

ORIGINAL ARTICLE

Effect of Direct and Indirect Ultrasonic Methods on Microbial Load and Chemical Quality of Raw Wheat Germ

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ABSTRACT

Background: Thermal methods such as pasteurization and sterilization are currently used to eliminate microorganisms, but they may reduce nutrition value or disturb texture, flavor, taste and color. This study assessed the effect of direct and indirect ultrasonic methods on microbial load and chemical quality of raw wheat germ.

Methods: The wheat germ was prepared from Shiraz, southern Iran. It was treated with two direct and indirect ultrasonic methods. In direct method using the frequency of 20 kHz and maximum time of 10 minutes, immersion of the device probe was undertaken in a reservoir containing wheat germ and in indirect way, ultrasound was applied at frequencies of 50-60 kHz for 30 minutes to the wall of the container with samples of wheat germ. The microbial and chemical characteristics of both treated groups were compared.

Results: The direct method reduced the microbial load of the product by 0.6 logarithms, while in the indirect method, the microbial load of the product was not significantly reduced.

Conclusion: It can be expected that the use of ultrasound waves in an appropriate manner for disinfecting the fresh edible plants, including sprouts, along with its remarkable benefits, in comparison with the traditional thermal methods such as pasteurization and sterilization as well as harmful environmental disinfectants, has a beneficial effect on reducing the microbial load of the germs and its effect on the micronutrients of this nutritious food is little or negligible.

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Introduction

Nowadays, the consumption of raw food materials, especially fruits and vegetables has a rising trend (1). Fresh vegetables play an important role in the diet for the health status of people, so in recent years, many people have been encouraged to consume

fresh vegetables (2). An important category of vegetables is the sprouts of various plants which has multiple uses as herbal foods containing the necessary ingredients for the body (2). Wheat germ makes up the 2.5 to 3% of the grain weight (3) which is a rich source of phytosterols, polyosanols,

unsaturated fatty acids, proteins, vitamins B group, dietary fiber and minerals such as zinc, copper, iron, and magnesium, and the most widely-known source of vitamin E as herbal origin (4, 5).

The importance and popularity of wheat germ in many countries as a food supplement is not a secret to any person. But the main point is that the sprouts can also be a good source of pathogenic and corrosive microorganisms which are mainly originating from the soil (6). The diseases caused by bacterial contamination in fresh vegetables such as wheat germ can occur in different stages of agriculture or after harvest and despite the advances in technology and the appropriate conditions for agriculture, it seems that the prevalence of these infections are still increasing (7).

The growth of the sprouts, within 3 to 7 days, at a temperature of 24-30 °C, and the presence of suitable pH and sufficient moisture provide a good condition for the growth of pathogens (2). Also, the presence of unsaturated fats and lipase oxidizing and hydrolyzing enzymes and lipoxygenase in the wheat germ have made it to become a compound with short efficient life and storage capacity (8). A number of bacterial agents have been reported in assessments carried out on the sprouts of different plants including *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Bacillus cereus* and *Aeromonas hydrophila* (9).

In this regard, few studies were conducted in Iran, which indicated the unfavorable condition of these food products. For instance, Bahonar et al. (2013) investigated the microbial contamination of clover, vetch and wheat germs in Tehran. It was determined that from a total of 27 samples of sprouts, one sample had a moderate status, 10 samples had poor quality, and the rest (16 samples) were spoilage (2). It seems necessary to pay special attention to food safety. Nowadays, thermal methods such as pasteurization and sterilization are used to eliminate the microorganisms, but these traditional methods can cause loss of vitamins as well as changes in the flavor, taste and color (10). Therefore, modern technologies are recommended to achieve a synergistic effect on microbial inactivation of food products and nutrient preservation (11).

Ultrasonic method is a non-thermal process that inactivate microorganisms in foods and enhance the quality and sensory properties of them. (12). One of the main advantages of this technique in comparison to thermal methods is the use of less energy, being economical and eco-friendly, and also minimizing the taste and flavor loss (13). Low frequencies of 20-100 kHz have high power in reducing microorganisms (11). This technique has already been used to reduce

the microbial loads of various fruits and vegetables such as plums, strawberries, lettuce, alfalfa seed, apple and red pepper (14). A major mechanism in reducing microorganisms is thinning of the cell membrane, local heating and the production of free radicals during the ultrasonic process (13). The purpose of this study was to investigate the effect of direct and indirect ultrasonic methods on microbial load and chemical quality of raw wheat germ.

Materials and Methods

In this study, all of the chemical materials were from Merck, Germany with high purity and all the used equipments had valid calibration. Sampling was taken from produced raw wheat germs (the first day of production) from a ready-to use vegetable production unit in Shiraz, southern Iran. Sample entry conditions were having the production date and expiration date on the product container and also having a healthy appearance. The transfer of specimens was carried out in a healthy condition and by a sample transfer bag.

In treatment of wheat germ samples by indirect method, an ultrasonic reservoir device (Elma Schmidbauer GmbH, Type: s60H, Germany) with a frequency of 50-60 kHz (ultrasound applied to the wall of the container containing samples of wheat germ) was used for 30 minutes. In the direct treatment method, the ultrasonic apparatus (Model BANDELIN -Typ: LS 8, UW 3200, Germany) with a frequency of 20 KHz with a 25% and 75% power in two times of 5 and 10 minutes (immersion of the probing rod of the ultrasound apparatus in a container containing samples) were used. Then, all the treated samples along with the control sample were assessed for microbial and chemical tests.

For evaluating of microbial quality of the control and treated samples, total count of microorganisms, Coliforms and *Escherichia coli* and mold and yeast were monitored. The total count of microorganisms were done based on National Iranian Standard No. 5272 (15). The mold and yeast count were done based on the National Iranian Standard No. 10899 by Pour Plate method by applying YGC culture media (16). In the present study, for the counting of the coliforms and *Escherichia coli*, the ECC CHROM agar culture media was used. The blue and red colors in colonies indicated *Escherichia coli* and other coliforms, respectively. The method was the same as the total count of aerobic microorganisms, but the plates were incubated at 37°C for 24 hours and then colonies were counted (17).

In order to evaluate the chemical characteristics of control and treatment samples, the antioxidant and fat oxidation properties were measured. The

antioxidant property was assessed by the FRAP method, and according to the process described by Biglari et al., and Silva et al. (18, 19). Measurement of fat oxidation by TBA was carried out according to Pokorny et al. (20). The research was conducted as a completely randomized design with three repetition. Statistical analysis of the data was performed using SPSS software (version 22, Chicago, IL, USA) by using univariate analysis of variance test, and One-way ANOVA. In order to measure the significant difference between the means, P-value less than 0.05 was considered statistically significant.

Results

Figure 1 shows that the indirect method was effective in reducing the coliforms, total amount of microorganisms, and total amount of mold and

yeast. It should be noted that the use of times more than 10 minutes, in spite of the use of a lot of ice around sample containers, the temperature of the sample increased and thus prevented to investigate the effect of the longer time. This fact is considered as limitations of the test. Figure 2 shows the mean reduction of the coliforms based on the power of ultrasonic treatment, and treatment time. The highest mean for reduction of coliforms was for ultrasonic treatment with a power of 75 kHz for 5 minutes and the least for ultrasonic treatment with a power of 25 kHz for 10 minutes. In Figure 3, the mean reduction of the total count of microorganisms has been presented based on the power of ultrasonic treatment and treatment time. The highest mean in terms of total count of microorganisms was related to ultrasonic treatment with a power of 75 kHz for

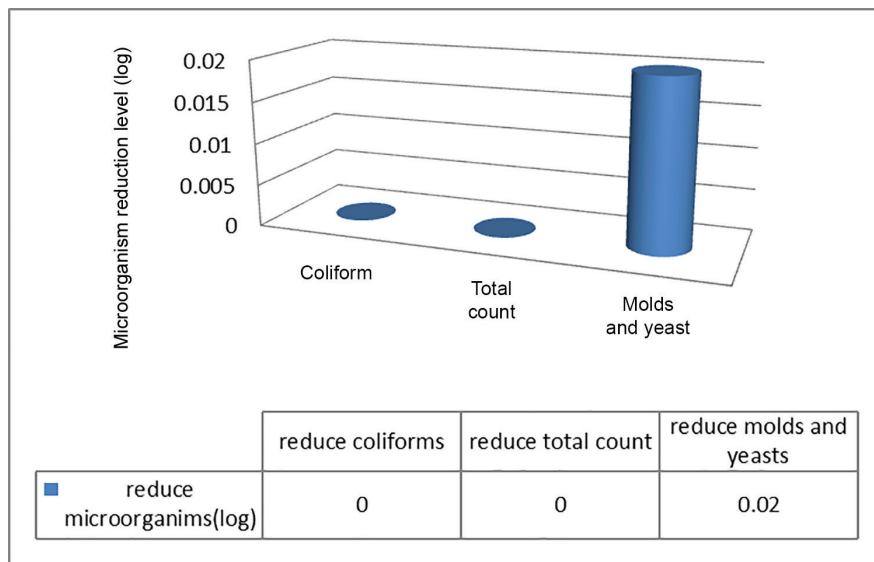


Figure 1: The results of microbial tests of indirect method treating wheat germ.

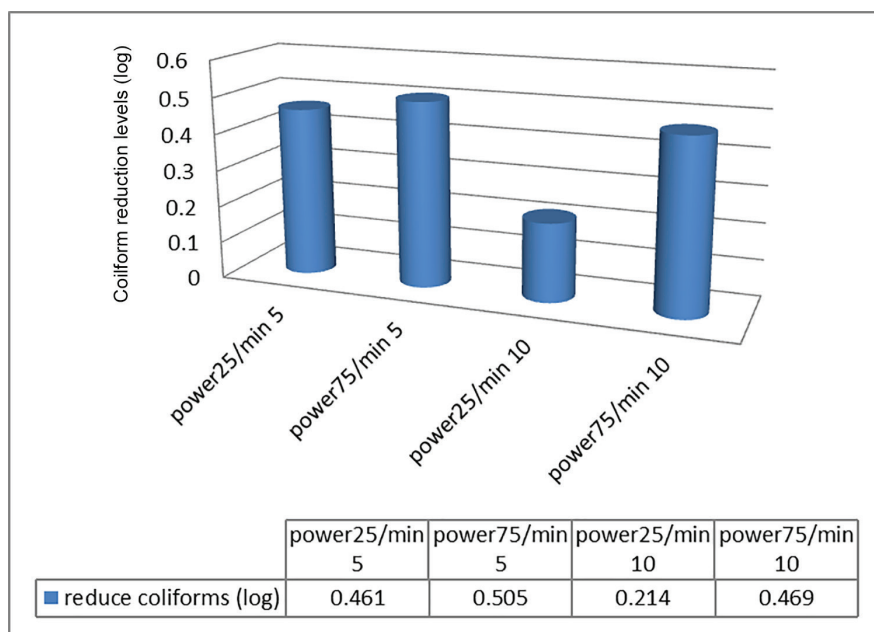


Figure 2: Mean reduction of coliforms (in terms of log cfu/g) based on the power of ultrasonic treatment and treatment time.

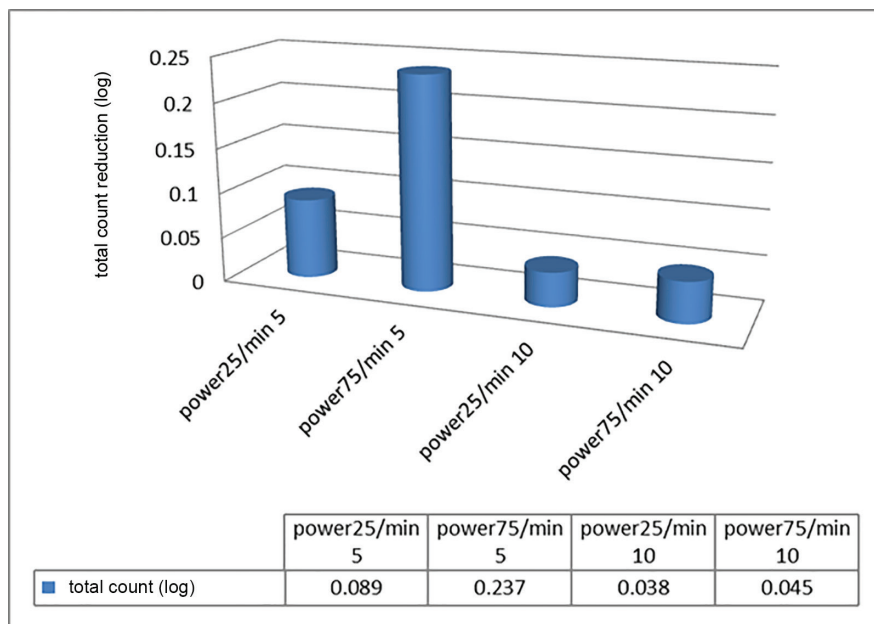


Figure 3: Mean of total microbial count (in terms of log cfu/g) based on the power of ultrasonic treatment and treatment time.

5 minutes, and the least was ultrasonic treatment with a power of 25 kHz for 10 minutes.

Figure 4 reveals the results related to the mean reduction of mold and yeast in terms of the power of the ultrasonic treatment and the treatment duration. As shown, ultrasonic treatment had a reversible effect on reducing the mold and yeast levels. The ultrasonic treatment led to an increase in the amount of mold and yeast levels. The highest mean in terms of mold and yeast increase was related to ultrasonic treatment, with a power of 25 kHz for 10 minutes, and the lowest was ultrasonic treatment, with a

power of 75 kHz for 5 minutes. Figure 5 denotes to the results related to the mean of antioxidant activity reduction based on the ultrasonic treatment power and treatment time. The highest mean in terms of antioxidant property was attributed to ultrasonic treatment with a power of 25 kHz for 10 minutes, and the least was related to ultrasonic treatment with a power of 25 kHz for 5 minutes.

The amount of lipid of raw wheat germ was very low and about 0.02%, however, the lipid oxidation of the treated samples was monitored. In Figure 6, the results related to the mean of lipid oxidation

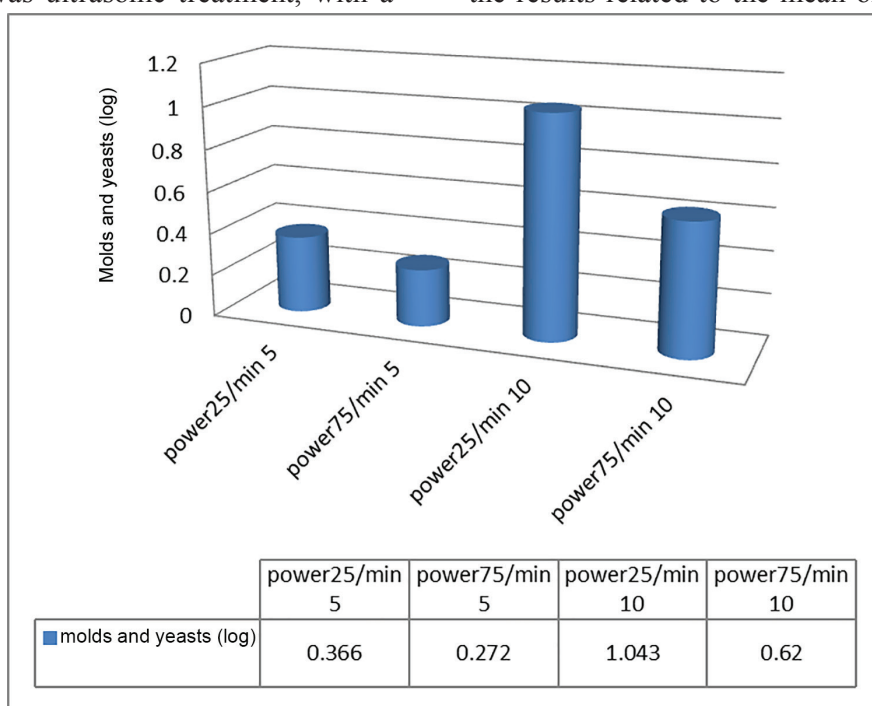


Figure 4: Mean of mold and yeast increase (in terms of log cfu/g) based on the power of ultrasonic treatment and treatment time.

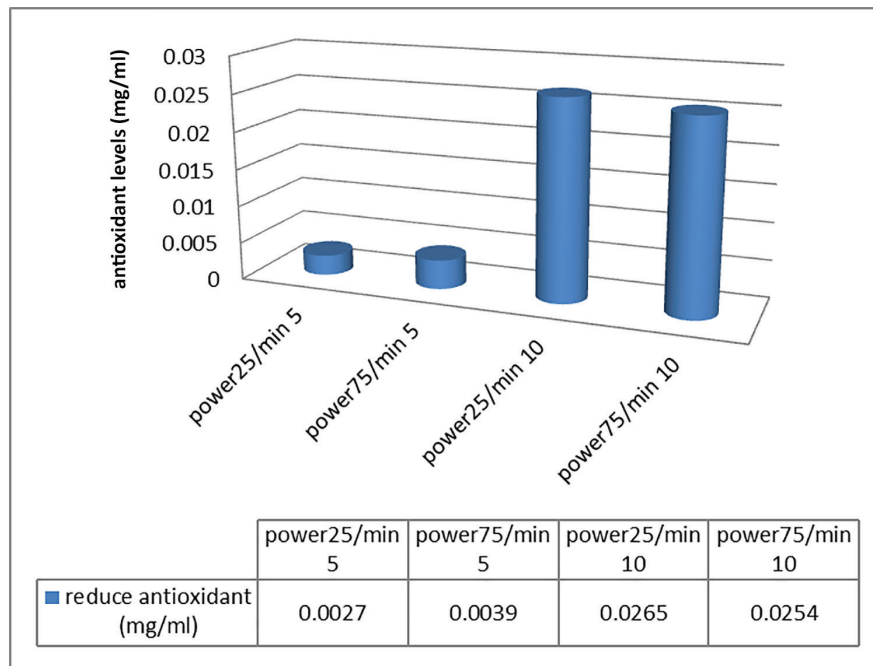


Figure 5: Mean of antioxidant property reduction (in terms of mg/ml) based on the power of ultrasonic treatment and treatment time.

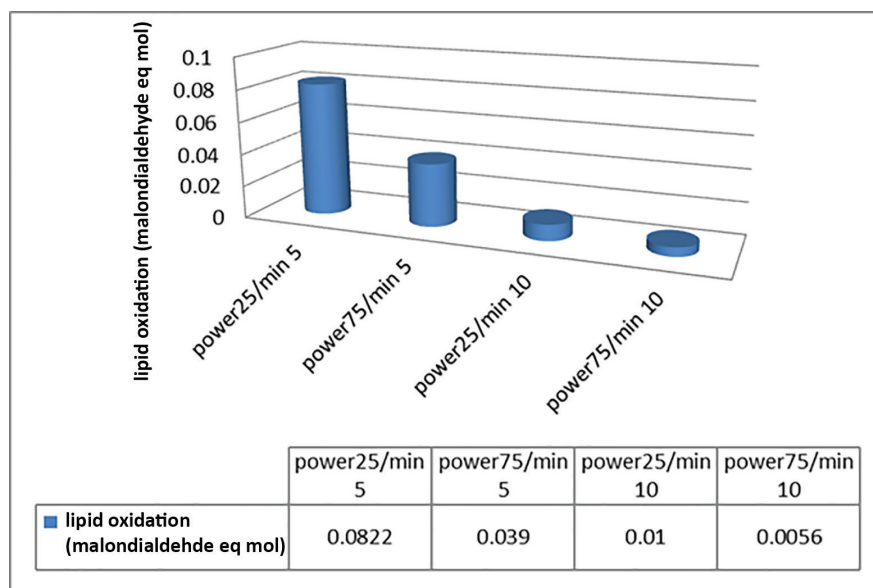


Figure 6: Mean of lipid oxidation increase (in terms of malondialdehyde) based on the power of ultrasonic treatment and treatment time.

increase based on the ultrasonic treatment power and the treatment time were expressed. The highest mean with regard to the increase in the fat oxidation was related to ultrasonic treatment with a power of 25 kHz for 5 minutes, and the least was attributed to the ultrasonic treatment with a power of 75 kHz for 10 minutes.

Discussion

Our results showed that in the indirect method, despite the use of waves with frequency of 50-60 kHz and even in 30 minutes, no reduction was observed in the count of coliforms and the total count of microorganisms in the wheat germ and

just the amount of mold and yeasts decreased by 0.02 log. However, in the direct method, i.e., the probe gauge was plunged in a reservoir containing a wheat germ sample at a frequency of 20 KHz and a maximum of 10 minutes, and a reduction in microbial load of up to 0.6 logarithms in the coliforms and total count was observed. While, in this method, the count of mold and yeast slightly increased. Therefore, an appropriate method should be combined with ultrasonic to cover this defect. There are several studies on combination methods with ultrasound for having a complementary effect on the inactivation of germs of food products (21, 22). Since the main reason for the use of sprouts is

the presence of valuable nutrients in these products, it is essential that these processes be carried out in such a way that they have the least harmful effect on these compounds (11, 12).

As it was seen in the results, there was no significant difference in the effect of treatment on the increase of lipid oxidation level, as well as in microbial tests, between different types of treatments, based on the power of ultrasonic treatment and treatment time. However, among all microbial and chemical tests, according to one-way ANOVA test results, the difference in the mean of antioxidant properties reduction in different treatments was significant. So in order to determine the best treatment we have to focus on this section. As it is indicated in the figure 5 and considering the fact that there is a significant difference between the applied treatments at the times of 5 and 10 minutes, therefore, the ten-minute treatments had no chance of selection and the best treatment should be introduced among the five-minute treatments.

Although there was no significant difference between different types of treatments in response to microbial tests for reduction of coliforms and total count of microorganisms, the status of ultrasonic treatment with the power of 75% and 5 minutes was more favorable. Therefore, between these two ultrasonic treatments; with the power of 75% in 5 minutes and with the power of 25% in 5 minutes, the best ultrasonic treatment was treatment with power of 75% in time of 5 minutes.

In this study, there was no possibility of using more ultrasonic power and temperature setting. Considering the benefits of using ultrasonic technology, and the results obtained from this study, further studies are needed. Studies that measure the effectiveness of this technology in reducing the microbial load and increase the final quality of the fresh products; as well as research that determine the impact of the combined technology of this method with other disinfectant methods.

It can be expected that the use of ultrasound waves in an appropriate manner for disinfecting the fresh edible plants, including sprouts, along with its remarkable benefits, in comparison with the traditional thermal methods such as pasteurization and sterilization as well as harmful environmental disinfectants, has a beneficial effect on reducing the microbial load of the germs and its effect on the micronutrients of this nutritious food is little or negligible.

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Conflict of Interest

None declared.

References

- 1 Jeddi MZ, Yunesian M, Gorji ME, et al. Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *J Health Popul Nutr.* 2014;32:391.
- 2 Bamonar A, Mokhtari A, Panahi P, et al. Investigation of microbial contamination of clover, mung and wheat germs in Tehran. *Comp Pathobiol.* 2013;10:937-942. (Persian)
- 3 Srivastava AK, Sudha ML, Baskaran V, et al. Studies on heat stabilized wheat germ and its influence on rheological characteristics of dough. *Eur Food Res Technol.* 2007;224:365-72. DOI:1007/s00217-006-0317-x.
- 4 Zarenejad F, Peighambari SH. Effect of stabilization on functional components and fatty acid profile of wheat germ. *Iran J Nutr Sci Food Technol.* 2014;8:93-100.
- 5 Sure B, Easterling L, Dowell J, et al. Improving the nutritive value of cereal grains. I. Improvement in the efficiency of the proteins in milled wheat flour with lysine, valine, threonine and an extract from condensed fish solubles. *J Nutr.* 1953;50:235-44. DOI:1093/jn/50.2.235. PMID:13070082.
- 6 Peles F, Györi Z, Bácskai T, et al. Microbiological quality of organic wheat grains and sprouts. *Analele Universității din Oradea, Fascicula Protectia Mediului.* 2012;18:53-60
- 7 Alexopoulos A, Plessas S, Ceciu S, et al. Evaluation of ozone efficacy on the reduction of microbial population of fresh cut lettuce (*Lactuca sativa*) and green bell pepper (*Capsicum annum*). *Food Control.* 2013;30:491-6. DOI:1016/j.foodcont.2012.09.018.
- 8 Srivastava AK, Sudha M, Baskaran V, et al. Studies on heat stabilized wheat germ and its influence on rheological characteristics of dough. *Euro Food Res Technol.* 2007;224:365-72. DOI:1007/s00217-006-0317-x.
- 9 Beuchat LR, Ryu JH. Produce handling and processing practices. *Emerg Infect Dis.* 1997;3:459-65. DOI:3201/eid0304.970407. PMID:9366597.
- 10 Gao S, Lewis GD, Ashokkumar M, et al. Inactivation of microorganisms by low-frequency high-power ultrasound: 2. A simple model for the inactivation mechanism. *Ultrason Sonochem.*

- 2014;21:454-60. DOI:1016/j.ultsonch.2013.06.007. PMID:23845410.
- 11 Ferrentino G, Spilimbergo S. High pressure carbon dioxide combined with high power ultrasound pasteurization of fresh cut carrot. *J Supercrit Fluids*. 2015;105:170-8. DOI:1016/j.supflu.2014.12.014.
 - 12 Silva F. Inactivation of *Byssochlamys nivea* ascospores in strawberry puree by high pressure, power ultrasound and thermal processing. *Int J Food Microbiol*. 2015;214:129-36. DOI:1016/j.ijfoodmicro.2015.07.031. PMID:26280285.
 - 13 Piyasena P, Mohareb E, McKellar R. Inactivation of microbes using ultrasound: a review. *Int J Food Microbiol*. 2003;87:207-16. PMID:14527793. DOI:1016/s01681605(03)00075-8.
 - 14 Bilek SE, Turantaş F. Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. *Int J Food Microbiol*. 2013;166:155-62. DOI:1016/j.ijfoodmicro.2013.06.028. PMID: 23867364.
 - 15 Institute of Standards and Industrial Research of Iran (Microbiology of the food chain Horizontal method for the enumeration of microorganisms Part 1: Colony count at 30 °C by the pour plate technique) ICS:07.100.30 2015. *Method No*. 5272-1.
 - 16 Institute of Standards and Industrial Research of Iran (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds -Part 2 : Colony count technique in products with water activity less than or equal to 0.95). *Method No*. 10899-2.
 - 17 Soltan Dallal MM, Vahedi S, Ebrahimi A, et al. Evaluation of three enrichment broths and six plating media for isolation and detection of salmonella in food stuffs. *J Kurdistan Uni Med Sci*. 2008;13:28-34.
 - 18 Biglari F, AlKarkhi FMA, Mat Ease A. Cluster analysis of antioxidant compounds in dates (*Phoenixdactylifera*): Effect of long-term cold storage. *Food Chem*. 2009;112:998-1001. DOI:1016/j.foodchem.2008.06.063.
 - 19 Silva EM, Souza JNS, Rogez H, et al. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chem*. 2007;101:1012-18. DOI:1016/j.foodchem.2006.02.055.
 - 20 Pokorny J, Dieffenbacher A. Determination of 2-thiobarbituric acid value: direct method-results of a collaborative study and the standardised method. *Pure Appl Chem*. 1989;61:1165-70. DOI:1351/pac198961061165.
 - 21 Al-Hashimi AM, Mason TJ, Joyce EM. Combined Effect of Ultrasound and Ozone on Bacteria in Water. *Environ Sci Technol*. 2015;49:11697-702. DOI: 1021/es5045437.
 - 22 Aday MS, Caner C. Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. *LWT Food Sci Technol*. 2014;57:344-51.