Study the Characterization of Spectral Absorbance on Irradiated Milk Protein

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Abstract. The milk has been adopted as a structural nature food for a long era since it is containing most of the growth factors, protective agents, and enzymes needed for the body. A few attempts have been conducted to treat the dairy products especially raw milk by the means of ionizing radiation. As its production has been an expanding industry for many years due to the high demands from the consumers worldwide, there is still some doubt about preserving these products by irradiation. In this work, a preliminary effort to describe the influences of ionizing radiation on raw milk’s protein will be devoted to measuring the spectral absorbance of the total protein (after subjected to varied radiation doses) by UV-VIS-NIR spectroscopy analysis. The absorbance spectrum then analyzed based on absorbance spectra of organic compounds. A comparison is made between the effects of different radiation doses to estimate the influence in milk’s structure.

1. Introduction

The distinguished nutritional value of milk basically originated from its unique compositions which include most of the substantial elements that required by the human body daily, milk structure cover almost all the necessary materials for the human body. Recent milk industry has gradually expanded to approximately 600 million tons annually, this massive productivity can be expressed for easily convert the milk into a wide variety of products which makes it strongly required [1].

For decades, many technologies had been used in attempting to preserve raw milk products during storage, starting from manual cooling of milk underground before electricity to the most popular method today which is the pasteurization of milk. Pasteurization of milk considers as one of the common methods used today for milk preservation.

Dairy research has shown that contamination of raw milk can come from several sources including cow’s mastitis, dirty udders or teats, and poorly cleaned milking and storage equipment [2]. These studies have proved that pasteurization of milk is not sufficient to kill all the microorganism inside the raw milk, several kinds of bacteria have found stayed alive after pasteurization process, these bacteria considered as a heat
resistant bacterium such as Coxiella burnetii and the one that Responsible for mad cow disease [3] & [4]. Subsequently, Milk irradiation has entered the picture to provide a spectacular aid, as proper alternative technology. Today’s technology is Photo purification – which uses ultraviolet (UV) light to inactivate pathogens – could outstrip pasteurization of dairy products in the future [5]. Although this technology still under research, milk such as cow milk after treated by irradiation has shown some changes in its protein structure due to the damaged occurred by radiation [6]. Hence, this study seeks to explain the rationales beyond the inability to irradiate the milk by interprets the characterization of irradiated raw milk using UV-VIS-NIR spectroscopy technology. The formed elements of milk have a wide range of major and minor ingredients that make up its peerless structure, the vast majority of the milk component is water in addition to the other ingredients including proteins, vitamins, enzymes, emulsified globules of fat, a diverse group of basic and secondary proteins, the carbohydrate lactose and minerals, altogether gave the milk its characteristic properties. According to the U.S. Department of Agriculture Nutrient Database; one cup of whole milk has 8 grams of protein. Whereas there are many types of proteins, each has its specific amino acid sequence that distinguished it from others, these amino acid sequences are linked together by peptide bonds. [7] Milk’s proteins are comprising of two major different groups of proteins which classified according to their solubility to insoluble caseins that makes up approximately 80% of the total milk protein and are thus the most abundant class of milk proteins, and the soluble whey proteins which represent about 20% of the total milk protein. Each of these proteins are consist of minor ingredients which contributing within different ratio. Table-1 shows of protein composition of cow’s milk. [8]

Table 1. Major groups of milk’s proteins.

| Major milk proteins | α-lactalbumin |
|---------------------|---------------|
| Casein              | α-s1-casein   |
|                     | α-s2-casein   |
|                     | β-casein      |
|                     | κ-casein      |
|                     | γ-casein      |
| Whey                | Immunoglobulin|
|                     | lactoferrin   |
|                     | β-lactoglobulin|
|                     | Albumin serum |

Specific food nutrients, such as vitamins and amino acids, were all found to be affected by ionizing radiations. The degree of effect was dependent on the concentration of the nutrient, its own relative radiosensitivity (specific inactivation dose, $D_{0}/C$), and the presence of other compounds. [9] Certain foods such as milk were found to be much more radiosensitive than some others, this may refer to the unique influence of ionizing radiation on milks ingredient. [10] According to (Choudhary et al., 2003) damage that has induced by the radiation on the protein can be resulting basically from the interaction of the ionizing radiation with protein structure, ionizing radiation initiates free radical oxidation and catalyzes other stages of the oxidation process. Lipid radicals, superoxide radicals (SOR), and $H_2O_2$ 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. Denaturation of components such as proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk may occur with radiation, thereby also bringing about textural changes. Water also absorbs radiation photons and produces OH-and $H^+$ radicals, which in turn aids changes in other food components.

2. Methodology and materials

I. Samples preparation

Raw goat’s milk used in this study as a target sample was collected from a local farm on the day of the experiment to ensure that they are fresh. The milk samples were divided into 30 individual cuvettes approximately 4 ml in each cuvette, each sample has been exposed to different X-irradiation energy. In this study, the milk sample was not diluted and only pure milk samples were used to study the actual effect of radiation on the natural milk’s protein structure.

II. Samples irradiation

X-irradiation exposures are divided into three categories; First, Second and Third category, the samples of the first group has exposed to the same value of kVp and different values of mAs, for the second category the kVp value has increased to 55, all the sample of this category have exposed to same kVp and different mAs while the last group has exposed for different kVp and same mAs values. Table 2 shows part of the samples and the exposure that has exposed.
Table 2. X-irradiation categories used in the experiment.

| Sample number | Exposure category | Sample name | Kvp | mAs |
|---------------|-------------------|-------------|-----|-----|
| 1             | First Category    | A1          | 40  | 0.45|
| 2             |                   | A2          | 40  | 140 |
| 3             | Second Category   | B1          | 55  | 50  |
| 4             |                   | B2          | 55  | 140 |
| 5             | Third Category    | G1          | 110 | 50  |
| 6             |                   | G2          | 140 | 50  |

III. Experiment Setup
A UV-VIS spectrophotometer used in this study is from model Carry 5000 UV-VIS-NIR, which consider as a high-performance UV-VIS-NIR spectrophotometer with superb photometric performance in the 175-3300 nm range. The measurements have been conducted within the wavelength of 200-400 nm with a scan rate of 100 nm/min. The absorbance has been measured for the specimens without irradiation and then after irradiation at the same range. The absorbance spectrum has been plotted by Cary WinUV software. The absorbance spectrum of the protein has analysed based on the chemistry of organic compound and its interaction with light.

3. Result and Discussion
Absorbance has been measured after irradiation for all sample, then compared to the spectrum of the control sample. the typical absorbance spectrum of the control sample is shown in Figure 1, where the protein’s wavelength ranged from 280-300 nm in pure samples. Basic amino acid tryptophan and tyrosine were observed at wavelength 280-290 nm. Other minor amino acids such as glutamine and aspartic acid have absorbed UV light at wavelength 295-305 nm. Both ingredients consider as a basic unit in the structure of milk’s protein which gave it the distinguished wavelength, they were found to be the most affected part by radiation exposure.

Proteins absorb ultraviolet light with absorbance maxima at 300 and 200 nm. Amino acids with aromatic rings are the primary reason for the absorbance peak at 290-300 nm. Peptide bonds are primarily responsible for the peak at 200 nm. Secondary, tertiary, and quaternary structure all affect absorbance, therefore factors such as pH, ionic strength, sample concentration can alter the absorbance spectrum. Commonly, the optical absorption of proteins is measured at 280 -300 nm. At this range, the absorption of proteins is mainly due to the amino acids tryptophan, tyrosine and cysteine with their molar absorption coefficients decreasing in that order. Of course, the molar absorption coefficient of the protein itself at 280 nm will depend upon the relative concentrations of each of these three amino acids. Therefore, different proteins can have different
absorption coefficients and even the wavelength of the maximum absorption may differ. This fact can be used to help identify different types of proteins by relatively fast and simple optical tests.

**Figure 1.** UV Absorbance of the milk before irradiation.

![Absorbance Spectrum](image)

For the first category of section one, the samples have exposed to 40 kVp and different (mAs) values. The protein structure after exposed for these exposures didn’t show any significant changes, the absorbance of major amino acids chain at wavelength 290 nm didn’t affect at all, while the absorbance of the minor amino acid chain which representing at wavelength 305 nm has gradually decreased by increasing the mAs value.

Figure 2. and Figure 3 shows the absorbance spectrum of the sample (A1) and (A2) which exposed to (40 kVp, 0.45 mAs), the graph shows literally, that is no change has occurred in the protein structure after exposed for this exposure.
Figure 2. UV absorbance spectrum for the sample A1.

Figure 3. UV absorbance spectrum for the sample A2.

Figure 4. Demonstrate the absorbance comparison between the protein in both sample A1 and A2 that have been exposed to the same kVp (40). The absorbance of the major amino acid chain remains the same while the peak at wavelength 295 nm, representing minor acids, has gradually decreased.

Figure 4. Absorbance value of the protein in sample A1 and A2.
For the second category, the samples have exposed for the same kVp 55 and different mAs, in this category, the protein structure has seriously affected by radiation compared to the first category. The absorbance value has gradually decreased by increasing the mAs which controls numbers of photons emitted from the generator. And since the kVp value has increased to 55 which means that the penetration power of these photons became higher so it passes more distance inside the sample. and lose more energy which in turn increases the absorbed dose.

**Figure 5.** UV absorbance spectrum for the sample B1.  
**Figure 6.** UV absorbance spectrum for the sample B2.

Figure 7. show comparison between the absorbance value of the protein in both sample B1 and B2 which have exposed for the same kVp (55), the absorbance of the major amino acid chain as well as minor group have significantly affected by these exposures the absorbance peak at wavelength 295 nm which representing major amino acids group has gradually decreased.
Figure 7. Absorbance value of the protein in sample B1 and B2

The coloured boxes representing the absorbance of the major amino acids group, it is obvious that the absorbance peak at the wavelength 295 nm has gradually decreased by rising the mAs value, while the green boxes representing the absorbance of the minor amino acid group and it have also affected in the same way as the major group.

Figure 8. and Figure 9. shows the absorbance spectrum of the sample (G1) and (G2) which respectively exposed to (110 kVp, 50 mAs), (140 kVp, 50 mAs) the graph shows that the absorbance peaks of sample G1 have totally disappeared while for sample G2 the absorbance has slightly affected at the region of minor amino acid.

For the last category, the samples have exposed to same mAs and different kVp values, the kVp that the samples have exposed to in this category is much higher than last two categories, the effect of radiation on the protein structure also different. In sample G1 which exposed to (110 kVp, 50 mAs) the absorbance peak at 295 nm has totally disappeared after irradiation which indicates that the protein structure has totally destructed. While in sample G2 which exposed to the same mAs (same number of photons have hit this sample as G1) but higher kVp (140), the protein structure did not show any change, and this can be explaining by; as the kVp increased for the same number of photon (same mAs) that’s means that the kinetic energy of these photons is also increased, which push these photon to penetrate the sample without do any significant interaction, they can still lose a trivial amount of its energy during its pathway, but these energy is not sufficient to make the change, in other words as the kVp value increase the absorbed dose going to decrease and so the effect on the protein structure.
**Figure 8.** UV absorbance spectrum for the sample G1.

**Figure 9.** UV absorbance spectrum for the sample G2.

**Figure 10.** Show comparison between the absorbance value of the protein in both sample G1 and G2 which have exposed for the same mAs (50), the influence of radiation on absorbance peaks of the major amino acid chain as well as minor group have been inversely proportional to kVp value.
The coloured boxes representing the absorbance of the major amino acids group, for sample G1, it is clear that the absorbance peak at the wavelength 295 nm has completely disappeared while by raising the kVp value it looks that it does not affect in sample G2, while the green boxes representing the absorbance of the minor amino acid group and it have also affected in the same way as the major group.

4. Conclusion

The damage that has induced by the radiation on the protein can be resulting basically from the interaction of the ionizing radiation with protein structure, 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 protein structure has affected gradually by increasing the mAs, and the damage has inversely proportional to kVp value. Denaturation of components such as proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk may occur with radiation, thereby also bringing about textural changes. Water also absorbs radiation photons and produces OH- and H+ radicals, which in turn aids changes in other food components. Denaturation of components such as proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk may occur with radiation, thereby also bringing about textural changes. Water also absorbs radiation photons and produces OH- and H+ radicals, which in turn aids changes in other food components.

The minor amino acids group has found more radiosensitive than the major amino acid, and this can be explaining by that the major amino acid is found in a large chain with a stronger peptide bond than that for the minor groups, so the energy need to break these bond is greater than that required to break up the bonds which links minor amino acid chain.

References
1. FAO, Ellen Muehlhoff, Anthony Bennett, Deirdre MacMahon, Milk and Dairy Products in Human Nutrition, 2014
2. Maricarmen Anzueto, Tracking Heat-Resistant, Sporeforming Bacteria in the Milk Chain: a Farn to Table Approach, 2014.
3. Hahn, G.Campylobacter jejuni. In: Monograph on The Significance of Pathogenic Microorganisms in Raw Milk. 1994a. Int. Dairy Fed., Brussels, Belgium. pp. 55-67
4. Rachel Arthur, UV radiation will light up milk's future, 2014
5. Srinivasarao Bandla, Ruplal Choudhary, et al., Impact of UV-C processing of raw cow milk treated in a continuous flow coiled tube ultraviolet reactor, 2012
6. Ruplal Choudhary, Srinivasarao Bandla, Ultraviolet Pasteurization for Food Industry, 2012.
7. Tetra Pak, Dairy processing- chapter 2, The Chemistry of Milk, 2014
8. Guetouache, Mourad; Guessas, Bettache and Medjekal, Samir, Composition and nutritional value of raw milk, 2014
9. FDA, Kim M. Morehouse, Vanee Komolprasert, Irradiation of food and packaging:an overview, 2004
10. Adriana Cristina de Oliveira Silva, Luiz A.T. de Oliveira, Edgar F.O. de Jesus, Effect of Gamma Irradiation on the Bacteriological and Sensory Analysis of Raw Whole Milk under Refrigeration, 2015