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The Effects of Low-dose X-ray Irradiation on Fermentation Products of Kefir

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ABSTRACT

Background: Fermented food products are an inherent element of the human diet throughout the world owing to their health benefits. Kefir grains are a starter culture containing yeast and bacteria, which are classified as fermented dairy products. The present study aimed to investigate the effects of low-dose X-ray irradiation on kefir grains and the fermentation constituents.
Methods: Five grams of kefir grains was added to nine dishes. Three dishes were irradiated to 40cGy, three dishes were irradiated to 80cGy X-ray, and three dishes remained non-irradiated as the control group. The chemical properties of the kefir samples (lactic acid, protein, fat, and lactose concentrations) were investigated after X-ray irradiation using standard methods.
Results: The contents of lactic acid, protein, and lactose increased in the samples irradiated to 80cGy X-ray ($P < 0.05$), while their fat content decreased ($P < 0.05$). No significant differences were observed between the 40cGy X-ray samples and controls.
Conclusion: According to the results, 80cGy X-ray irradiation affected the kefir grains and increased their protein, lactose, and lactic acid, while decreasing the fat content, which makes milk consumption more convenient for those with high blood lipid levels.

1. Introduction

Kefir is a commonly consumed traditional drink in the Middle East owing to its favorable properties. The origin of this beverage is the Caucasus Mountains in Central Asia, and it has been consumed historically for thousands of years [1]. Kefir seeds contain lactic acid bacteria, acetic acid bacteria, a combination of yeasts with casein, and a combination of sugars [2]. Considering the high nutritional value and health benefits of kefir, it is recommended to the general population, especially premature infants, children, pregnant and lactating women, and patients. Kefir is nontoxic and abundant and could be used efficiently in radioprotection to exert preventive and/or therapeutic effects [3]. Due to the different properties and compositions of this product, the fermentation conditions in the kefir composition could be altered depending on the properties of kefir grains and the fermentation conditions [4-8].

In recent decades, researchers have reported the positive effects of low-dose ionizing radiation [9-11].

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Radiation hormesis generally refers to the physiological positive effects of low-dose ionizing radiation within the absorption dose range of 1-50 cGy [12, 13]. The hormesis theory applies its effects by controlling intracellular, intercellular, hormonal, and growth factors, as well as calcium and protein kinase signals [14]. Several studies have confirmed the improvement of metabolism in the microorganisms, plants, invertebrates, and laboratory animals receiving low-dose ionizing radiation [15, 16].

By designing different types of studies, researchers have been attempting to increase the biomass of the bacteria present in kefir or modify the compositions of kefir in order to optimize the fermentation conditions and provide optimal products in the most stable conditions.

The present study aimed to investigate the effects of low-dose X-ray on modulating the concentration of the products derived from fermented milk using kefir seeds.

2. Materials and Methods

The linear accelerator facility (Siemens, PRIMUS^{TM}, and Germany) was used for the generation of the X-ray beams in this study. The irradiation conditions were designed with the source surface distance (SSD) of 100 cm, field of 10 × 10 cm2, and energy of 6 MV. Prior to irradiation, the dosimetry and calibration steps were performed in accordance with the International Atomic Energy Agency Technical Reports Series No.398 (IAEA TRS-398) to ensure the output of the accelerator. To this end, nine 500-milliliter dishes containing five grams of kefir were prepared, and every three dishes were divided into three groups, including kefir with 40 cGy X-ray irradiation (group one), kefir with 80 cGy X-ray irradiation (group two), and kefir with sham irradiation (no irradiation; group three/control).

After irradiation, the fermentation process was initiated using 250 milliliters of pasteurized milk, which was obtained from Zanjan Pegah Dairy Company, Iran. The pasteurized cow milk was added to the study groups and incubated at the temperature of 25°C for 24 hours. The concentrations of the fermented milk products (fat, protein, lactic acid, and lactose) were measured before and 48 hours after the radiation of the kefir seeds.

2.1. Fat Measurement

The Gerber method was applied for the measurement of the fat content in the samples using Gerber fat gauge centrifuge (model: Nova Safety). After the temperature of the specimens reached 20 degrees, the fat gauge was inserted, and special pipettes were used to slowly add the milk and sulfuric acid to the fatty gauge. Following that, the fat gauge was placed in the centrifuge device for five minutes, and the fat percentage was read from the calibrated fat column and

fat percentage was read from the calibrated fat column and compared with the standard values [17].

2.2. Protein Measurement

In this study, the measurement of the protein content was performed in three stages, the first of which was the digestion stage where milk was boiled in sulfuric acid, and heat accelerated digestion. Following that, the distillation stage was carried out where the nitrogen content in the valve was released by the ammonia gas, and after entering the liquid, it entered boric acid and formed ammonium borate. At the third stage, titration was performed, so that the ammonium borate formed in the previous stage would be titrated with normal hydrochloric acid. The PECO FOOD device was used for protein distillation, and the nitrogen level in the samples was measured using the following formula:

(Acid Consumption × Acid Normality × 14 × 100) / (1,000 × Warm Weight Sample)

To measure the protein content, the determined nitrogen level was multiplied by the protein factor [17].

2.3. Lactic Acid Measurement

Fresh milk does not contain lactic acid; through fermentation, lactose in lactating acid increases milk acidity. At this stage, one gram of the milk samples was poured into an Erlenmeyer and added to the same amount of water. Following that, five drops of phenol was added to phthalene and titrated with standardized sodium hydroxide. With the appearance of a purple color, titration was discontinued, and the amount of hydroxide consumed was noted. Acidity in terms of the percentage of lactic acid was obtained using the following equation [17]:

Acidity in Lactic Acid Percentage =100 g Sample Weight × ml Sodium Hydroxide × 0.009

2.4. Lactose Measurement

At this stage, the samples were filtered by a sediment solution. After adding phenylhydrazine, the samples were placed inside a hot water bath. After determining their optical density, the following calculations were obtained [17]:

Lactose in Standard Solution × Density Difference of Sample and Control = mg of Lactose per ml of Milk

2.5. Statistical Analysis

Data analysis was performed in SPSS version 18, and the obtained data were expressed as mean and standard error of mean of at least three independent experiments per group. In addition, one-way analysis of variance (ANOVA) was performed to compare the study groups, followed by Tukey's multiple comparison tests. In all the statistical analyses, the *P*-value of less than 0.05 was considered significant.

3. Results and Discussion

A significant increase was observed in the lactose concentration in the group with the irradiation of 80cGy compared to the control group (from 4.01 ± 0.09% to 4.38 ± 0.03%; $P < 0.05$). Furthermore, 80cGy irradiation increased the protein content of the fermented milk compared to the control group (from 2.76 ± 0.06% to 3.17 ± 0.10%; $P < 0.05$).

A significant reduction was observed in the fat content of the fermented milk in the samples with the irradiation of 80cGy compared to the control group (from 1.06 ± 0.02% to 0.86 ± 0.06%; $P < 0.05$). In addition, 80cGy irradiation significantly increased the concentration of lactic acid in the fermented milk compared to the control group (from 0.24 ± 0.01% to 0.28 ± 0.02%; $P < 0.05$). Changes in the lactose, protein, fat, and lactic acid concentrations in the samples with 40cGy irradiation had no significant difference with the non-irradiated samples ($P > 0.05$) (Table 1).

Kefir is a natural probiotic and a viable, active culture medium with normal microbial activity, which is induced by the intense activity of microorganisms and is the primary digestion for proteins, resulting in the final digestion and absorption of proteins in humans [18]. Recently, some reports have indicated that kefir is a potential antioxidant, which directly interacts with multiple species that cause oxidative damage [3].

Radiation hormesis is a hypothesis suggesting the benefits of low-dose radiation for the improvement of metabolism in various microorganisms, plants, invertebrates, and laboratory animals. For the first time, we investigated the effects of low-dose X-ray (<100cGy) on modulating the concentration of the products derived from fermented milk using kefir seeds 48 hours post-radiation. According to our findings, irradiation of 40 cGy X-ray 48 hours post-radiation caused no significant changes in the nutritional parameters of the control and radiation groups. On the other hand, 80cGy irradiation 48 hours post-radiation increased lactose, protein, and lactic acid concentrations compared to the control group, while the fat concentration of the fermented milk decreased compared to the control group, thereby making milk consumption more convenient for those with high blood lipid levels.

Table 1; Effects of radiation on chemical parameters

Chemical characteristics	Groups		
	Control group	40cGy	80cGy
Lactose (%)	4.01 ± 0.09	4.03 ± 0.02	4.38 ± 0.03
Protein (%)	2.76 ± 0.06	2.85 ± 0.12	3.17 ± 0.10
Fat (%)	1.06 ± 0.02	1.03 ± 0.02	0.86 ± 0.06
lactic acid (%)	0.24 ± 0.01	0.24 ± 0.01	0.28 ± 0.02
Lactose (%)	4.01 ± 0.09	4.03 ± 0.02	4.38 ± 0.03

4. Conclusion

Kefir has been reported to strengthen the nervous system, exert anti-cancer effects, accelerate the recovery of tuberculosis patients, lower cholesterol and blood pressure, reduce fatigue, relieve depression, cure gastrointestinal disorders, and improve anti-diabetic effects. To the best of our knowledge, this was the first study to exhibit the effects of low-dose X-ray on modulating the concentration of the products derived from fermented milk using kefir seeds. According to the results, 80cGy X-ray irradiation with kefir grains decreased fat, while increasing the lactose, protein, and lactic acid concentrations of the fermented milk 48 hours post-radiation. Nevertheless, further investigations are required to validate the observed effect, and it is recommended that the effects of physical parameters such as temperature, incubation time, and milk acidity be evaluated as they influence the probabilistic bacterial viability.

Authors' Contributions

All the authors contributed to the preparation of this article. F.S., conducted the field work and drafted the manuscript; O.S., contributed to the statistical analysis; F.S., and H.R., contributed to data collection. All the authors revised and approved the final manuscript.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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