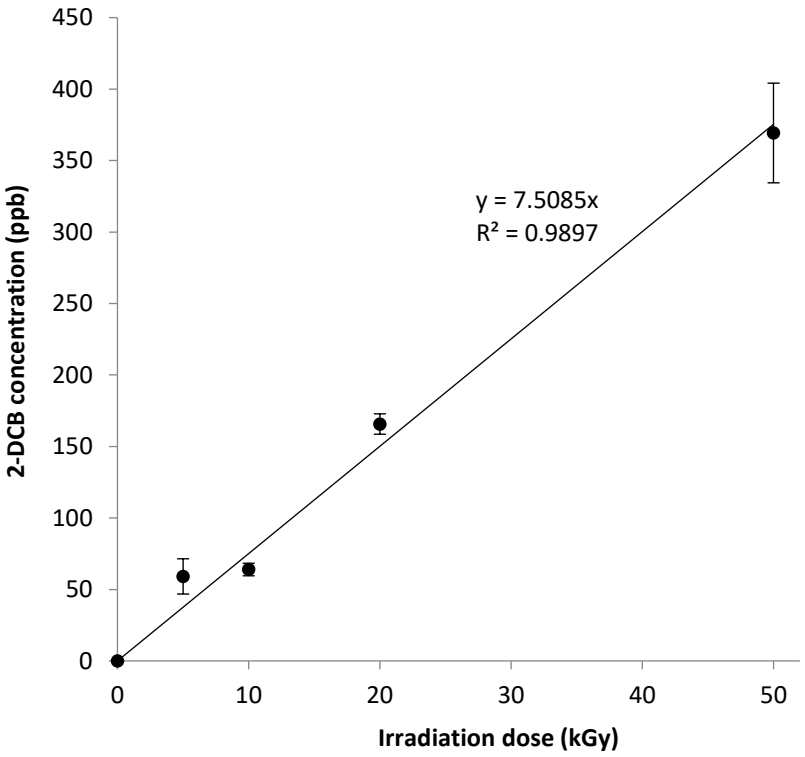


**Figure 4.6 Irradiation dose response of 2-DCB in chicken jerky treated with glycerol. The data is average of three replicates, and bars indicate standard deviation. This experiment was not performed blinded (doses were known by the examiner).**

## Conclusion

Food matrix, incubation temperature, diluting solvent, dilution rate, and fiber stationary phase are the most important factors that should be taken into account for the extraction of 2-DCB. The critical part of this method is the transition of 2-DCB from liquid to gas phase during incubation and regarding the possible influence of food matrix on either this transition or signal suppression in MS, it is important that the standard curve is made using the same food matrix as the sample that is analyzed for quantitation. However, the addition of glycerol to the formulation of chicken jerky does not affect the extraction rate.

## Acknowledgment

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# Chapter 5 : Quantification of 2-dodecylcyclobutanone (2-DCB) in γ-irradiated chicken jerky treats using SPME-coupled GC-MS technique[1]


**Abstract:**

There have been health concerns regarding the consumption of irradiated chicken jerky treats (CJT), and there is a lack of an established method to quantify the irradiation dose within the legal limits. In this study, we developed an environmental-friendly and sensitive method based on SPME-coupled GC-MS analysis. The formation of an irradiation-specific lipid decomposition compound, 2-dodecylcyclobutanone (2-DCB), was measured in CJT samples prepared with or without glycerol and irradiated at 0 to 50 kGy using Cesium-137. SPME was performed at 80 °C for hydrated CJT (1:5 ratio). The quantification of irradiation dose was based on GC-MS analysis of 2-DCB-spiked CJT (0 to 640 ng/g CJT) for the selected ions m/z 84, 98, 112. The concentration of 2-DCB increased linearly (10.8 ppb/kGy, R2 = 0.999) from 0 to 543 ng/g CJT with irradiation from 0 to 50 kGy. Addition of glycerol to the formulation of chicken jerkies did not significantly affect the formation or measurement of 2-DCB. SPME-coupled GC-MS analysis can serve as a suitable technique to quantify 2-DCB concentrations in CJT as irradiation marker.


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1. Taghvaei, M., Sommers, C., Ceric, O., Hussain, F., Smith, J. S., Yucel, U. (2019) Quantification of 2-dodecylcyclobutanone (2-DCB) in γ-irradiated chicken jerky treats using SPME-coupled GC-MS technique. Manuscript submitted.

# Introduction

Ionizing radiation, commonly known as irradiation, is a non-thermal process used for sterilization, disinfection, decontamination, shelf-life extension and pathogen destruction of both human and pet food products (Smith & Pillai, 2004). The irradiation process is approved as safe below the determined doses (FDA, 2015). However, over the last decade or so there have been concerns for the safety of irradiated pet food products due to numerous cases of illness and death in dogs and cats associated with the consumption of chicken jerky treats (CJT) in USA and dry cat food in Australia, respectively (FDA, 2019; Burke, 2009). Despite detailed biological and chemical analyses of the affected animals and related products by official and private institutions, the causation is still unclear. Ensuring the products are irradiated within the legal limits remain as an important checkpoint. The current available standard method for detection of 2-DCB in foodstuff (European committee for standardization, 2003) only shows the presence/absence of irradiation treatment and does not measure the irradiation dose used for the product. This method also consists of timely Soxhlet extraction of fat with hexane and several steps of cleanup and drying. There is, therefore, a large need for a suitable method for quantification of the irradiation dose in pet food products, such as CJT, after treatment. Such method should be universal, precise, accurate, fast and easy. A suitable method should also be able to measure compounds that are specific to irradiation, are formed more at higher irradiation doses, and are stable throughout the shelf life of the product. Amongst these, the specificity, stability and formation range of the analyte being monitored is utmost importance that determine the reliability of the analytical method. In low-moisture food matrixes, irradiation-specific lipid decomposition products such as hydrocarbons and alkylcyclobutanones (2-ACBs) are shown a linear dose response (Gadgil, Hachmeister, Smith, & Kropf, 2002; Hijaz, & Smith, 2010).

Specifically, 2-ACBs are irradiation-specific ketones formed from the fatty acid moieties of lipids through cyclisation of fatty acid with cleavage of ester bond, followed by a formation of a cyclobutanone ring that is attached to the hydrocarbon chain (Fan, 2013; Stewart, 2001). CJT contain small amounts of lipid (ca. 3%) with a 20% palmitic acid (Boschetti, Bordoni, Meluzzi, Castellini, Dal Bosco, & Sirri, 2015). 2-dodecylcyclobutanone (2-DCB) is known to form from palmitic acid moieties in irradiated CJT, and it can serve as an suitable analyte to monitor the irradiation history of the products (Fan, 2013).

Typically, 2-DCB forms at low (i.e., ppb levels) concentrations in lipid-containing food matrixes, such as meat products (Soncin, Panseri, Rusconi, Mariani, & Chiesa, 2012). Its detection is challenging and specific to the composition of food matrix. An ideal method should be user-friendly and clean while it is sensitive and repeatable. GC-MS can serve as a suitable method for analyzing low molecular weight compounds that are present in the food at low concentration, for which the sample preparation usually involves long and strenuous series of solvent extraction and clean up steps. Solid phase micro extraction (SPME) is an alternative sample preparation method to eliminate the use of solvent (i.e., except for solvent for mobilization of the analyte). The air in equilibrium with the sample serve as a solvent for the volatiles while a specific fiber captures and stabilizes them. It is known to be a fast and reliable technique to analyze volatiles at low concentrations (i.e., lower than ppt levels) without the need of sophisticated instrumentation and sample preparation. (Solid phase microextraction, 2019)

The SPME technique previously used for detection of 2-DCB in irradiated ground beef (Caja, Ruiz del Castillo, & Blanch, 2008; Soncin, Panseri, Rusconi, Mariani, & Chiesa, 2012) and dry-cured ham (Blanch, Caja, Flores, & Ruiz del Castillo, 2009). One study so far aimed for quantitation of 2-DCB in irradiated meat using SPME (Soncin, Panseri, Rusconi, Mariani, &

Chiesa, 2012). The performance of SPME extraction depends on extraction temperature, chemical properties of analyte and fiber material, and sample matrix.

The objective of this study was to develop a user-friendly, reliable and sensitive method based on SPME-coupled GC-MS analysis for quantification of irradiation dose in CJT prepared with different glycerol composition. The results of this study can serve as guidelines for regulatory agencies, such as Food and Drug Administration (FDA), for surveillance of the irradiated of pet food products, including CJT.

# Materials and Methods

## Materials

Analytical standards (2-DCB, 2,4-di(tert-pentyl)cyclohexanone, and 2-cyclohexylcyclohexanone) and food-grade glycerol (purity > 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The polydimethylsiloxane/divinyl benzene SPME fiber with stationary phase coating diameter of 65 µm (PDMS/DVB 65 µm) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Optima grade methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA). Raw chicken tenderloins (Tyson, Kings Mountain, NC, USA) were purchased from a local supermarket.

## Sample preparation

CJT samples were prepared based on the method developed by the FDA Veterinary Laboratory Investigation and Response Network scientists (FDA VetLIRN). Briefly, the chicken breast tenderloins (ca. 4kg) first coated with glycerol (150 mL) by mixing in a food grade container and massaging into the meat for 2 min prior to drying. Coated and non-coated samples were then separately dried at 68 °C for 48 hours in a food dehydrator (Sedona SD-P9000, Korea)

to obtain CJT. CJT samples were irradiated at 0, 5, 10, 20, and 50 kGy using a Cesium-137 source in individual LDPE bags (Ziplock) at the USDA Eastern Regional Research Center (Windmore, USA). The samples were over-night shipped under refrigerated conditions, and stored at -80 °C until used.

## Standard solutions

A stock solution of 2-DCB (1000 ppm) was prepared by dissolving the entire bottle of pure standard (5 mg) in methanol (5 mL). Similarly, a stock solution of 2,4-di(tert-pentyl)cyclohexanone and 2-cyclohexylcyclohexanone (1000 ppm) was prepared by dissolving entire bottle of pure standard (250 mg) was dissolved in methanol (250 mL). A working standard solution (1 ppm) was prepared daily by subsequent dilution of stock solution in water. Stock solutions were kept at -80 °C for a maximum of 1 month until used.

## Standard curve and sample preparation

For making the standard curve, Standard curves were prepared using non irradiated CJT (i.e., control samples) and 2-DCB analytical standard. The CJT samples (3 g) were first ground to a fine powder with a Waring blender (model 34FL97, Waring, Stamford) (i.e., particle size < 100 µm) for ca. 1 minute. Ground sample (1.25 g) was transferred into a siliconized glass vial (20 mL), and mixed with water (5 mL) and aliquots of 2-DCB standard solution were added to final concentrations of 5, 10, 25, 50, 75, 200, and 400 ng/g CJT. The IS (2,4-di(tert-pentyl)cyclohexanone or 2-cyclohexylcyclohexanone) was also added to the concentration of 80 ng/g CJT. The irradiated CJT prepared the same way but without adding 2-DCB. The mixture was homogenized in a high-shear mixer (Omni International TH01, USA) at the highest speed (35,000 rpm) for at least 1 minute. Then, a magnetic stirring bar (15×8 mm) was added to the mixture, and the vials were sealed with aluminum crimp cap interfaced with

polytetrafluoroethylene/white silicon liner for the subsequent SPME. The conditions for the SPME method were determined in a parallel study (Taghvaei, Sommers, Ceric, Yucel, Hussain, & Smith, 2019). Briefly, the sealed vials were incubated at 80 °C in a heating/stirring block (Pierce Heating/Stirring Module, USA) with stirring for 30 minutes to allow temperature equilibrium. The SPME fiber (PDMS/DVB 65 µm) was then inserted and equilibrated with headspace for 15 min to adsorb volatile analyte.

## Gas chromatography mass spectroscopy analysis

Following the extraction, the SPME fiber was exposed to a GC-MS chromatograph's (HP 5890, Agilent Technologies, USA) equipped with an HP-5MS (60 m × 0.25 mm × 0.25 µm) column. The GC-MS conditions were adapted from Hijaz and Smith (2010) with some modifications. The analyte was desorbed from the SPME fiber at the injector port (for 1 minute at 250 °C). The oven temperature initially was kept at 55 °C for 0.5 minutes and then brought up to 200 °C at a rate of 20 °C per minute, follow by 15 °C per minute up to a final temperature of 270 °C. The fragment ions with m/z of 139, 153, and 168 were monitored between 0 to 13 minutes for IS, and fragment ions with m/z of 84, 98, and 112 were monitored after that for the detection of 2-DCB. The ions were quantified by HP MS 5972 mass detector at 280 °C. The initial identification of the retention times and ion fragments were confirmed with monitoring m/z of 30 to 450 in scan mode, and compounds were identified by comparing their mass spectrum with those of the National Institute of Standards and Technology (NIST) database.

## Quantification of the irradiation dose

The area under the curve (AUC) was obtained by manual integration of peaks (valley to valley). A standard curve was prepared by dividing the AUC of the 2-DCB peak (0-400 ng/g CJT) with the AUC of IS (80 ng/g CJT) peak. Similarly, the concentration of 2-DCB in

irradiated samples were determined by using the ratio of AUC of the 2-DCB peak (0-50 kGy) with the AUC of IS (80 ng/g CJT) peak (i.e., concentration = ratio x slope of the standard curve). The concentration of 2-DCB was then plotted against samples irradiated from 0 to 50 kGy.

### Statistical analysis

Figures and tables were prepared in MS Excel (Microsoft Office, 2010). The significance of difference was analyzed using ANOVA, and individual treatments were compared using post-ANOVA Tukey's multiple compassion test using Minitab (v17, Minitab Inc).

## Results and discussion

### Mass chromatogram

As the first step, methanol solutions of 2-DCB analytical standard (100 ppm) and IS (2,4-di(tert-pentyl)cyclohexanone, 100 ppm) was analyzed by direct injection to the GC-MS setup, and characterized for their gas chromatogram and mass spectra for ion fingerprinting between 30 to 450 m/z (Figure 1). Figure 5.1A shows the ion fragments obtained from 2-DCB peak, and Figure 5.1B shows the ion fragments from internal standard IS peak. Three ion fragments for 2-DCB (84, 98, and 112 m/z) and IS (139, 153, and 168 m/z) were selected and monitored by MS for subsequent quantification for improving the level of detection and quantification. Selection of ion fragments was based on abundance (i.e., selection of ion fragments with higher abundance to improve detection) and molecular weight (i.e., selection of ion fragments with higher molecular weight to lower the probability of having the same ion fragment from another compound). In the selected ion mode (SIM) analysis the MS detector was set to monitor the fragment ions of 139, 153, and 168 for internal standard IS between 0 to 13 minutes, and the ion fragments of 84, 98,

and 112 were monitored for 2-DCB from 13 to 14.6 minutes (Figure 5.1C). The IS and 2-DCB were well-separated with respective retention times of 12.22 and 13.45 minutes.

In order to confirm the identity of the analyte and IS, and the measurement efficiency, ion ratios were monitored for all experimental conditions (Figure 5.2). Figure 5.2A shows the relative abundance of 2-DCB ions obtained from CJT spiked with 5 to 400 ng/g CJT 2-DCB. For 2-DCB at all concentrations, ion fragment 98 had the highest relative abundance, with ions 84 and 112 having similar but lower relative abundance. This ratio provides a valuable criteria complementary to the retention time for identification and approval of the peaks used in quantification. Figure 5.2B shows the relative abundance of IS ions for internal standard at the constant concertation (80 ng/g CJT) for the same CJT spiked with 2-DCB (from 5 to 400 ng/g CJT). For all samples, the ion fragment 153 had the highest relative abundance followed by ions 139 and 168. The relative abundance of ions for internal standard did not significantly changed by increasing the concentration of 2-DCB from 5 to 400 ng/g CJT. This shows that increasing the concentration of 2-DCB in samples does not significantly affect the adsorption of internal standard by competing for fiber's adsorption sites.

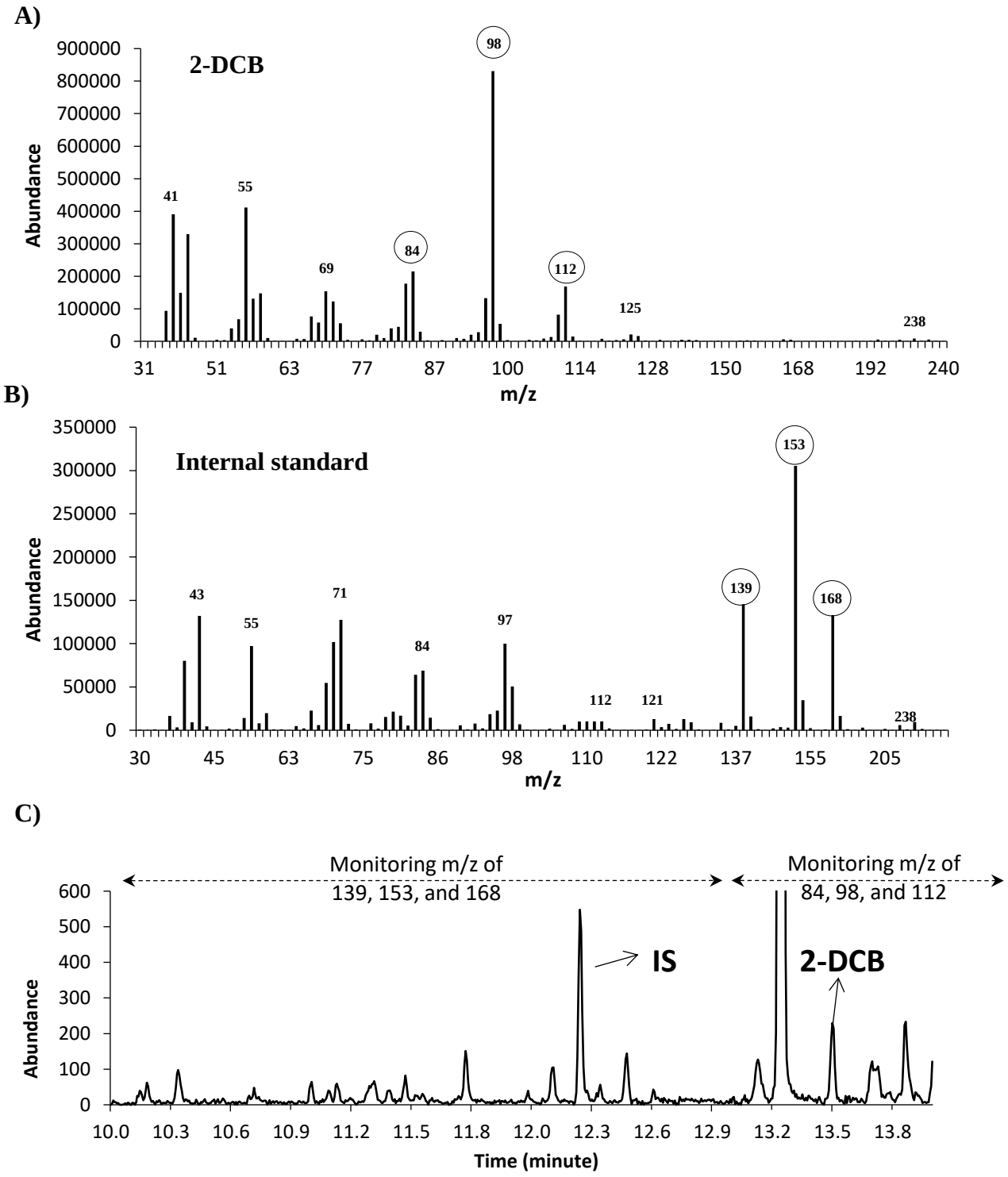
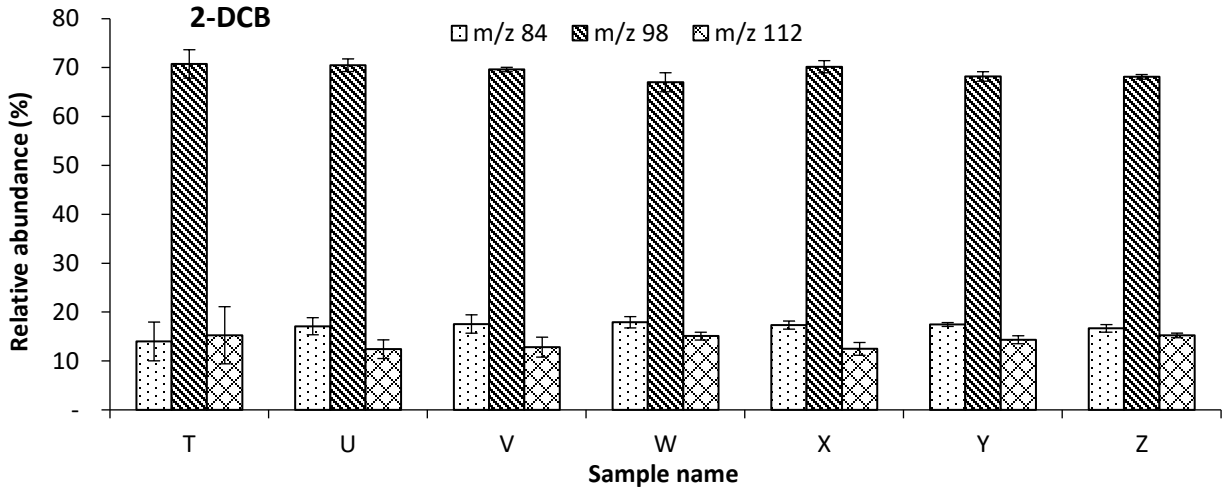



**Figure 5.1 The ion fragments obtained from a direct injection of A: 2-DCB (100 ppm) and B: IS (100 ppm) in hexane monitoring m/z of 30 to 450. C: The chromatogram obtained from adding 5 ppb of 2-DCB and IS to the chicken jerky in SIM mode. Ion fragments of 139, 153, and 168 were monitored up to minute 13, and from that point on ion fragments of 84, 98, and 112 were monitored.**

**A)**




**B)**

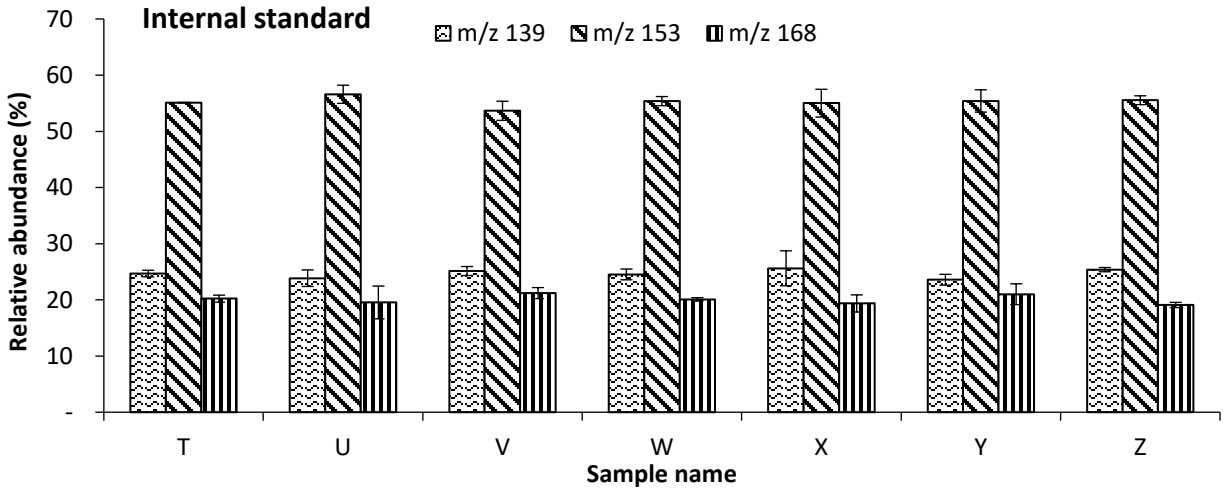


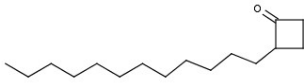
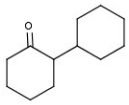
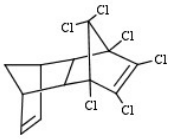
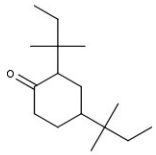

**Figure 5.2 The relative abundance of A: 2-DCB and B: internal standard ions obtained from CJT spiked with 5ppb IS and 5 (T), 10 (U), 25 (V), 50 (W), 75 (X), 200 (Y), and 400 (Z) ng/g CJT 2-DCB. Data is the average of three replicates, and bars indicate standard deviation.**

## IS selection and efficiency

We compared the efficiency of 2,4-di(tert-pentyl)cyclohexanone as IS to that of the 2-cyclohexylcyclohexanone internal standard as suggested by European committee for standardization (2003). 2-cyclohexylcyclohexanone was shown to be as a suitable IS for quantification of 2-DCB in previous studies based on solvent extraction methods (D'Oca et al.,

2009; Kwon, Akram, Nam, Lee, & Ahn, 2011). SPME technique extraction entails migration of compounds from liquid to gas phase (headspace) and parameters such as molecular weight and flash point (volatility) that are less important in solvent extraction, play more crucial role in SPME extraction technique. Table 5.1 shows the physicochemical properties 3 potential internal standards in comparison to 2-DCB. Isodrin was another IS suggested by Tewfik (2008), who used it in a rapid solvent extraction method. However, the physicochemical properties of isodrin was not similar to 2-DCB, and was not considered further (Table 5.1).

**Table 5.1 The physical properties of three different compounds considered to be used as internal standard compared with 2-DCB.**

| Name | Molecular weight | Log (p) | Flash point (˚C) | Boiling point (˚C) | Reference |
|---|---|---|---|---|---|
| **2-DCB**  | 238 | 5.27 | 134 | 311 | ChemSrc (2018a) |
| **2-cyclohexylcyclohexanone**  | 180 | 3.32 | 121 | 269 | ChemSrc (2018b) |
| **Isodrin**   | 364 | 5.32 | 186 | 384 | ChemSrc (2018c) |
| **2,4-di(tert-pentyl)cyclohexanone**  | 238 | 5.27 | 129 | 303 | Chemspider (2018) |

The same irradiated CJT samples (0, 5, 10, and 30 kGy) were prepared with the 2-cyclohexylcyclohexanone (i.e., EU-suggested IS) the same way for comparison with the 2,4-

di(tert-pentyl)cyclohexanone. The efficiencies of the two IS were evaluated by comparing the ratio of 2-DCB AUC to AUC of two IS. The dose-response increase linearly for both cases; however, the slope of the curve with 2,4-di(tert-pentyl)cyclohexanone (0.0219) was an order of magnitude higher than that of 2-cyclohexylcyclohexanone (0.0034). The higher slope yields a better differentiation of samples irradiated at different doses. Therefore, we can conclude that using 2,4-di(tert-pentyl)cyclohexanone as IS for SPME analysis of 2-DCB is a better choice than the IS originally introduced by European method for quantification purposes.

2-DCB was added to pure water and a mixture of CJT and water at two concentration of 50 and 100 ng/g CJT. AUC of 2-DCB peak in CJT was three orders of magnitude lower than the 2-DCB in water at the same concentration (Figure 5.3). The smaller headspace concentrations in CJT are due to interaction of the analyte with the complex food environment. Therefore, we prepared the standard curves for dose-quantification by spiking non-irradiated CJT with 2-DCB and IS (Figure 5.4).

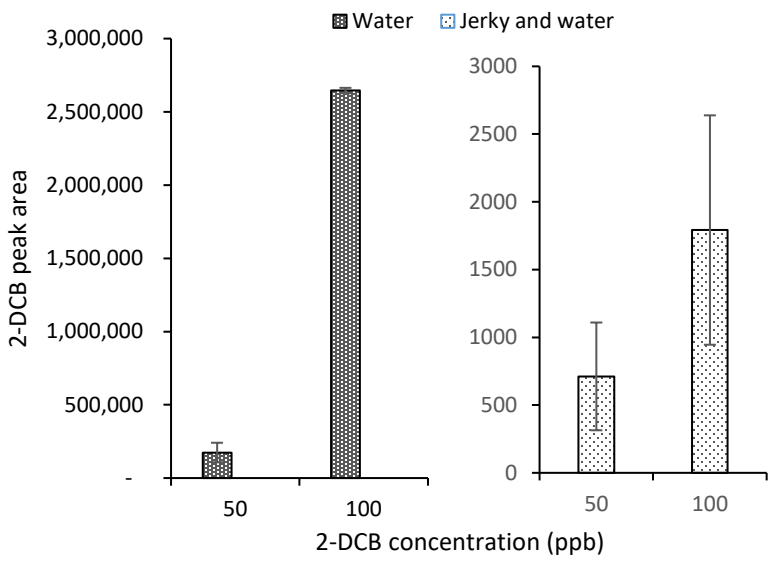
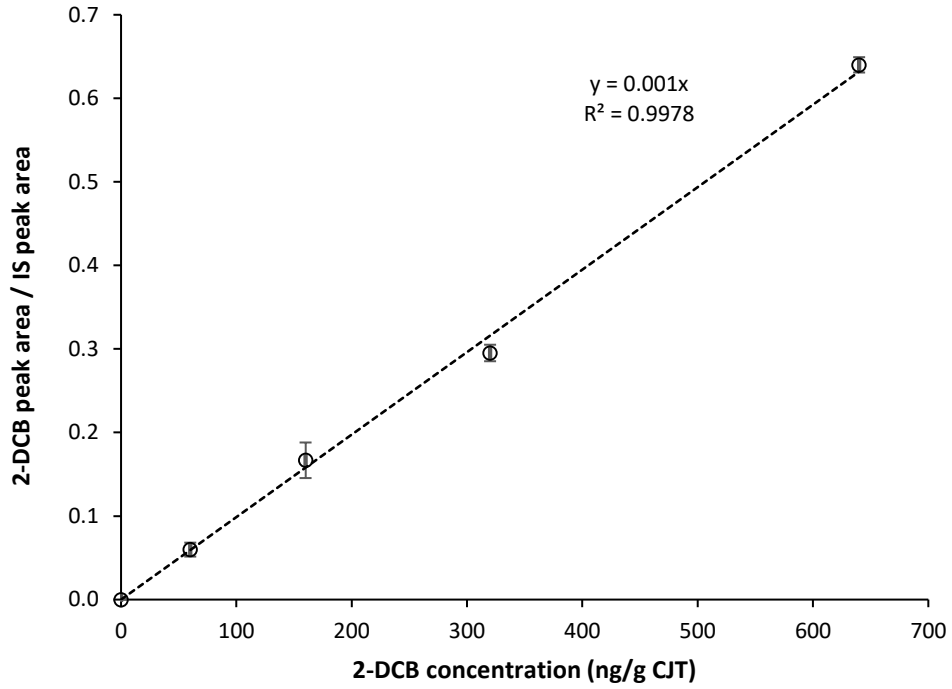



**Figure 5.3 The 2-DCB peak area obtained from spiking water and water-jerky slurry at 50 and 100 ppb. The data is the average of four replicates, and bars indicate standard deviation.**

Two different concentration of IS was used to prepare the standard curve. Figure 5.4A shows the standard curve obtained from 80 ng/g CJT of internal standard. Figure 4B shows the standard curve obtained from 5 ng/g CJT of internal standard. The addition of IS at 80 ng/g CJT improved the linear regression coefficient from 0.996 to 0.998. This could also be due to the fact that using closer concentration of IS to the middle range of 2-DCB concentration helps for better correction of various experimental errors by IS. The standard curve using 80 ng/g CJT of IS was chosen to quantitate the concentration of 2-DCB in irradiated samples.

**A)**




**B)**

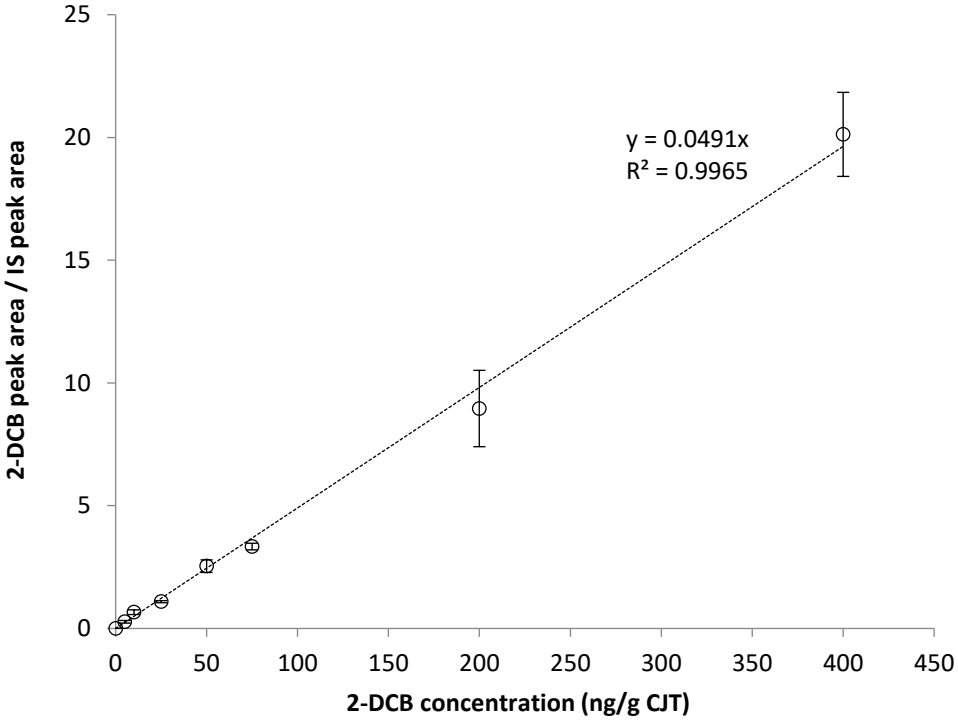



**Figure 5.4 The standard curve obtained from A: 80 ng/g CJT B: 5 ng/g CJT of internal standard. Data is the average of three replicates and bars indicate standard deviation. The dash line shows linear regression line.**

## Quantification of 2-DCB in irradiated CJT

The ratio of AUC 2-DCB to AUC IS was obtained in irradiated samples. The concentration of 2-DCB was calculated using the trendline from figure 5.4A. The addition of the IS to the CJT samples is a critical step in method development. In order to account for the effects of grinding step in potential analyte losses, we compared the effect of adding IS before and after this step. Figure 5.5A shows the results obtained from spiking CJT (with and without glycerol) with IS after grinding during homogenization by adding 10 µL to the 6.25 mL slurry to a final concentration of 80 ng/g CJT. Figure 5.5B shows the results obtained from spiking of IS directly into the CJT powder during grinding. A higher trendline slope was obtained by adding IS to slurry (e. g. 8.10 vs 12.89 ppb/kGy for CJT without glycerol). A higher slope corresponds to better distinction of doses to measure irradiation history of unknown samples. For this reason, addition of IS to slurry was used to quantitate 2-DCB in irradiated samples.

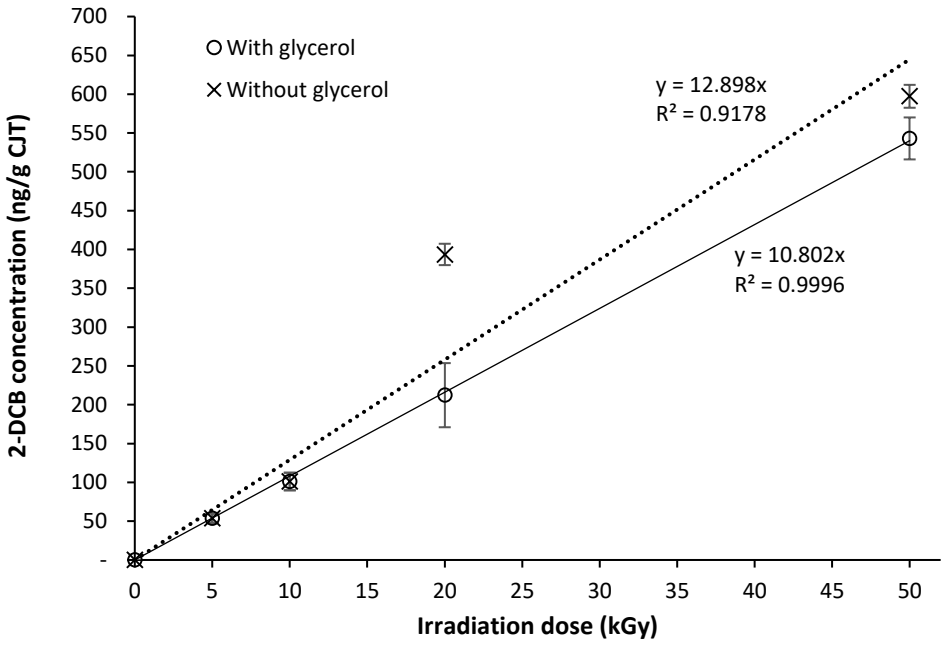
The 2-DCB concentration in irradiated CJT (0 to 50 kGy) increased linearly with a slope of 10.8 ppb/kGy and linear regression coefficient of 0.999 in samples with glycerol (Figure 5.5A). This increasing trend was expected since 2-DCB is a compound produced specifically by irradiation. Soncin, Panseri, Rusconi, Mariani, and Chiesa (2012) also showed that 2-DCB's concentration linearly increases in irradiated ground beef at higher irradiation dosages from 0.5 to 8 kGy. Although using a different sample, the 2-DCB production rate in their study was close to our results at 13.87 ppb/kGy.

The presence of glycerol didn't affect the dose-response ($p < 0.05$). Figure 5.5B, for example, shows a similar trendline slope of 8.1506 for samples with glycerol and 8.106 for samples without glycerol. Some manufacturers treat chicken meat with glycerol before drying to achieve desired organoleptic properties. Glycerol is a sugar alcohol with three hydroxyl groups

that provide a potential site for hydrogen-bonding with water (Ockerman, 1991). But since hygroscopic effect of glycerol results in higher moisture content in samples treated with glycerol, the effect of water's radiolytic products on occurrence of 2-DCB was a concern. It could be concluded that the occurrence of 2-DCB in chicken jerky is not affected by the presence of glycerol or little variation in moisture content.

2-DCB was not observed in the control (i.e., untreated samples) as expected. This is similar to what is reported before. Moreover, Caja, Ruiz del Castillo, and Blanch (2008) showed that the detection limit of 2-DCB was 2 kGy in irradiated ground beef. Although Variyar, Chatterjee, Sajilata, Singhal, and Sharma (2008) showed the occurrence of 2-DCB in not irradiated cashew nut and nutmeg samples, their study has not been confirmed by another independent study. Further investigation of the Joint Research Centre (JRC) of the European Commission did not approve the existence of 2-DCB in not irradiated cashew nut and nutmeg (Breidbach, & Ulberth, 2015).

**A)**




**B)**

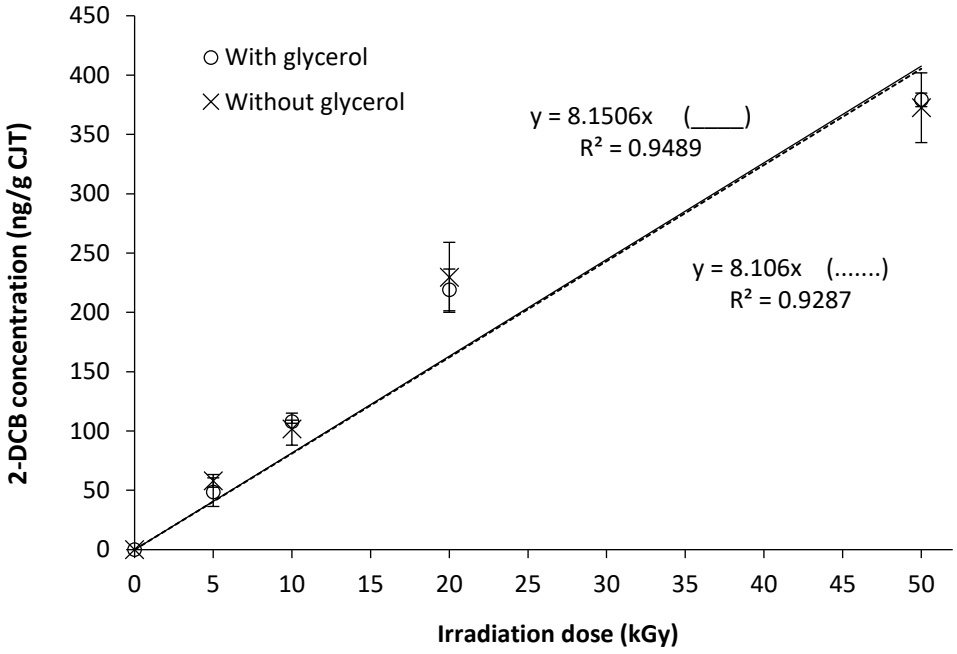



**Figure 5.5 A: 2-DCB concentration in irradiated CJT with glycerol and without glycerol (0 to 50 kGy) obtained from the addition of IS to A: the slurry of CJT and water and B: CJT powder during grinding. The data is the average of three replicates and bars indicate standard deviation. The solid line and dash line show the linear trendline of CJT with and without glycerol, respectively.**

## Detection limit

An analysis of jerky sample mixed with 2-DCB with a concentration as low as 50 part per trillion (ppt) was performed and part of the resulting chromatogram is shown in figure 5.6. The obtained signal from this analysis is still eight times higher than noise in blank analysis. Although the occurrence of 2-DCB in irradiated chicken jerkies are much higher than 50ppt, this shows the ability of the proposed method in this study to detect 2-DCB. Concentrating the analyte on a fiber, combined with lowering the noise in MS by using SIM mode, probably allowed for such a high detection capability.

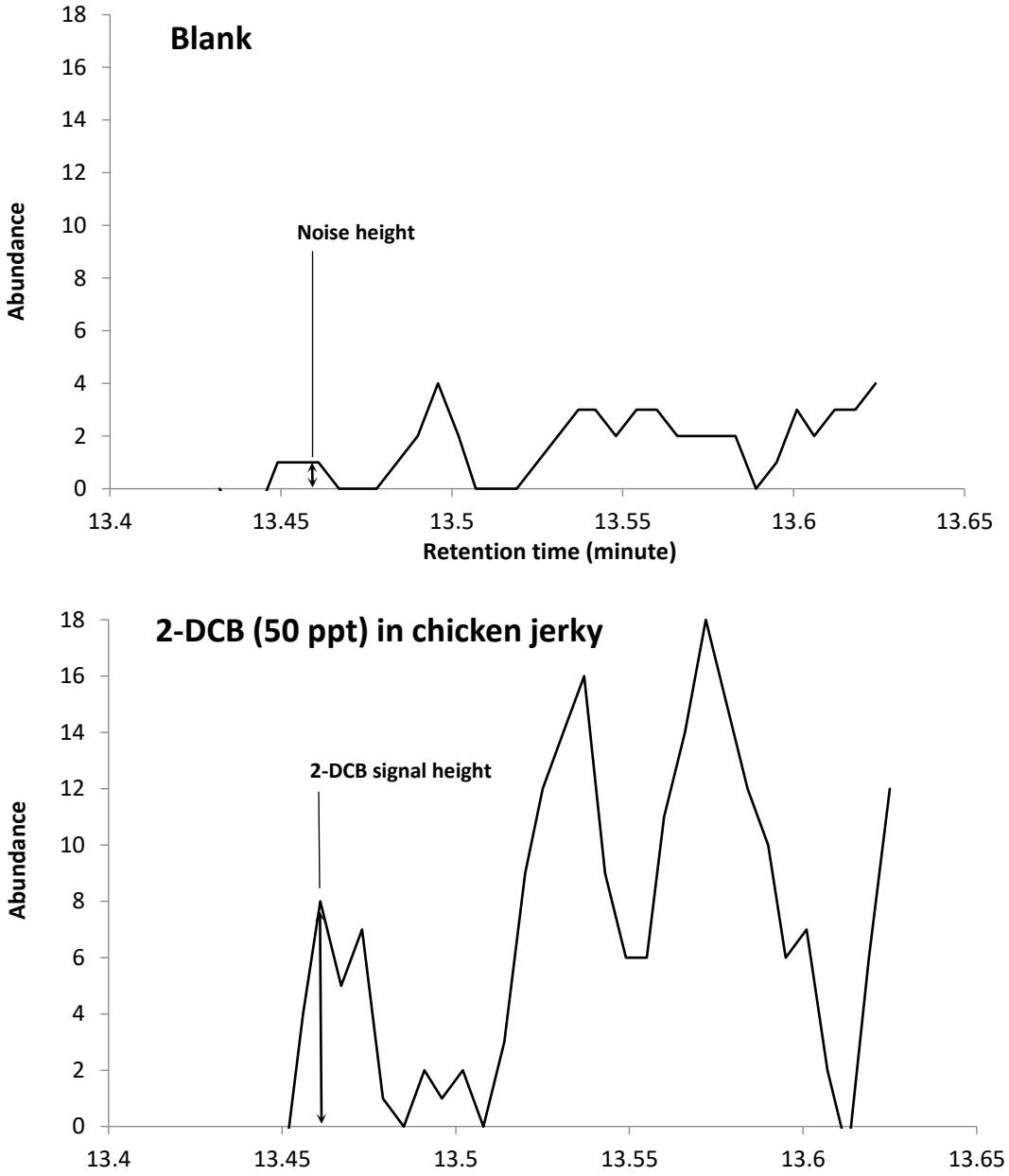



**Figure 5.6 The comparison of the signal obtained from 2-DCB (sum of 84, 98, and 112 ion fragments) at 50 part per trillion (ppt) with noise signal.**

## Conclusion

The use of SPME technique for the detection of 2-DCB provides a faster and universal method for the estimation of irradiation history without using organic solvents that facilitates laboratory work in an environmentally friendly manner. The addition of glycerol to the formulation of chicken jerky does not affect the occurrence of 2-DCB. Combination of using a suitable internal standard and SIM mode in MS is effective in lowering the variability and improving the detection. This technique provides a fast and easy way to the regulatory agencies to control the irradiation of chicken jerky products.

## Acknowledgment

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# Chapter 6 : Gas chromatography-mass spectrometry of major hydrocarbons induced by gamma irradiation[1]


## Abstract

Chicken jerky pet treats are one of the products that are more frequently processed with irradiation. To better monitor the irradiation of chicken jerky treats, the objective of this study was to develop a fast and easy analytical testing method to measure the irradiation history of these products. A solid phase micro extraction (SPME) technique was applied to extract volatile compounds from the headspace of samples irradiated from 0 to 50 kGy, and later these compounds were detected using gas chromatography-mass spectrometry (GC-MS). Fifteen different hydrocarbons, aldehydes, and ketones were identified, among which fourteen showed increasing trend at higher irradiation doses. Pentadecane, that is derived from palmitic acid, was the best hydrocarbon that could be used as irradiation marker showing the highest concentration and a linear regression of 0.95. The SPME technique provides a fast and simple alternative to the existing solvent extraction methods for analyzing irradiation markers.


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1. Taghvaei, M., Sommers, C., Ceric, O., Linghu, Z., Yucel, U., Smith, J. S. (2019) Gas chromatography-mass spectrometry of major hydrocarbons induced by gamma irradiation. Manuscript under preparation. Manuscript submitted.

# Introduction

Gamma irradiation is commonly used in food manufacturing, primarily with purpose to reduce the levels of parasites and pathogenic bacteria, as well as to extend the shelf life of products. For regulatory agencies to monitor the use of food irradiation, a validated analytical method is needed to quantify the dose that was used during production. A better understanding of formation of irradiation markers and their dose response could help for measuring the irradiation history of food products. Up to this point, all the suggested compounds to be used as the irradiation marker are derived from food lipids (Fernades, Pereira, Antonio, & Ferreira, 2018). The structure of fatty acids or triglycerides breaks down to a number of different free radical fragments under irradiation depending on the position of the cleavage. Such fragments could accept hydrogen radicals, which results in formation of a stable hydrocarbon, aldehyde, ketone, mono and di-glyceride (LeTellier & Nawar, 1972).

Nawar and Balboni (1970) showed that irradiation of pork produces mainly six hydrocarbons of pentadecane, 1-tetradecene, heptadecane, 1-hexadecene, 8-heptadecene, and 1,8-hexadecadiene. These hydrocarbons are derived from palmitic, stearic, and oleic acid, which are the main fatty acids found in pork fat. The concentration of such hydrocarbons proved to increase at higher irradiation doses, while they are absent or found in very small quantity in non-irradiated foods (Nawar, & Balboni, 1970). Their occurrence is proved to be independent of the presence of air, moisture, and mild heating during irradiation; however, presence of a specific free fatty acid would highly increase the production of resulting hydrocarbons from that fatty acid (Dubravcic, & Nawar, 1976). Dubravcic and Nawar (1976) showed that the amount of tridecane produced from myristic acid (free fatty acid) was 34 times higher than that from trimyristin (triglyceride).

Many other similar compounds such as aldehydes and shorter chain saturated and unsaturated hydrocarbons could also be produced in lower quantity during irradiation of animal fat (Champagne, & Nawar, 1969). An aldehyde with the same carbon number as the original fatty acid was shown to be the most predominant in this category of compounds (Dubravcic, & Nawar, 1976). While the most of the previous work in this area was done in pure lipids or model systems, separation and detection of such compounds at low concentration in different food matrixes has been a challenging task for food scientists.

The current available standard method for detection of irradiation markers in foodstuff (European committee for standardization, 2003) consists of timely extraction of fat and several steps of cleanup and drying. However, solid phase micro extraction (SPME) is a fast and simple extraction process using a fiber that is coated with adsorbent materials. With this technique, it is possible to concentrate organic compounds in the air or aqueous solutions (even at very low concentration) on the fiber coating, and later analyze them by gas or liquid chromatography. The SPME technique could provide a faster and simpler extraction without using organic solvents compared with the current standard method.

The application of SPME technique for detection of irradiation induced hydrocarbons was previously examined by Hwang et al. (2014) where the major extracted hydrocarbons from irradiated ground beef were pentadecane and 1-tetradecene from palmitic acid, heptadecane, and 1-hexadecene from stearic acid, and 8-heptadecene and 1,7-hexadecadiene from oleic acid. Li, Ha, Wang, and Li (2010) also studied SPME of irradiated chilled beef using GC-MS to quantify irradiation-induced hydrocarbons 1,7-hexadecadiene and 8-heptadecene. Their results showed a good linear correlation of both hydrocarbons with irradiation doses from 0.1 to 8 kGy. Panseri et al. (2015) identified 25 volatile compounds with dose response to irradiation of beef patties,

among which 1-nonene, 1-decene, 1-decyne and 1,7-hexadecadiene showed higher potential to be used as an irradiation marker. These results show that SPME coupled with GC-MS is an effective technique to detect irradiation markers at low concentration, and it might be possible to use this technique to develop an analytical testing method to quantify irradiation markers and measure the irradiation history of foods in the market.

Glycerol is a sugar alcohol that is added to food to improve taste, appearance, and the texture of product by absorbing water (Ockerman, 1991). Three hydroxyl groups in glycerol provide a potential site for hydrogen-bonding with water resulting in lower water activity on the surface of jerkies. A layer of glycerol on the surface of the product also helps to maintain the moisture content inside the treats at the desired level. However, the potential variation of moisture content among samples that are produced using glycerol and samples that are produced without using glycerol could affect the production of irradiation markers through water's radiolytic products. As some of the irradiated chicken jerky treats in the market are treated with glycerol and some do not contain glycerol as an ingredient, it is also important to understand how the presence of glycerol may affect the final concentration of irradiation markers.

Chicken jerky pet treats are one of the products that are more frequently processed with irradiation. To better monitor the irradiation of chicken jerky treats, the objective of this study was to develop a fast and easy analytical testing method to measure the irradiation history of these products based on the formation of different hydrocarbons under irradiation.

# Materials and Methods

## Sample preparation

Samples were prepared by drying raw chicken breast tenderloins (Tyson Foods Inc, USA) at 68 °C for 48 hours in a commercial food dehydrator (Sedona SD-P9000, Korea). Another batch of chicken tenderloins (4 kg) was mixed with 150 mL of food grade glycerol followed by 2 minutes of massaging the glycerol into the meat, before drying. Both chicken jerkies dried with and without glycerol were irradiated by a Cesium-137 source, at doses of 5, 10, 20, 30, and 50 kGy at the USDA Eastern Regional Research Center (Windmore, USA). Irradiated samples were kept at -80 °C.

## Standard solutions

All standard compounds (tridecane, tetradecane, pentadecane, heptadecane, and 2,4-di(tert-pentyl)cyclohexanone as internal standard) were purchased from Sigma-Aldrich (USA). A 10000 ppm stock solution of standards were prepared by dissolving appropriate amount of pure standard into 10 mL of ethanol (except for internal standard which was dissolved in methanol). A 1ppm working standard solution was prepared by subsequent dilution of stock solution with water.

## Standard curves

For making the standard curve, Control (not irradiated) jerky strips were ground for 1 minute (Waring blender, model 34FL97). 1.25 g of ground sample was transferred into a 20 mL siliconized glass vial and diluted with 5 mL of water. The appropriate amount of working standard and internal standard solution was added to the vial. The mixture was homogenized (Omni International TH01, USA) at the highest speed for 1 minute, and a 15×8 mm magnetic stirring bar was added to the mixture. Vials were sealed with silver crimp cap having

Polytetrafluoroethylene/white silicon liner and incubated at 80 °C (Pierce Heating/Stirring Module, USA) with high speed magnetic stirring for 30 minutes, followed by exposing to a polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase micro extraction fiber (Supelco, USA) to the vials' headspace for 15 minutes.

### Solid phase micro extraction

Irradiated jerky strips were ground for 1 minute and 1.25 g of ground sample was transferred into a 20 mL siliconized glass vial and diluted with 5 mL of water. The appropriate amount of working internal standard solution was added to the vial. The mixture was homogenized (Omni International TH01, USA) at the highest speed for 1.5 minute, and a 15×8mm magnetic stirring bar was added to the mixture. Vials were sealed with silver crimp cap having Polytetrafluoroethylene/white silicon liner and incubated at 80 °C (Pierce Heating/Stirring Module, USA) with high speed magnetic stirring for 30 minutes, followed by exposing to a polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase micro extraction fiber (Supelco, USA) to the vials' headspace for 15 minutes.

### Gas chromatography-mass spectroscopy

### Full scan analysis

To identify all the volatile compounds produced in jerkies, an initial GC-MS analysis was made using the MS in scan mode. After sampling from headspace, SPME fiber was exposed to a GC-MS chromatograph's (HP 5890, Agilent Technologies, USA) injector for 1 minute at 250 °C. The initial oven temperature was 40 °C (kept for 0.5 minutes) and then brought to 200°C at a rate of 5 °C per minute. Later, the temperature was increased to 270°C at a rate of 20°C per minute that kept for 4 minutes. An HP-5MS (60 m × 0.25 mm × 0.25 µm) GC column was used to separate the headspace compounds. The detection was performed by HP MS 5970 mass

detector at 280 °C. The ions with m/z of 30 to 400 were monitored in scan mode and compounds were identified by comparing their mass spectrum with those of the National Institute of Standards and Technology (NIST) database.

## Selective ion mode analysis

Compounds that were selected from full scan GC-MS analysis, were later quantitated using GC-MS in Selective Ion Mode (SIM) and analytical standards. The GC-MS conditions were adapted from Hijaz and Smith (2010) with little modifications. The oven temperature initially was kept at 55 °C for 0.5 minutes and then brought up to 200 °C at a rate of 20 °C per minute. A milder temperature gradient was applied at a rate of 15 °C per minute up to 270 °C, which was the final temperature. An HP-5MS (60 m × 0.25 mm × 0.25 µm) GC column was used to separate the headspace compounds. Since all saturated linear hydrocarbons break down into the same smaller ion fragments under MS, the ion fragments with m/z of 71, 85, and 99 were monitored for all four hydrocarbons of tridecane, tetradecane, pentadecane, and heptadecane. The ion fragments with m/z of 139, 153, and 168 were monitored for internal standard. The detection was performed by HP MS 5970 mass detector at 280 °C.

## Statistical analysis

Creation of all tables and graphs as well as analysis of linear regression and standard deviation were done using Microsoft Excel (Microsoft Office 365 ProPlus). The Tukey test for analysis of means was performed using Minitab 17 software (Minitab Inc.).

# Results and discussion

## Solid phase micro extraction

One of the important factors that affect the SPME efficiency is the affinity of analyte to fiber's stationary phase. A few different stationary phases with various coating diameters are available, and selection of fiber is based on polarity and molecular weight of analyte (Selection guide, 2018). Polydimethylsiloxane (PDMS) with two coating diameters of 7 and 100 µm, carboxen/polydimethylsiloxane (CAR/PDMS) with coating diameter of 75 µm, and polydimethylsiloxane/divinylbenzene (PDMS/DVB) with coating diameter of 65 µm were initially tested for their affinity to hydrocarbons (figure 6.1). Since fiber with PDMS/DVB-65 µm stationary phase showed the highest affinity to both pentadecane and heptadecane, this fiber was chosen for this study. The rest of the extraction parameters were adopted from previous work on the detection and quantitation of 2-dodecylcyclobutanone (2-DCB) from chicken jerky treats (unpublished).

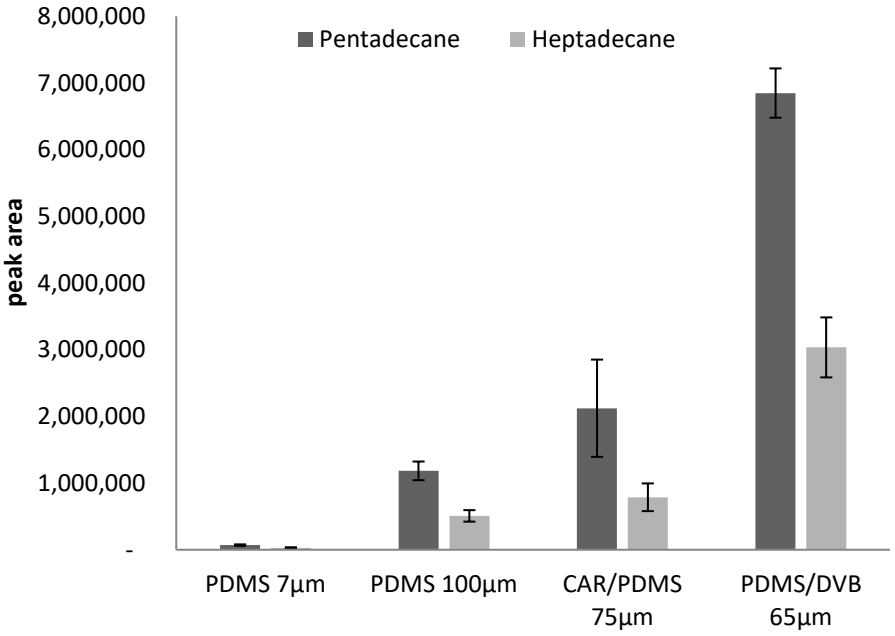

**Figure 6.1 The peak area of pentadecane and heptadecane obtained from the analysis of irradiated jerky at 20 kGy using four different stationary phases/thickness in scan mode. Data is the average of three replicates and error bars represent standard deviation.**

## Full scan analysis

The main issue with the identification of hydrocarbons with similar structure by mass spectrometry is that most linear hydrocarbons break down to similar low molecular weight ion fragments in MS and the identification should be made by considering the parent ion and longer ion fragments. However, the parent ion is not necessarily present in all peaks and NIST library identifies compounds based on their existing lower molecular weight ion fragments. For this reason, the following approaches were taken into account to identify hydrocarbons:

1. We accepted the NIST library identification if the probability was more than 90% and the parent ion matched with the molecular weight of identified compound.

2. If NIST library gave a 90% or higher probability but the parent ion was absent, we considered the order of elution. For example, if the library identifies a peak between tetradecane and hexadecane as pentadecane more than 90% but without the parent ion, we accepted it since the order of elution was correct.

3. If the above criteria were not met, we compared the retention time with that of standard compound solution (were available for hexanal, nonanal, tridecane, tetradecane, pentadecane, and heptadecane).

4. If none of the above criteria were met, we did not identify the compound.

Based on the above criteria, 15 different compounds were identified in irradiated chicken jerky samples, among which 14 show increasing trend at higher irradiation doses (table 6.1). Figure 6.2 provides sample chromatograms of irradiated chicken jerky at 50 kGy including the identified compounds and their structures.

Several linear hydrocarbons with a saturated structure or unsaturation on first carbon position that are known to be related to irradiation, including heptadecane, pentadecane, tetradecane, 1-tetradecene, teridecane, and dodecane, were identified. This concurs with Stewart (2001) where it is explained that two major hydrocarbons resulting from a fatty acid under irradiation are one with a carbon less and the other with two carbons less and a double bond at first carbon position. This means heptadecane (C17) is derived from stearic acid (C18), pentadecane (C15) and 1-tetradecane (C14:1) are derived from palmitic acid (C16), and so on.

Many linear aldehydes and alcohols with shorter chain lengths could also be observed. Dubravcic and Nawar (1968) showed that irradiation of pure tripalmitin at 6 megarad (60 kGy) will mainly promote the production of pentadecane (88.4 mmol/100 g) and tetradecane (19.4 mmol/100 g) with all shorter chain hydrocarbons being less than 3 mmol/100 g. The reason for less production of shorter chain hydrocarbons and the lack of aldehydes and ketones might be irradiation under vacuum in that study, while the present study samples were irradiated in plastic bags with the presence of little air inside the bags. However, the effect that the presence of oxygen has on the production of aldehydes and ketones may make them unsuitable to serve as an irradiation marker.

Surprisingly no hydrocarbon with unsaturation inside the chain was found, suggesting that either unsaturated fatty acids do not go through the same reaction as saturated fatty acids, or their products have less stability. As unsaturated fatty acids are more prone to autooxidation, they might be oxidized faster under irradiation and instead produce shorter chain aldehydes resulting from lipid oxidation reactions. To further support this hypothesis, it is worthy to mention that oleic acid is the most abundant fatty acid in chicken fat (Boschetti, Bordoni, Meluzzi, Castellini, Dal Bosco, & Sirri, 2015) while its main irradiation induced products such

as 9-octadecene is absent in our results. Additionally, a relatively large peak was found for nonanal and hexanal, which are the oxidation products of oleic acid and linoleic acid, respectively.

Benzaldehyde is the only identified volatile compound that does not show increasing trend at higher irradiation doses, suggesting that its production is not related to irradiation. This compound is naturally occurring in many plants, as well as dried or cooked meat, causing an almond like aroma (Tao, Wu, Zhou, Gu, & Wu, 2014; Garcia-Gonzalez, Tena, Aparicio-Ruiz, & Morales, 2008). 3-cyclohepten-1-one is also a naturally occurring compound in meat (Wang, Li, Ding, Zhang, Wang, Reed, & Zhang, 2019). Although there is little information on its production in food, its seven-membered ring suggests formation through cyclization of aldehydes or ketones containing seven carbons. Such reaction could be catalyzed by irradiation, as its concentration showed increase at higher irradiation doses.

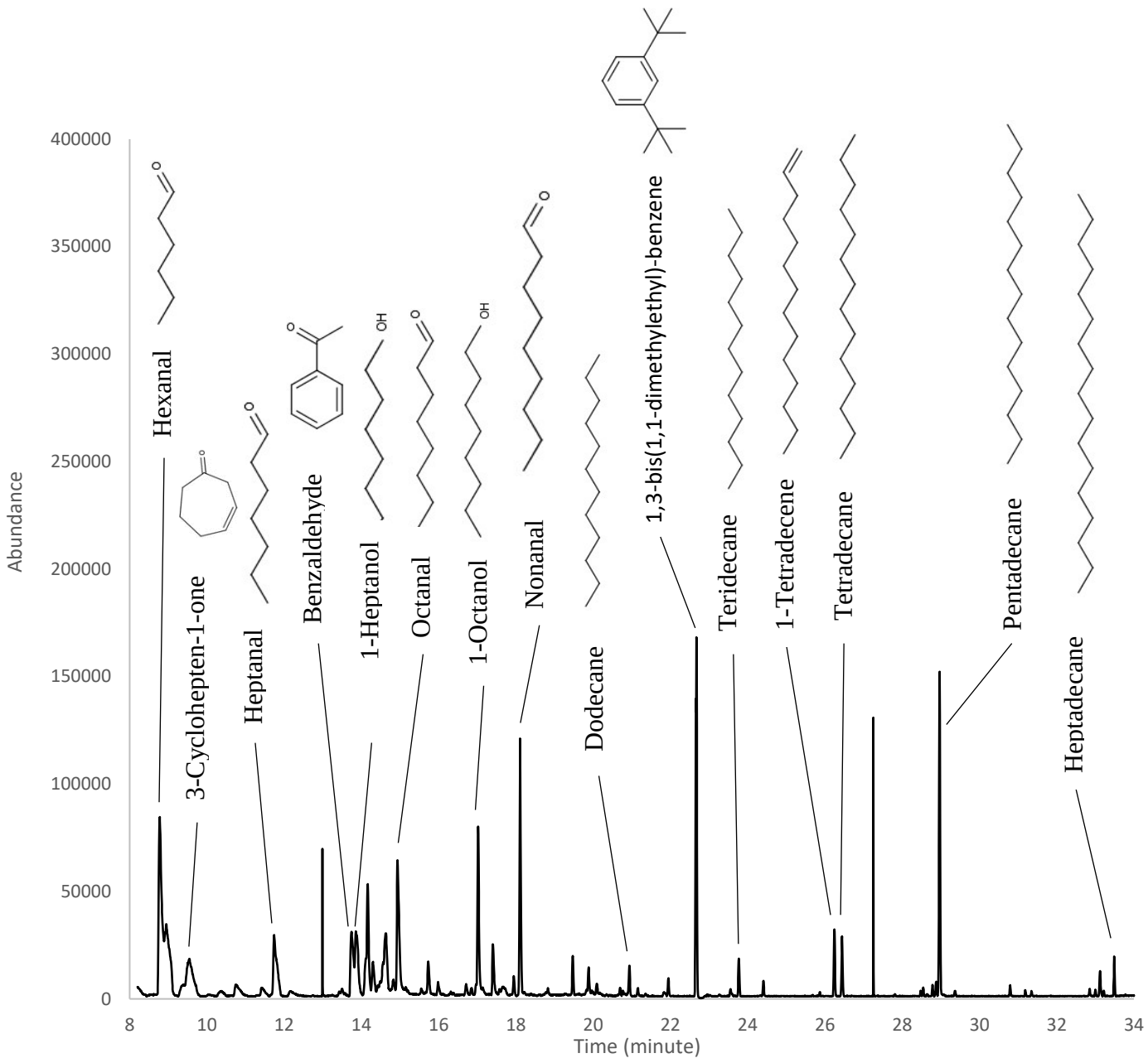


**Figure 6.2 An example of chromatogram obtained from chicken jerky treat irradiated at 50 kGy in MS scan mode with indication of identified compounds and their structure.**

The highest peak area belonged to 1,3-bis(1,1-dimethylethyl)-benzene which also showed an increasing trend with high linear regression. However, this compound is probably derived from a stabilizer/antioxidant known as Irgafos-168 that is commonly used in polymers such as

polyethylene and polypropylene (food package, plastic bag, ziplock, etc). Stoffers, Linssen, Franz, and Welle (2004) showed a reduction of Irgafos-168 concentration parallel with production of 1,3-bis(1,1-dimethylethyl)-benzene at higher irradiation doses of polyethylene plastic. As the suggested reaction is shown in figure 6.3, this volatile that is formed in the jerky package in our study probably migrated from the package to the chicken jerky. However, this fact might be overlooked in similar studies that falsely reported 1,3-bis(1,1-dimethylethyl)-benzene as a suitable food irradiation marker. Studies such as Shim et al. (2009) that identified 1,3-bis(1,1-dimethylethyl)-benzene as an irradiation induced compound in medicinal herb. While the authors were uncertain of its formation mechanism, they suggested further study of its potential use as an irradiation marker. Kim, Cho, Ahn, Cho, and Cha (2005) also used 1,3-bis(1,1-dimethylethyl)-benzene as an irradiation marker in their study on irradiated beef extract powder and concluded this compound could serve as a suitable irradiation marker. On the contrary to the previous two studies, our conclusion is that since 1,3-bis(1,1-dimethylethyl)-benzene is not derived from a food component and is simply produced and migrated from food packaging, it could not be used as a food irradiation marker.

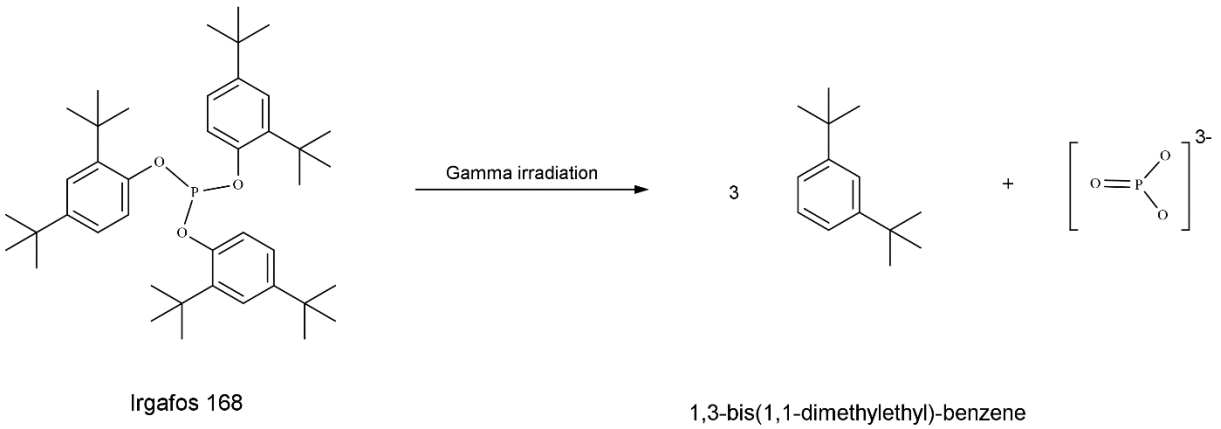


**Figure 6.3 The suggested formation reaction of 1,3-bis(1,1-dimethylethyl)-benzene from irgafos-168**

To choose a suitable irradiation marker for chicken products we considered the occurrence, detection (higher peak area), dose response (higher linear regression), and variability (standard deviation) of all identified compounds. A low variability and high linear regression, for example, will lead to higher precision in predicting the irradiation dose used for samples. Table 6.1 provides the linear regression for all identified compounds. From these results, four hydrocarbons of tridecane, tetradecane, pentadecane, and heptadecane were selected for analytical quantitation in irradiated chicken jerky and their potential use as irradiation marker.

**Table 6.1 The identified compounds in control and irradiated chicken jerky treats produced with and without glycerol. The $R^2$ value represents the linear trend regression of the data from 0 to 50 kGy and data is the average of two replicates ± of the standard deviation.**

| RT (min) | Compound | Jerky without glycerol (total ion count × $10^{-4}$) | | | | | | | Jerky with glycerol (total ion count × $10^{-4}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0kGy | 5kGy | 10kGy | 20kGy | 30kGy | 50kGy | $R^2$ | 0kGy | 5kGy | 10kGy | 20kGy | 30kGy | 50kGy | $R^2$ |
| **8.77** | **Hexanal** | 148 | 194 ± 30 | 510 ± 51 | 956 ± 108 | 864 ± 86 | 892 ± 112 | 0.6625 | 130 | 239 ± 0 | 504 ± 147 | 407 ± 86 | 639 ± 130 | 849 ± 79 | 0.8846 |
| **9.55** | **3-cyclohepten-1-one** | - | 119 ± 6 | 175 ± 1 | 337 ± 25 | 410 ± 78 | 385 ± 24 | 0.6033 | - | 121 ± 5 | 159 ± 20 | 139 ± 15 | 180 ± 24 | 200 ± 1 | -0.025 |
| **11.74** | **Heptanal** | - | 25 ± 4 | 75 ± 10 | 238 ± 29 | 258 ±76 | 291 ± 47 | 0.8154 | - | 45 ± 2 | 87 ± 20 | 90 ± 22 | 138 ± 38 | 204 ± 22 | 0.8956 |
| **13.73** | **Benzaldehyde** | 230 | 210 ± 14 | 288 ± 13 | 248 ± 2 | 301 ± 39 | 274 ± 15 | 0.3334 | 163 | 160 ± 7 | 161 ± 4 | 155 ± 10 | 144 ± 7 | 144 ± 4 | 0.8878 |
| **13.86** | **1-heptanol** | - | - | - | 130 ± 23 | 88 ± 18 | 151 ± 30 | 0.7655 | - | 66 ± 6 | 68 ± 0 | 93 ± 4 | 133 ± 18 | 187 ± 12 | 0.834 |
| **14.94** | **Octanal** | 47 | 35 ± 0 | 71 ± 8 | 284 ± 25 | 302 ± 62 | 345 ± 23 | 0.8209 | 34 | 86 ± 9 | 320 ± 5 | 730 ± 133 | 371 ± 10 | 383 ± 104 | -0.055 |
| **17.03** | **1-octanol** | 18 | 8 ± 4 | 17 ± 2 | 136 ± 30 | 267 ± 76 | 274 ± 31 | 0.8629 | 16 | 16 ± 3 | 25 ± 6 | 73 ± 1 | 106 ± 13 | 237 ± 22 | 0.9504 |
| **18.12** | **Nonanal** | 66 | 43 ± 4 | 100 ± 16 | 323 ± 13 | 368 ± 109 | 439 ± 103 | 0.8716 | 92 | 72 ± 13 | 128 ± 27 | 158 ± 57 | 220 ± 77 | 310 ± 41 | 0.971 |
| **20.94** | **Dodecane** | - | 5 ± 3 | 4 ± 1 | 14 ± 3 | 21 ± 5 | 54 ± 4 | 0.9236 | - | 2 ± 1 | 5 ± 3 | 19 ± 2 | 15 ± 1 | 30 ± 4 | 0.92 |
| **22.67** | **1,3-bis(1,1-dimethylethyl)-benzene** | - | 49 ± 16 | 104 ± 14 | 206 ± 16 | 188 ± 11 | 262 ± 4 | 0.7785 | - | 31 ± 5 | 80 ± 26 | 270 ± 4 | 173 ± 2 | 351 ± 31 | 0.817 |
| **23.78** | **Tridecane** | - | 5 ± 3 | 5 ± 1 | 12 ± 2 | 36 ± 10 | 92 ± 10 | 0.868 | - | 2 ± 1 | 5 ± 4 | 14 ± 2 | 21 ± 3 | 31 ± 5 | 0.9872 |
| **26.25** | **1-tetradecene** | - | 1 ± 1 | - | 6 ± 1 | 13 ± 3 | 24 ± 3 | 0.9237 | - | 0 | 3 ± 3 | 19 ± 3 | 28 ± 5 | 56 ± 8 | 0.9534 |
| **26.45** | **Tetradecane** | - | 13 ± 8 | 12 ± 1 | 26 ± 5 | 63 ± 17 | 187 ± 21 | 0.8549 | - | 6 ± 3 | 9 ± 6 | 39 ± 23 | 30 ± 13 | 41 ± 15 | 0.7312 |
| **28.97** | **Pentadecane** | - | 37 ± 25 | 38 ± 6 | 66 ± 6 | 237 ± 38 | 381 ± 21 | 0.9284 | - | 25 ± 15 | 54 ± 39 | 113 ± 16 | 260 ± 41 | 280 ± 16 | 0.9105 |
| **33.49** | **Heptadecane** | - | 2 ± 2 | 2 ± 0 | 4 ± 0 | 19 ± 1 | 37 ± 2 | 0.8854 | - | 1 ± 1 | 3 ± 2 | 8 ± 2 | 24 ± 5 | 29 ± 1 | 0.9157 |

## SIM analysis

Four selected hydrocarbons from full scan study were analyzed for quantitation with analytical standards and 2,4-di(tert-pentyl)cyclohexanone as internal standard. The retention times of tridecane, tetradecane, pentadecane, and heptadecane were 9.19, 9.95, 10.74, and 12.30 minutes, respectively. The internal standard eluted after 12.22 minutes.

As figure 6.4 shows, the lowest and highest linear regression with irradiation dose were observed with tridecane and tetradecane, respectively. Heptadecane is also showed a good linear regression of 0.9116, however, the highest concentration belonged to pentadecane. The distinction among values from different irradiation doses is also easier to see with pentadecane. This distinction results a more precise dose measurement for unknown samples and higher concentration of pentadecane in the jerky samples helps with better detection at lower irradiation doses. For these reasons, pentadecane, which is one of the irradiation products of palmitic acid, could be the best candidate to be used as an irradiation marker.

Another observation is that using full scan, all four selected hydrocarbons were only seen in irradiated samples and no corresponding peak were observed in not irradiated samples (table 1). Whereas using SIM all four hydrocarbons showed up also in not irradiated samples (figure 6.4). Although their concentrations were very low compared with irradiated samples, this shows the natural occurrence of such hydrocarbons at very low concentration in chicken jerky.

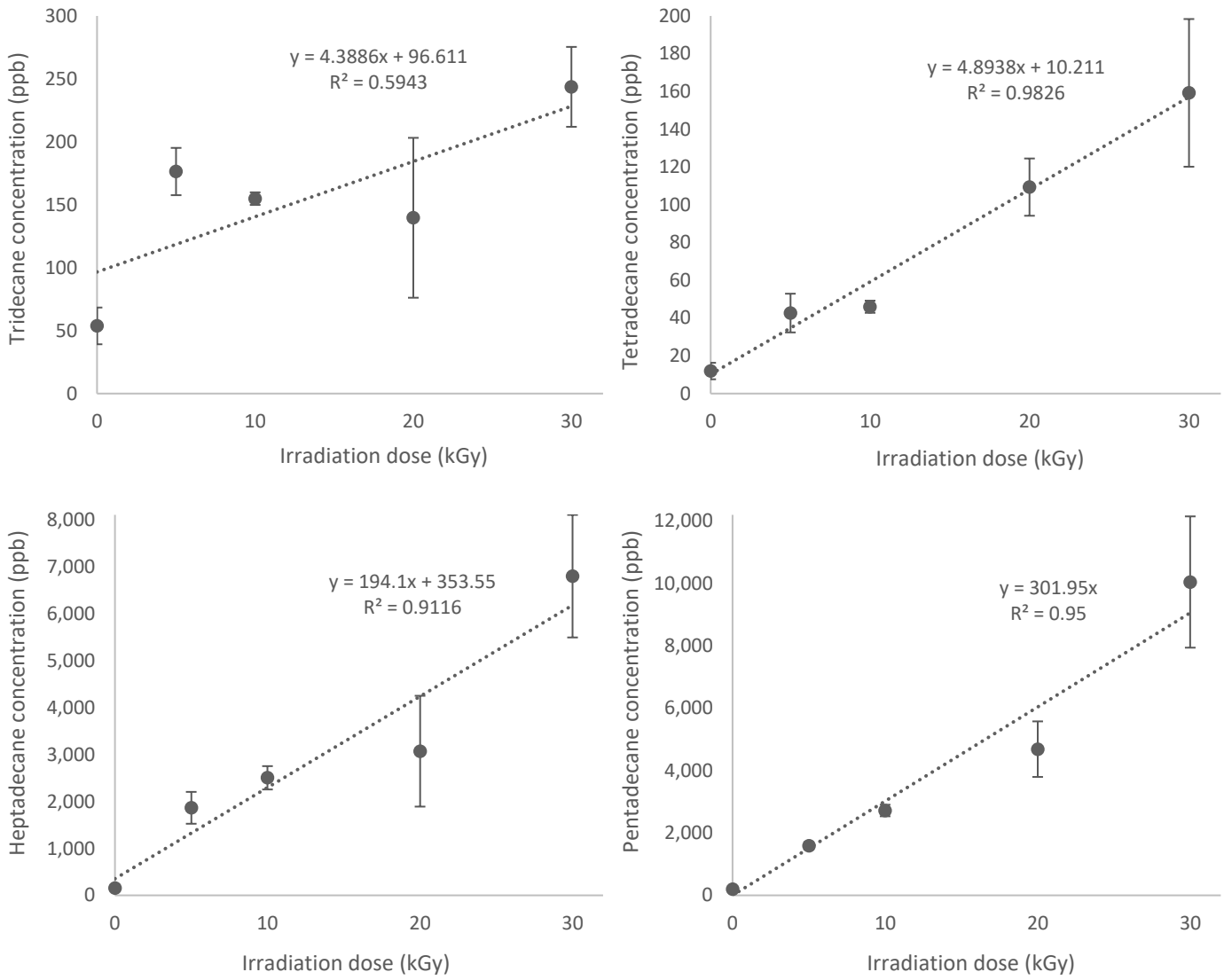


**Figure 6.4 The dose response of tridecane, tetradecane, heptadecane, and pentadecane with irradiation dose using MS in selected ion mode. Data is the average of three replicates and error bars represent standard deviation.**

## Effect of glycerol

The initial full scan study of hydrocarbons was done for two batch of samples separately, first batch was made without using glycerol and second batch had glycerol in the formula. As some of chicken jerky pet treat manufacturers use glycerol to improve product's charachteristics, it was important for us to examine whether addition of glycerol would affect the production of irradiation markers or not. The data in table 1 is presented into two categories of samples without glycerol (on the left) and samples with glycerol (on the right). Comparing each irradiation dose results, it could be observed that some are significantly different in samples with and without glycerol while the other irradiation doses of same sample are not. To better understand the effect glycerol, the peak areas obtained from heptadecane as an example is plotted against irradiation doses (figure 6.5). While some irradiation doses show difference between samples with and without glycerol, the general linear trend for the production of heptadecane does not differ. It is important to note that only two samples are being compared as X axis is showing different irradiation doses applied to the same two samples of with and without glycerol. Hence, the comparison of linear trends would give a more comprehensive understanding about the effect of glycerol, and difference in some data points could be related to other experimental variations between two samples rather than glycerol. Additionally, another study on the effect of glycerol on 2-dodecylcyclobutanone (2-DCB), which is an irradiation marker, revealed that glycerol has also no significant effect on the production of 2-DCB (unpublished). Based on these observations it could be concluded that the irradiation dose measurement of chicken jerky samples could be done using hydrocarbons without considering the presence or absence of glycerol in the formula.

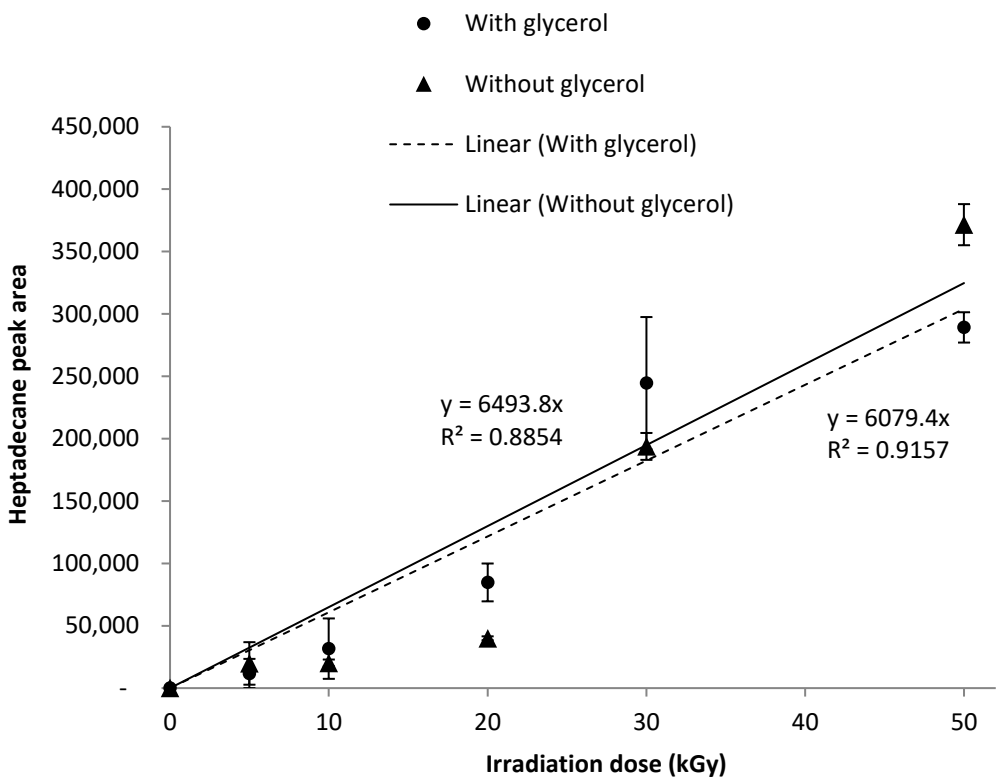


**Figure 6.5 The dose response of heptadecane in samples made with and without using glycerol. Data is the average of three replicates and error bars represent standard deviation.**

## Conclusion

Irradiation of chicken jerky treats from 0 to 50 kGy leads to formation of many linear hydrocarbons, aldehydes, and ketones. The concentration of these compounds increases linearly at higher irradiation doses, which makes them suitable to be used to measure the irradiation history of samples. Among all, pentadecane, that is derived from palmitic acid, showed the highest concentration and could be the best option to be used as irradiation marker. The presence of glycerol in the formula of chicken jerky does not significantly affect the formation of linear hydrocarbons. The use of SPME technique for the detection of irradiation markers provides a

faster and easier method for the estimation of irradiation history without using organic solvents that facilitates laboratory work in an environmentally friendly manner.

## Acknowledgment

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# Appendix A – Blinded Method Test

Method validation parameters measured using blinded samples at two different days and three batches of irradiated, irradiated and spiked, irradiated mixed with non-irradiated samples.

| Day-1 | | | | | | | 2-DCB (ppb) | | Pentadecane (ppb) | | Heptadecane (ppb) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample ID | 20kGy (g) | Control CJT (g) | 2-DCB (ng/g CJT) | pentadecane (ng/g CJT) | heptadecane (ng/g CJT) | | Reported | Expected | Reported | Expected | Reported | Expected |
| TS-40 | 1.25 | | | | | Average | 181 | 181 | 1,289 | 1,289 | 190 | 190 |
| TS-22 | 1.25 | | | | | Accuracy% | 100 | | 100 | | 100 | |
| TS-24 | 1.25 | | | | | STDEV | 22 | | 155 | | 23 | |
| TS-38 | 1.25 | | 420 | 400 | 400 | RSD% | 12 | | 12 | | 12 | |
| TS-36 | 1.25 | | 420 | 400 | 400 | Average | 549 | 601 | 1,538 | 1,689 | 582 | 590 |
| TS-39 | 1.25 | | 420 | 400 | 400 | Accuracy% | 91 | | 91 | | 99 | |
| TS-29 | 0.5 | 0.75 | | | | STDEV | 45 | | 156 | | 48 | |
| TS-33 | 0.5 | 0.75 | | | | RSD% | 8 | | 10 | | 8 | |
| TS-25 | 0.5 | 0.75 | | | | Average | 81 | 72 | 747 | 516 | 83 | 76 |
| | | | | | | Accuracy% | 111 | | 145 | | 109 | |
| | | | | | | STDEV | 8 | | 201 | | 9 | |
| | | | | | | RSD% | 10 | | 27 | | 11 | |

| Day-2 | | | | | | | 2DCB (ppb) | | Pentadecane (ppb) | | Heptadecane (ppb) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample ID | 30kGy (g) | Control CJT (g) | 2-DCB (ng/g CJT) | pentadecane (ng/g CJT) | heptadecane (ng/g CJT) | | Reported | Expected | Reported | Expected | Reported | Expected |
| TS-17 | 1.25 | | | | | Average | 144 | 144 | 3,159 | 3,159 | 1,515 | 1,515 |
| TS-13 | 1.25 | | | | | Accuracy% | 100 | | 100 | | 100 | |
| TS-11 | 1.25 | | | | | STDEV | 20 | | 1,827 | | 819 | |
| TS-20 | 1.25 | | | | | RSD% | 14 | | 58 | | 54 | |
| TS-15 | 1.25 | | 230 | 300 | 440 | Average | 319 | 374 | 2,740 | 3,459 | 1,380 | 1,955 |
| TS-19 | 1.25 | | 230 | 300 | 440 | Accuracy% | 85 | | 79 | | 71 | |
| TS-14 | 1.25 | | 230 | 300 | 440 | STDEV | 76 | | 1,077 | | 633 | |
| TS-16 | 0.625 | 0.625 | | | | RSD% | 24 | | 39 | | 46 | |
| TS-12 | 0.625 | 0.625 | | | | Average | 54 | 72 | 914 | 1,579 | 443 | 757 |
| TS-18 | 0.625 | 0.625 | | | | Accuracy% | 75 | | 58 | | 58 | |
| | | | | | | STDEV | 9 | | 94 | | 65 | |
| | | | | | | RSD% | 16 | | 10 | | 15 | |