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A COMPARATIVE STUDY OF THE EFFECTS OF X-RADIATION AND GAMMA-RADIATION ON STRUCTURAL CHARACTERISTICS OF RAW MILK PROTEIN (STUDY CASE OF GOAT'S MILK)

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ABSTRACT

Milk has been adopted as a structural nature food for a long era, it is containing most of the growth factors, protective agents, and enzymes needed for the body. The wide demand for fresh milk with the desired organoleptic characteristics and health benefits led to developing non-thermal processing technology aiming to retain all the product qualities and nutrients. Irradiation is an emerging non-thermal technology used to deactivate micro- and macroorganisms that might exist in food. A few attempts have been conducted to treat dairy products especially raw milk by the means of ionizing radiation. With the endorsement of many international food and health organizations such as the Food and Agriculture Organization (FAO) and World Health Organization (WHO), food irradiation is becoming more widely researched as a process to maintain quality, improve safety and reduce quarantine and post-harvest loss. In this role, this study going to investigate the impact of x-radiation and gamma radiation on raw milk construction by evaluating the changes in the protein structure after exposing to certain energies of gamma and x-irradiation.

Keywords: Milk preservation; Food irradiation; Protein denaturation; UV-VIS-NIR Spectroscopy

INTRODUCTION

Basically, milk described as a colloidal organic fluid that contains more than 100 individual chemical compounds mainly water, whey proteins, casein proteins, minerals, lactose, and fat. It is secreted by the mammary gland of the females of all mammals as an action after parturition for feeding newborns of the species throughout their initial period of growth (1). Application of ionizing radiation treatment of foods on an industrial scale started in the early 1980s after the joint Food and Agriculture Organization/International Atomic Energy Agency (IAEA)/World Health Organization (WHO) expert committee accepted the application of a 10- kGy overall average dose for foods (2). Vast knowledge has now accumulated on the chemical and biological

effects of ionizing irradiation, which has contributed to the promotion of its utilization (3,4).

In 2015, the World Health Organizations classified food safety as a major concern. Milk is considered one of the major food classes for human beings. Milk and other dairy products play an important role in the human's nutrition. They are used as raw products like milk or supporting with other foods such as cheese, cakes, and pies to improve the food characterizations including flavor, texture, color, and nutrition, thus, its safety has gained immense importance in the last decade (5). Since the beginning of food preservation technologies; several methods have been developed to conserve food products. Initially, the cool temperature had used as a basic process for preservation of dairy products (6). Recently, pasteurization is considered widely common process to preserve dairy products.

Treatments methods on dairy products are carried out to extend its shelf life, but above all to ensure its safety for consumers. Thermal technologies including milk pasteurization are the most widely applied to achieve these purposes, but these treatments have a negative effect on certain components of the milk itself, reducing its vitamin content and other nutrients, as well as sensory features that make them less attractive in terms of color and textural properties. Non-thermal technologies are an alternative to thermal treatment that is being studied and developed in order to obtain a better final product sensory quality but without neglecting microbial safety. In this way, these alternatives to thermal technologies can produce food products with maintaining nutritional characteristics and minimizing the loss of quality in terms of flavor, color, and nutritional value. One of these innovative technologies is dairy products irradiation (7).

In 2014, Brownstein has proved that commercial pasteurization of milk is not sufficient to kill all the pathogenic microorganism including thermoduric bacteria such as Coxiella Burnetii, Mycobacterium avium complex as well as Escherichia coli O157:H7 which can naturally exist in raw milk and responsible for many diseases (8). Thus, the attention turned into a non-thermal technology for milk preservation such as irradiation. The use of ionizing radiation for food preservation is not a new technology; ionizing radiations were used for food preservation since the discovery of the radioactivity by Henri Becquerel. It has been officially approved by the Food and Drug Administration (FDA) for the first time in 1963; recently, different kinds of food products including meats, species, have successfully treated by radiation (9).

Irradiation is used as a food preservation method to destroy microorganisms and increase shelf life. The radiation dose unit, previously referred to as the rad, is currently known as the Gray (Gy). The Gy is the absorption of 1 J of energy per kilogram irradiated material and is equivalent to 100 rads (10,11). Irradiation doses usually range from 10 Gy to 1 kGy for sprouting inhibition of potatoes, onions, garlic, etc.; 1 to 10 kGy for fresh meat and seafood as well as vegetables and fruits; and 10 to 100 kGy mainly for food sterilization (IAEA, 2002) (12).

The recommended dose levels are as follows: low level at 1 kGy to inhibit insect infestation and delay ripening; medium-level at 1–10 kGy to reduce bacterial load (particularly of pathogens); and high level at 10–50 kGy for commercial sterilization and elimination of viruses (WHO, 1981, 1999) (13, 14). Irradiation of foodstuffs has an additional advantage: it can be used in prepackaged foodstuffs to avoid microbial recontamination, in addition to allowing the use of different packaging materials (15, 16).

According to the Codex General Standard for Irradiated Foods, (17) ionizing radiations for food processing are limited to high-energy photons (ϒ-rays of radionuclides ^{60}Co and, to a much smaller extent, ^{137}Cs, or X-rays from machine sources with energies up to 5 MeV or accelerated electrons with energies up to 10 MeV). In the United States, the FDA amended the food additive regulations by establishing a new maximum permitted energy level of X-rays for treating food of 7.5 MeV provided that the X-rays are generated from machine sources that use tantalum or gold as the target material (U.S. FDA, 2004) (18).

These types of radiation are chosen for the following reasons: They produce the desired food preservative effects, they do not induce radioactivity in foods or packaging materials, and they are available in quantities and at costs that allow commercial use of the irradiation process (19).

The chemical changes resulting from the irradiation of proteins food have been the subject of many studies (20-22). These studies indicate that in addition to protein structure, the state of protein - whether it is native or denatured, dry or wet, liquid or frozen, or present with other substances - affects the radiolytic products formed. Irradiation condition (e.g., absorbed dose, dose rate, and the presence or absence of oxygen) also plays an important role (23).

When proteins were treated with ionizing energy, large protein molecules were cleaved into smaller ones that upon digestion yielded the same amino acids as the original proteins. Some of these studies demonstrated the occurrence of both fragmentation and aggregation. Moreover, minimal changes were reported in total amino acid profile as a result of treatment with irradiation; consequently, no effects of major nutritional significance were found (24, 25).
In this role, Fohely, 2017 (26) has explained the effects of X-irradiation on raw milk proteins. The current study is a comparative study aimed to show up the effects of gamma-irradiation from different radioactive sources on raw milk's structure and compare this effect with the impact of X-radiation on raw milk structure.

UV Absorbance Spectrum of Protein
The proteins are basically giant molecules built up of smaller units called amino acids. The protein molecule consists of one or more interlinked chains of amino acids, where the amino acids are arranged in a specific order as presented in figure 1 (27).

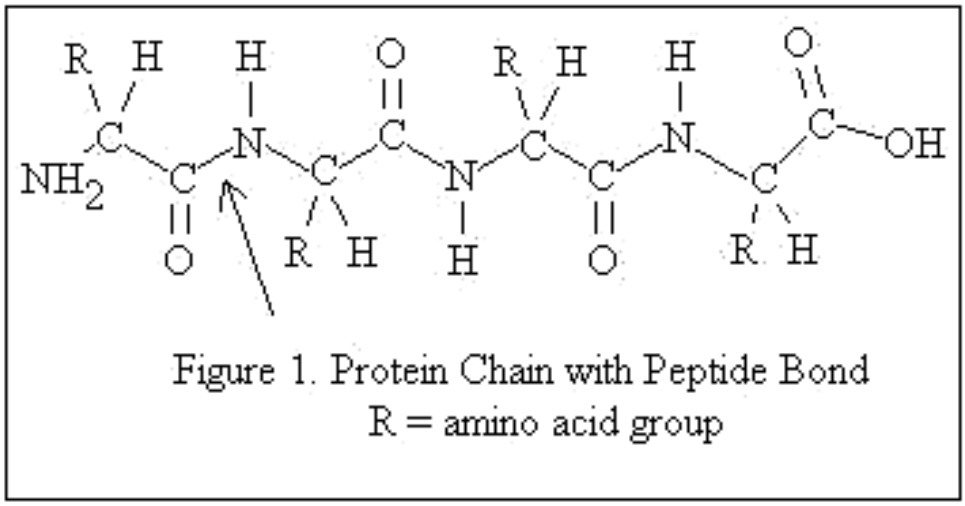

Figure 1: Protein Chain with Peptide Bond, the R= amino Acid group

Microspectroscopy allows the user to learn more about the optical features and the chemical structure of the protein. Additionally, microspectroscopy also allows for the determination of the concentration of protein. Protein has a unique ultraviolet absorption spectrum (UV spectrum) ranging from 280 nm to 300 nm depending on the protein's concentration in the targeted sample (28). The UV absorbance value of protein greatly varies between different proteins; the ultraviolet absorption spectrum of proteins is depending on the concentration of Tyrosine (Tyr), Tryptophan (Trp) and Phenylalanine (Phe) and disulfide bonds in the protein structure (29).

Figure 2 shows the UV absorbance spectrum of native goat's milk protein. The highlighted area that ranged from 280 nm to 300 nm represents the amino acid chain Tryptophan (Trp) and Tyrosine (Tyr) including the peptide bonds. Amino acids with aromatic rings are the primary reason for the absorbance peak at 295 nm (30).

The typical UV spectra of raw milk before irradiation are shown in Figure 2, where protein's absorbance spectra ranged from 280-305 nm in pure samples. The amino acid chains tryptophan at 295 nm and tyrosine was observed at wavelength 280 nm (represent in primary peak) (32). Other minor amino acids such as glutamine and aspartic acid have absorbed UV light at wavelength 270-275 nm (represent in the second peak). Both are considered primary units in milk's protein structure that gives it the unique absorbance wavelength, they were found to be the most sensitive components to radiation exposure (33).

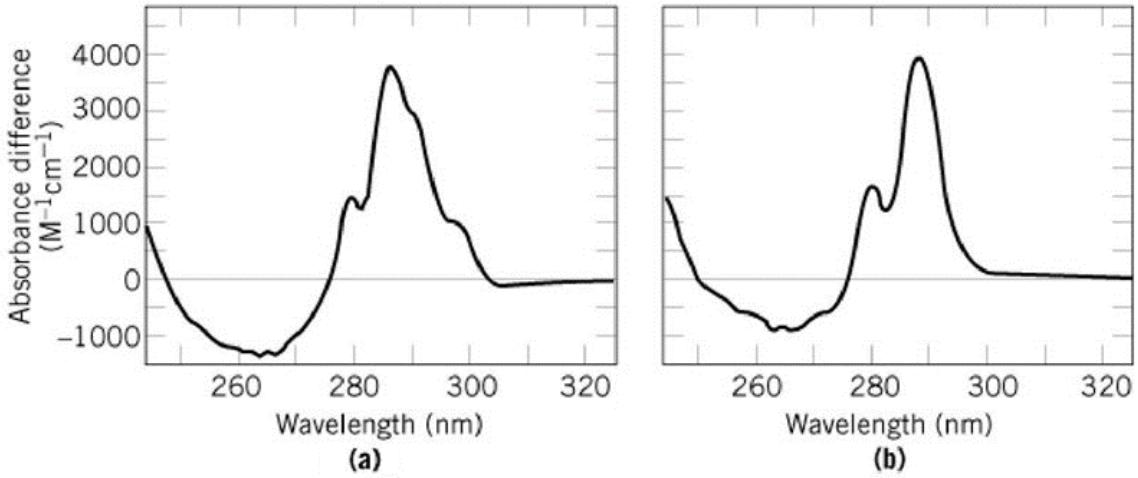

Figure 2: UV Absorbance spectrum of different native milk protein (31)

EXPERIMENTAL

Sample collection

Fresh goat's milk was mainly used as a target sample to study the impact of X-radiation from x-ray machine within the medical diagnostic range and gamma-ray of radionuclides sources (^{60}Co, ^{137}Cs, ^{226}Ra, and ^{241}Am) on the UV absorbance of milk's protein.

330 ml raw goat milk sample was collected from a local farm (Peace Love Healthy Dairy Farm - Penang, Malaysia) on the day of the experiment to ensure they are fresh. The milk samples were kept in the refrigerator (4°C) for two hours before the experiment.

Measurement of the UV Absorbance Spectra of Raw Milk Samples

Cary 5000 UV-VIS-NIR spectrophotometer was used in this study. It is considered a high-performance UV-VIS-NIR spectrophotometer with superb photometric performance in the 175-3300 nm range. By using a special detector (PbSmart), the Cary 5000 extends its NIR range to 3300 nm making it a powerful device for analysis of non-dilute and pure samples materials. The UV-VIS spectrophotometer operated by Cary WinUV software (2011), modular Windows-based software, makes it easy to perform powerful analysis and control a number of optional accessories. The large sample compartment can be expanded to hold large accessories and integrating spheres for spectral and diffuse reflectance. The lockdown mechanism makes it possible to quickly change and position accessories for reproducible results. Practically many features distinguish this generation of spectrophotometer from others including the ability to measure beyond 8.0 absorbance units with reference beam attenuation as well as the wide photometric range that arranged from 175 to 3300 nm.

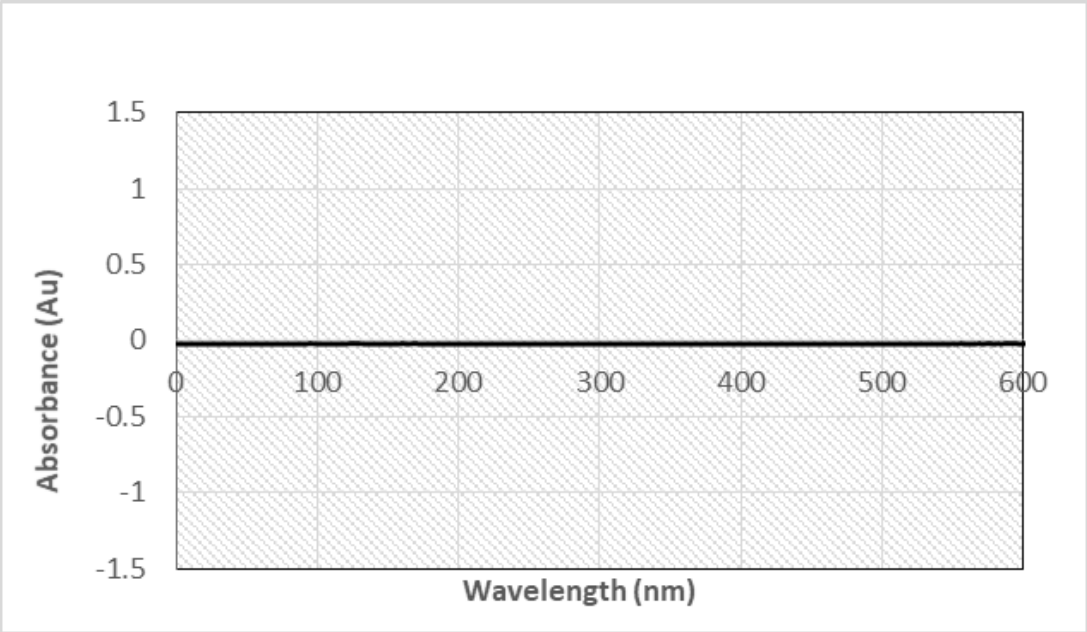

Figure 3: Baseline correction of the UV-VIS-NIR spectrophotometer

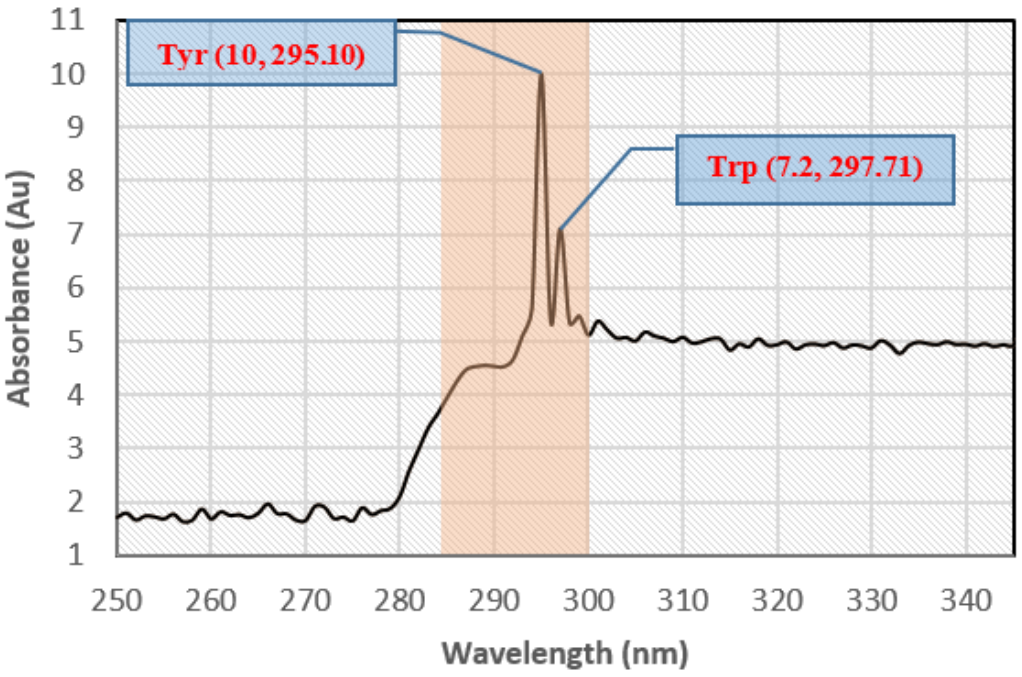

Figure 4: UV Absorbance of native milk protein

Before measuring the UV absorbance spectra for milk samples, the baseline correction of the machine has measured without any sample reference. Figure 3 shows the baseline correction of the UV-VIS-NIR spectrophotometer.

After measuring the baseline, proteins show absorption maxima between 280 and 295 nm (Figure 4), which are caused by the absorbance of the two aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and, to a small extent, by the absorbance of cystine (i.e. of disulfide bonds). The absorbances of Trp and Tyr depend on the microenvironment of their chromophores.

Sample Irradiation

Milk samples basically divided into two main sections, the first section consists of 40 samples, this samples group exposed to x-radiation from diagnostic x-ray machine (Toshiba KXO-50S, 2012) while the second section consists of 30 samples, this samples group exposed to gamma radiation from different radioactive materials. Table 1 shows samples group of section one that exposed to x-radiation, while table 2 shows samples group of section two that exposed to gamma radiation.

Table 1: Samples group of section one that exposed to x-radiation

Sample number	Exposure Category	Sample name	kV	mAs
1	First Category	A1	40	0.45
3		A10	40	140
4	Second Category	B4	55	10
6		B10	55	140
7	Third Category	G6	110	50
9		G9	150	50

Table 2: Samples group of section two that exposed to gamma radiation

Sample Number	Sample Name	Source type	Chemical Symbol	Half-life (Years)	Activity (μCi)	Energy emitted (MeV)
1	M1	Cesium – 137	$_{55}Cs^{137}$	30.05	5.00	0.6617 γ ray
2	M2	Cobalt – 60	$_{27}Co^{60}$	5.26	5.00	1.1732, 1.3325 γ ray
3	M3	Radium – 226	$_{88}Ra^{226}$	1600	5.00	4.77 α particles 0.186 γ ray
4	M4	Americium–241	$_{95}Am^{241}$	432.2	5.00	5.486 α particles 0.0595409 γ ray

RESULTS AND DISCUSSION

The UV-VIS-NIR spectrophotometer scan range adjusted to wavelength 200 – 450 nm

(since the native protein has absorbance value within this range). Figure 5 – 11 present the UV-VIS absorbance spectra of irradiated milk samples of section one that exposed for X-irradiation. Sample A1 exposed to X-radiation (40 kV, 0.45 mAs). Figure 5 presents the UV absorbance spectrum of this sample which shows no shifting in the absorbance wavelength and slightly changes in the absorbance unit value of the aromatic acid chain which indicates no major change observed on the structure of the protein. While sample A10 exposed to (40 kV, 140 mAs). Figure 6 presents the UV absorbance spectrum of this sample which shows a slightly change on the absorbance unit value and no shifting in the absorbance wavelength value for both aromatic acid Tyr and Trp. This indicates no change on the structure of the protein. Protein unfolding transitions can thus be measured by following the absorbance wavelength changes at 287–292nm as a function of temperature or denaturant concentration (34).

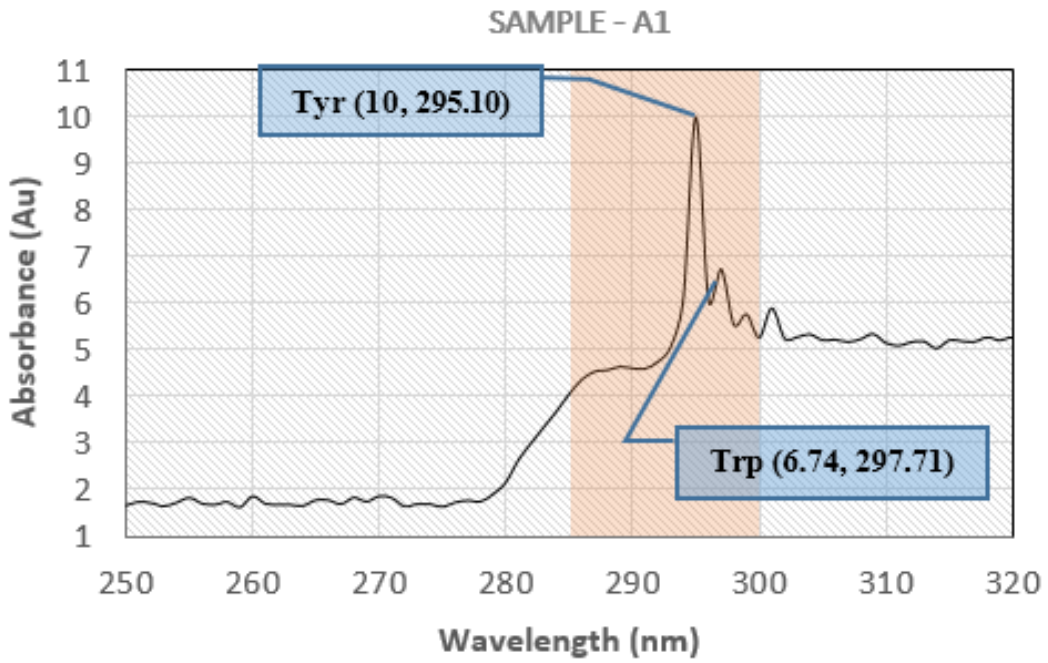

Figure 5: Absorbance spectrum of sample A1

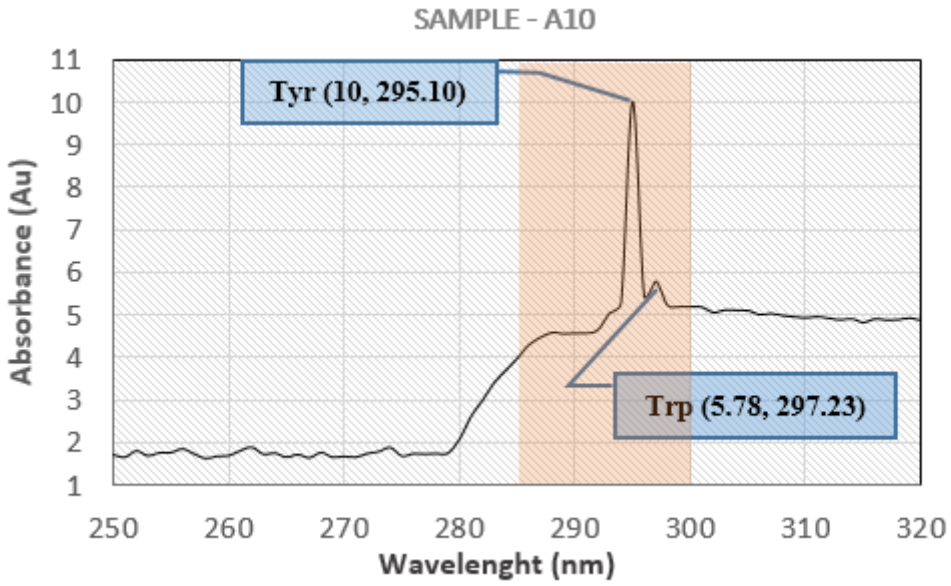

Figure 6: Absorbance spectrum of sample A10

Samples (B4, B10) exposed to (B4: 55 kV, 10 mAs) (B10: 55 kV, 140 mAs). Figure 7 shows the absorbance spectrum of sample B4, It is clear that the absorbance unit value for aromatic acid tyrosine stay not changed at (10 Au) while the absorbance of tryptophan is slightly reduced compared to the sample before irradiation. Since there is no change in the absorbance wavelength of both aromatic acids this also indicates that there is no change observed in the protein structure. Figure 8 shows the absorbance spectrum of sample B10, It is obvious that the absorbance value for both tyrosine and tryptophan markedly reduced and this refers to the effect of radiation on minor aromatic acids while there is no change in the absorbance wavelength which indicates no change on the protein structure.

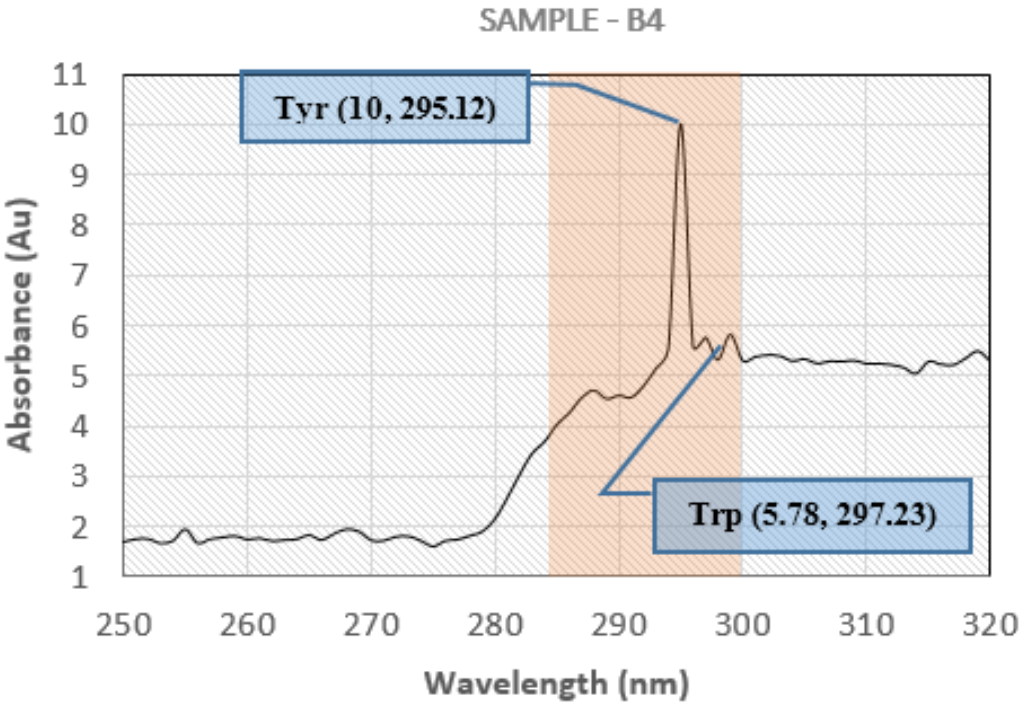

Figure 7: Absorbance spectrum of sample B4

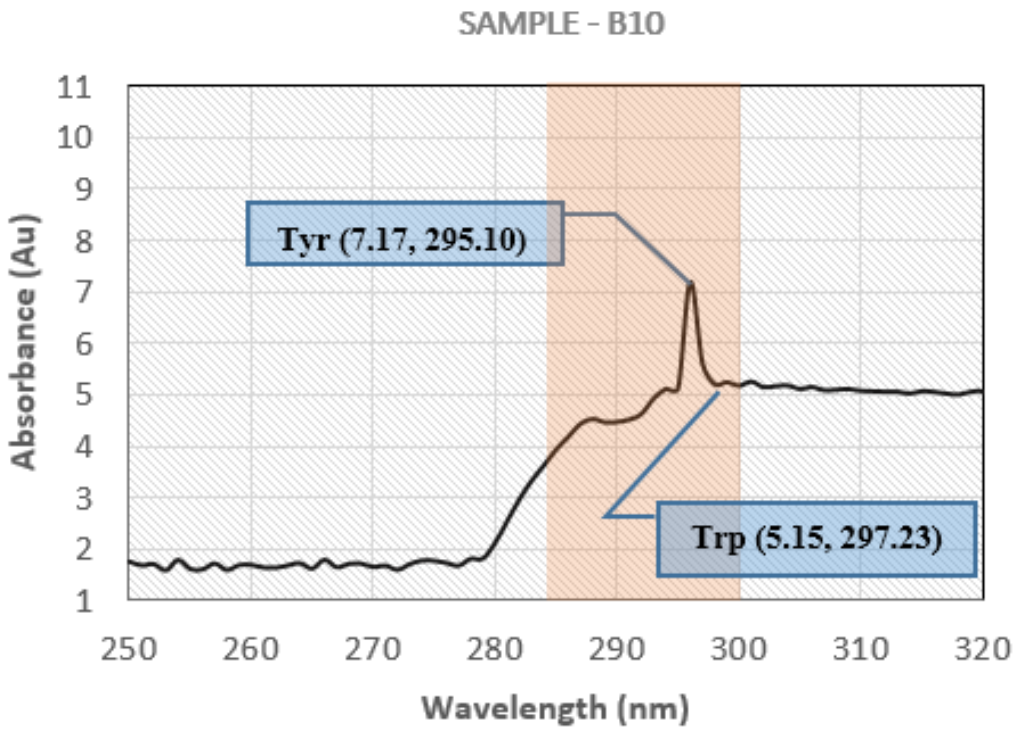

Figure 8: Absorbance spectrum of sample B10

The second section included samples group that exposed to gamma radiation. These radioactive materials have been characterized based on the half-life and activity as well

as the emitted radiation energy as shown in table 2. These radioactive materials included Cs^{137}, Co^{60}, Ra^{226}, and Am^{241}. These sources have placed inside a shield box alongside raw milk samples (every individual sample has fixed with a single source) as shown in figure 9. The samples were kept in the refrigerator (4.0 °C) for 7 hours then the UV absorbance measured again after irradiation.

Figure 9: Shows the shield box that contains milk sample and the radioactive source

Sample M1 exposed to 0.6617 MeV gamma radiations from Cs^{137}, sample M2 exposed to 2.5 MeV from Co^{60}. Figure 10 shows the absorbance spectrum of sample M1. The absorbance wavelength of tyrosine is shifted from 295 nm to 305 nm, it is clear that there is shifting in the absorbance wavelength which indicates a major change in the protein structure. while figure 11 shows the absorbance spectrum of sample M2, it is obvious that there is a shifting in the absorbance wavelength of tyrosine while the absorbance peak of tryptophan has completely faded away, this is demonstrated a major defect in the protein structure.

Sample M3 exposed to 0.186 MeV γ ray while sample M4 exposed to 0.059 MeV. The same effect appeared in sample M3 and sample M4, figure 12 shows absorbance spectrum of sample M3, the absorbance wavelength of tyrosine shifted from 295 nm to 304 nm the absorbance unit value also dramatically reduced. In sample M4 the absorbance wavelength shifted from 295 nm to 307 nm.

Native proteins unfolded by radiation (reactive oxygen spices resulting from the interaction of ionizing radiation with aromatic acids). Protein unfolding transitions can thus be measured by following the absorbance changes at 295–310 nm as a function of

radiation dose or denaturant concentration. The absorbances of native and of unfolded protein molecules can also depend on temperature. The refractive index decreases slightly with temperature, as well as the protein concentration (due to the thermal expansion of the solution), and the ionization of dissociable groups can change. Together, these effects influence protein absorbance only to a minor extent and therefore the dependence on temperature is usually small in the absence of structural transitions. When denaturants are added, the absorbances of tyrosine and tryptophan at 295 nm and at 297 nm, respectively, increase slightly, even in the absence of structural transitions.

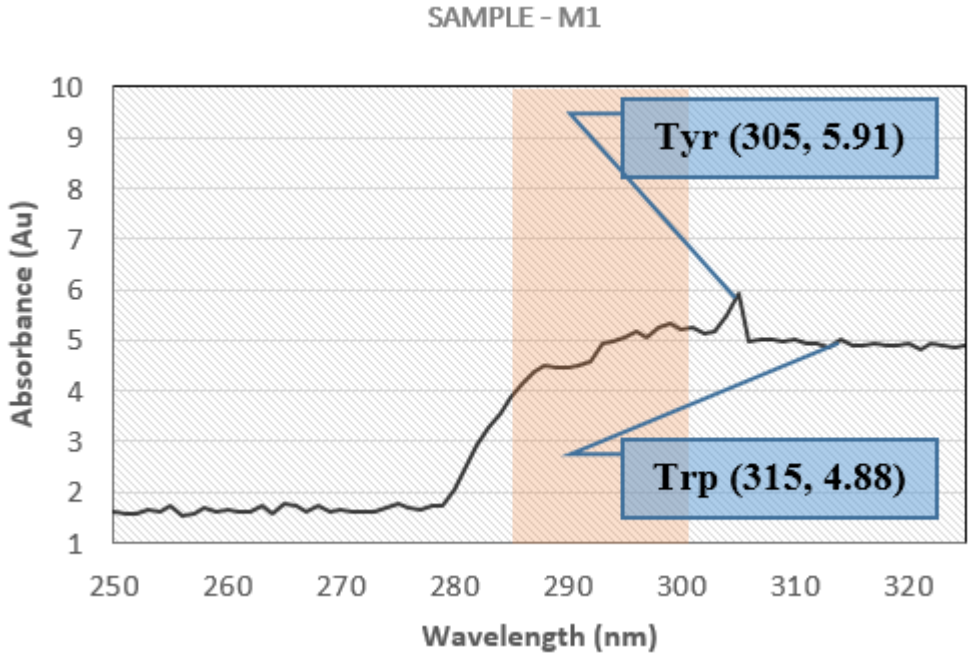

Figure 10: Absorbance spectrum of sample M1

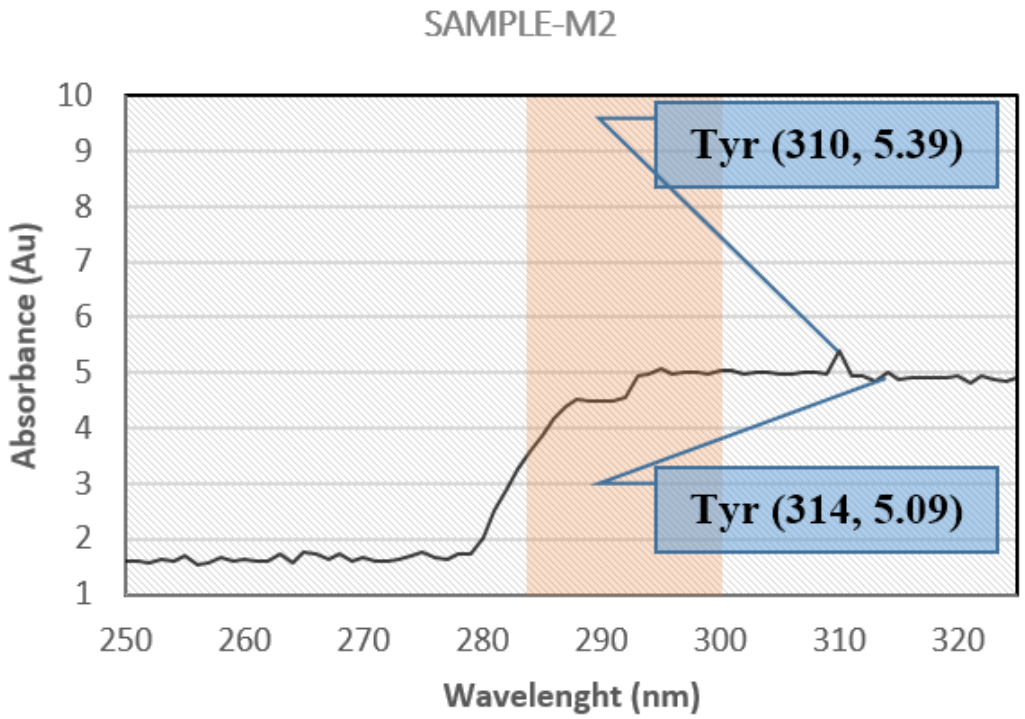

Figure 11: Absorbance spectrum of sample M2

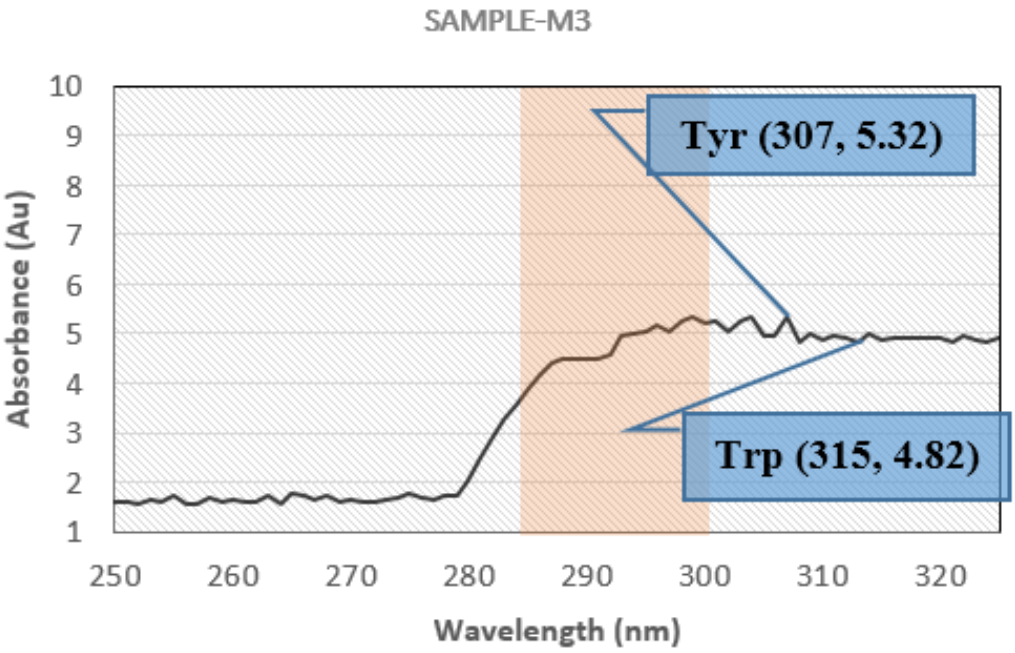

Figure 12: Absorbance spectrum of sample M3

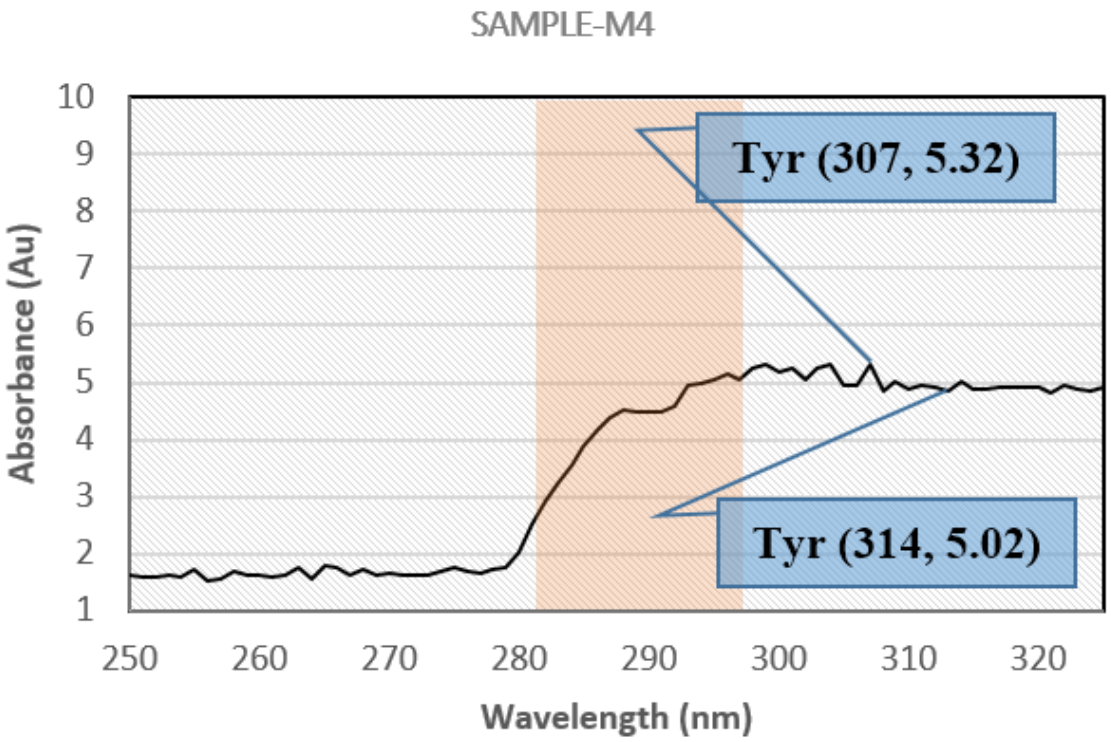

Figure 13: Absorbance spectrum of sample M4

DISCUSSION

Although exposing food to ionizing radiation does not kill pathogens, but basically it damages their DNA enough to prevent them from reproducing.The severity of gamma radiation effect was gradually increased by increasing radiation energy. Samples M3 and M4 were exposed to gamma radiation respectively from Rn^{226}, Am^{241}, sample M3 exposed to 0.168 MeV and sample M4 exposed to 0.05954 MeV. The radiation effect on protein's structure is less than M1 and M2 samples since the radiation energy they have exposed less and thus absorbed dose by these samples also less too.

Gamma radiation was more damaging to protein structure and this is related to the higher energetic "free radicals", it is classified as unstable compounds, its uncharged molecule (typically highly reactive and short-lived) having an unpaired valence electron. These free radicals can be resulted from radiation interaction with amino acids in the protein molecules leading to a chain of events which is likely responsible for the formation of the undesirable side effect including off-flavor (35).

Native proteins can be unfolded by denaturants such as ionizing radiation. Protein unfolding transitions can thus be measured by following the absorbance changes at 295–305 nm as a function of radiation or denaturant concentration.

Basically, the damage that was induced by the radiation on the protein resulting from

the interaction of the ionizing radiation with protein structure, the ionizing radiation initiates free radical oxidation and catalyzes other stages of the oxidation process. Lipid radicals, superoxide radicals (SOR), and H2O2 are formed due to radiation. SOR can further induce carbohydrate cross-linking, protein cross-linking, protein fragmentation peroxidation of unsaturated fatty acid, and loss of membrane fluidity function. The denaturation of components such as proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk may occur after radiation exposure. Milk is considering a radiosensitive compound; this is due to the milk composition; the majority of milk is water (which presents more oxygen area) this is can up the radiation effect to three-time greater than non-oxygenated regions (36).

In our findings, the study shows that the impact of gamma radiation on the protein structure especially on the basic aromatic acids tyrosine and tryptophan was greater compared to x-radiation effects. It is clear that the absorbance wavelength of both aromatic acids does not change with all of the x-radiation exposures which indicates no changes in the protein structure after these exposures, while dramatic changes observed on the absorbance wavelength and absorbance unit value after the exposure to gamma radiation which indicates a major change in the structure of the protein, that explained previously as protein unfolding. Figure 14 shows a comparison between the effect of both X and gamma radiation on the absorbance of tyrosine and tryptophan.

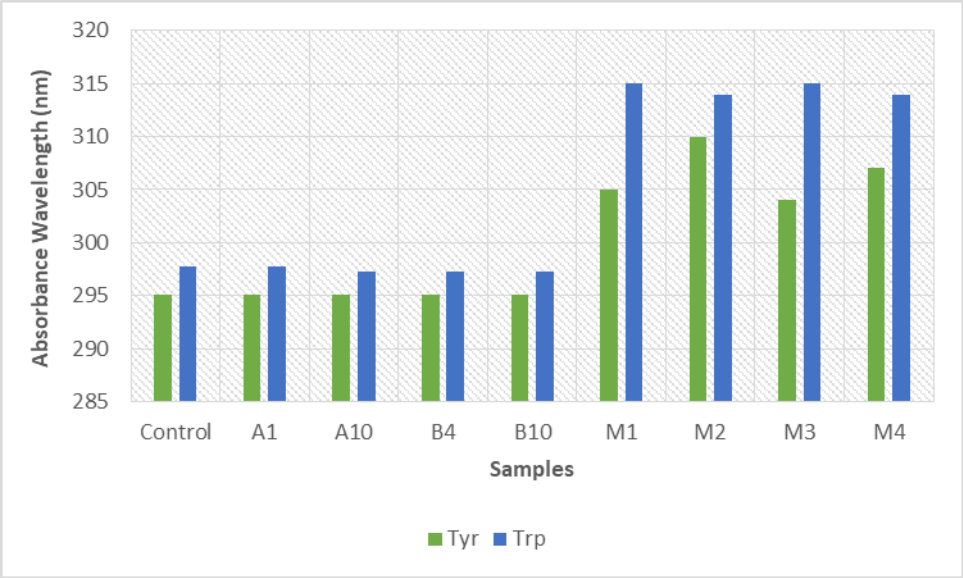

Figure 14: Comparison between X-irradiation and Gamma irradiation effects on the UV absorbance of raw milk protein

CONCLUSION

This study confirmed that gamma irradiation from the radioactive sources used in this

study was more damaging to the structure of milk's protein. All methods used were able to detect damages of proteins as a consequence of preceding irradiation. No principal difference in the observed effects could be found for X- or gamma-irradiation but the damage size obviously varied through the radiation dose scale. With X-irradiation exposures, it is clear that major protein's structure was not affected by X-irradiation exposures, while the effect took step gradually by increasing the kilovoltage and the milliamperes. A series of functional and structural changes could be found with proteins in samples that exposed to gamma radiation; it shows significant damage to the protein's structure in both major and minor amino acids group. It is an interesting observation that the nature of primary damages induced in various proteins by X or gamma irradiation is very similar. However, when using gamma-ray, some of the radiation effects, especially inactivation phenomena, intensified considerably in the post-irradiation period (due to formed H_2O_2) while with X-ray post-irradiation effects will be less pronounced due to the lower ionizing energy. Our findings do not include identifying the specific damage of other nutrients group present. However, in evaluating the radiation exposure range examined in this study, we can propose that the higher the irradiation energy, the higher the actual damage size resulting in other milk's components. Hence, it is recommended to focus on finding a suitable irradiation technique that might replace commercial dairy products pasteurization together with ensuring food safety.

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