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The effect of ultrasound and pure apple vinegar as pretreatments on the functional and antibacterial properties of drying garlic slices in hot air

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ABSTRACT

Drying is one of the most effective ways to increase the shelf life and preserve the valuable compounds of Foodstuffs, which can be improved by using different methods and materials. Using bioactivecompounds as a pre-treatment can be one of the effective ways to increase their bioactive and antibacterial properties.This research was conducted to simultaneously apply ultrasonic pretreatment and pure apple vinegar before drying garlic slices, and investigate their effect on the bioactive and antibacterial properties of garlic slices dried by hot air.Slices of garlic were subjected to pure apple vinegar and ultrasonic treatment for ten minutes at 35 ºC and they were placed before the drying process and dried using a hot air displacement dryer at a temperature of 50 ºC to a final moisture content of 6% on a wet basis.After evaluating chemical properties, including the content of total phenolic and flavonoid compounds, the determination of functional properties, antioxidant and antibacterial properties were evaluated.The results showed that the simultaneous use of ultrasound and pure apple vinegar had a significant impact on the amount of bioactive compounds and antibacterial properties of dried garlic slices. Pre-treated samples with pure apple vinegar had a more significant impact on increasing functional properties than the ultrasound pretreatment, and it eventually became clear that the use of ultrasonic pretreatments and pure apple vinegar improved the quality, bioactive, and antibacterial properties of sliced dry garlic samples.

1-Introduction

Garlic, with the scientific name " *Allium Sativum*.L "is a plant cultivated all over the world due to its medicinal and food properties and is used in the medical industry to treat many diseases and in the food industry as a spice. Its ingredients include anthocyanins, glycosides, essential oils, flavonoids, fructans, lectins, adenosines, pectin, vitamins B1, B2, B6, C, E, and biotin, essential amino acids, nicotinic acid, glycolipids, fatty acids and phospholipids [1-3]. In recent decades, many studies have been conducted on the biological properties of garlic, which include antioxidant, anti-inflammatory, antibacterial, antifungal, anti-cancer, anti-diabetes, and anti-obesity properties [4-7].

Fresh garlic has a moisture content of 65-75% (wet basis), which reduces its quality over time.Therefore, drying is one of the best methods to increase shelf life and preserve bioactive compounds [1, 8].Drying materials reduce their water content by 90%, which reduces spoilage by microorganisms, reduces transportation costs, and prevents harmful reactions from water [9-11].

Apple vinegar is a natural product of apple fermentation, containing polyphenols, vitamins, minerals, and organic acids. The acetic acid in apple vinegar has an excellent ability to destroy toxic substances and bacterial organisms. This substance increases the effectiveness of digestive enzymes, increases absorption capacity, and plays a role in reducing blood lipids and improving body immunity [12, 13]. Many studies have shown the therapeutic effects of vinegar due to the presence of acetic acid [14]. The application of ultrasound as a non-thermal process can prevent the harmful effects of heat on the nutritional and sensory properties of Foodstuffs and improve the efficiency of drying and inactivating enzymes [15, 16]. The ultrasonic process accelerates chemical reactions and shortens the reaction time [17]. According to the studies, the use of ultrasound as a pre-treatment can reduce the water activity, improve the color of the product, and preserve the nutrients [18]. It seems that using these two pretreatments has a high effect on improving the characteristics of dried garlic slices.

In examining the qualitative characteristics of dried garlic slices pretreated with sonication, osmosonication, and osmosonication under vacuum, it has been reported that the best quality is related to samples pretreated with osmosonication under vacuum, osmosonication, sonication, and samples without pretreatment, respectively [1]. In another study that examined the quality characteristics of garlic slices dried by infrared under the influence of alcohol-ultrasonic pretreatments, it was found that with this method, the quality characteristics of the productand the microbial properties are improved except for allicin content [19, 20].In another study, the effect of ultrasound pretreatment on garlic slices dried by microwave and convection methods was investigated.According to the results, this pretreatment increased the drying speed and decreased the rehydration ratio, yellowness and redness index, and density of each sample [21].In another study, the use of ultrasound treatment along with hot air drying of garlic slices improved the quality characteristics of the product and preserved functional compounds [22, 23].

Despite studies on drying garlic slices using ultrasound and non-thermal pretreatments, the use of acidic substances containing functional compounds has not been given much attention in this field.Therefore, in the current research, the effect of ultrasound pretreatment and pure apple vinegar on the functional and antibacterial properties of garlic slices dried under hot air has been investigated.

2-Material and methods

2.1. Chemicals and Fresh garlic samples

The chemicals used in this research include: ethanol (Arvin Chemical), NaOH (Merck), Folin-Ciocalthio reagent (Sigma), gallic acid (Sigma), 2,2-diphenyl-1-picrylhydrazine (DPPH) (Sigma), quercetin (Sigma), $AlCl_3$ (Merck), sodium nitrite (Merck), sodium carbonate (Merck), nutrient broth (Merck), nutrient agar (Merck), and apple vinegar with 5% acetic acid (organic).The required raw purple garlic was purchased from the local market in Urmia and stored in the refrigerator at a temperature of 4±1°C for two weeks.

2.2. Preparation of samples

First, the garlic samples were washed, then peeled, and cut into 2±0.5 mm thick. The initial moisture content of the samples was calculated in an oven (SDON-301, made in Korea) with a temperature of 105 ± 2 °C using the AOAC (2000) method until a constant final weight was reached. This value for fresh garlic samples was 68.96±0.24% (wet basis). Garlic slices were subjected to ultrasonic pretreatments (RoHS, DSA100-SK_2-4.0L, made in Korea) with a frequency of 40 kHz for 10 minutes at a temperature of 35 °C [1, 24]. They were kept at 35 °Cin the pure apple vinegar treatment for ten minutes. Then, the surface of the samples was dried with filter paper. The samples were dried at a temperature of 50 °C with a speed of 1 m/s under hot air flow until reaching the final moisture content of 6% (wet basis).

Considering that garlic has a spicy taste, it is not consumed alone, and it is used as a flavoring powder in combination with other food ingredients. On the other hand, according to the studies, it was already knownthat the application of ultrasound pretreatment caused the color of the samples to become lighter, and the use of vinegar under certain conditions caused the green color in the samples. Therefore, by considering all of these cases, different conditions of temperature and timewere used toproduce samples with minimal colorchanges. Finally, the optimal conditions were mentioned.

2.3. Preparation of an alcoholic extract

To prepare the extract, 0.5 g of powdered garlic was added to 10 ml of 80% ethanol and mixed at 25 °C for 30 minutes under ultrasound treatment (RoHS, DSA100-SK_2-4.0L, made in Korea) with the frequency set to 40 kHz. Then, the resulting mixture was centrifuged at 10000 rpm for 10 minutes, and the upper part was separated [22].

2.4. Determination of total phenol content(TPC)

5 ml of FolinCiocaltio reagent (1:10 with water) was mixed with 1 ml of garlic extract and 4 ml of 7.5% sodium carbonate for 10 minutes and kept at 30°C for 30 minutes. It is absorbance was measured by a spectrophotometer (Pharmacia, LKB Biochrom, made in England) at 760 nm.Phenol content was reported in $mg/g_{\text{gallic acid (GAE)}}$ of the dry weight of the material [1].To draw the gallic acid standard curve, 25 mg of gallic acid was mixed with 250 ml of distilled water, and different concentrations of gallic acid (0-100 mg/lit) were prepared.Moreover, according to the method of measuring the phenol content of the extract, the absorption of gallic acid solution samples was measured.

2.5. Determination of total flavonoid content(TFC).

0.5 ml of the extract was added to 2 ml of distilled water and 0.15 ml of NaNO2 solution (5%). Six minutes later, 0.15 ml of AlCl3 solution (10%) was added to the mixture. After 6 minutes, 2 ml of NaOH (4%) was added to the mixture and immediately made up to 5 ml with distilled water. The absorbance of the samples was measured by a spectrophotometer after 15 minutes of storage at 510 nm, and the result was reported in terms of $mg/g_{\text{quercetin}}$ of the dry weight of the sample [1]. In order to draw the standard curve of quercetin, 5 mg of quercetin was added with 80% ethanol in

a 10 ml flask, and different concentrations of quercetin were prepared. The steps explained to measure the absorption of the extract for different concentrations of quercetin were repeated.

2.6. Antioxidant activities

The DPPH method was used to measure the antioxidant capacity of dried garlic slices. 3 mL of garlic extract was mixed with 1 mL of a 0.1 mM DPPH solution and placed in the dark at 25°C for 30 minutes. The absorbance was read at 517 nm [1]. Formula (2) was used to calculate the antioxidant activities, where A0 is the absorption rate of the control sample (80% ethanol + DPPH), and A is the absorption rate of the sample (extract + DPPH).

$$\text{redical scavengingDPPH} = \frac{A_0 - A}{A_0} \times 100\% \quad (2)$$

2.7.Fourier-transform infrared spectroscopy (FT-IR)

Some dried garlic was powdered and mixed with potassium bromide and pressed into a thin sheet, then scanned (at 400-4000 cm^{-1}) with the Fourier transform infrared spectrophotometer (IASCO, FT/IR-4100, Japan) [1].

2.8. Determination of hydroxymethyl furfural content (HMF)

1 g of dried garlic powder with 1 ml of clarifying solutions, Carrez I (10.6 g of potassium hexaferrocyanide in 100 ml of water), and Carrez II (21.9 g of zinc acetate with 3 ml of aceticacid were added to the volume of 100 ml of distilled water) to precipitate fat, protein, and carotenoids brought to 15 ml with distilled water.After 30 minutes of storage, the samples were centrifuged at 3500 rpm for 15 minutes at room temperature, and the supernatant was passed through a 0.45 µm filter.The samples were kept at -20 degrees until use.From the HPLC device (KNAUER D-14163, Germany), equipped with a UV detector in the form of a C_{18} column (250 mm long, 4.6 mm internal diameter, and 5 µm particle size at room temperature) to inject the sample in a volume of 200 µl. It was used at 283 nm and a mobile phase (a mixture of acetonitrile and methanol at a ratio of 4% and 6% in HPLC-grade distilled water) at a speed of 0.8 ml/min.The HMF standard sample with a concentration of 1 ppm was injectedinto the device to determine the time and place of exit.The HMF standard curve was drawn using its different concentrations (5-100 mg/lit) [25].

2.9. Determination of antibacterial activity

Four types of bacteria, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Listeria monocytogenes, were used by the paper disk diffusion method, and the non-growth halo was investigated. Activation of bacteria was done by nutrient broth culture medium. then a suspension equal to 0.5 McFarland (1.5×10^8) cfu/ml was prepared by dilution method and determination of turbidity at 625 nm, and a dilution suspension equivalent to 1.5×10^6cfu/ml was prepared by physiology serum and cultured on nutrient agar culture medium. For sterilization, the extracts were passed through a 0.45 µm filter, and sterile paper discs were added to it and placed on a nutrient agar culture medium. The culture medium was kept at 37 °C for 18 hours inside the incubator [26].

1. Statistical analysis

The results were designed as a factorial experiment in a completely random design and analyzed using SPSS Statistics 25 software. Graphical analyseswere achieved usingExcel 2019.

3-Results and discussion

4.1. Total phenol content(TPC)

The resulting equation of the gallic acid curve was y=0.0269x-0.0026 with R^2=0.998, which was used to determine the total phenol content.The results of the analysis of the samples according to Table 1 showed that there was a significant difference between all the samples in terms of phenol content ($p<0.05$).The highest value was in the samples pre-treated with ultrasound and pure apple vinegar, and the lowest was in the control sample.

According to the results of research articles, apple vinegar samples contain gallic acid, catechin, epicatechin, chlorogenic acid, and p-coumaric acid, and the main phenolic substances in apple vinegar are gallic acid and chlorogenic acid.During the processing according to the mentioned method, compoundsof apple vinegar entered the garlic slices.As a result, it has caused an increase in the content of phenolic compounds.Furthermore, due to the acidic state of apple vinegar, some of the compounds present in the cell wall of garlic slices have been decomposed, and the possibility of transferring the mass of phenolic and antioxidant compounds in vinegar into the garlic slices has increased. On the other hand, some compounds in the reaction with acid have changed and turned into compounds with an OH group and are phenolic.According to the research, it has been observed that the amounts of antioxidant and phenolic compounds increase with the release of phenolic compounds bound to sugar in the fruit (garlic slices) [27, 28].

Ma et al. (2021) have stated in their reports about the use of 5% acetic acid in the production of traditional green garlic "Laba" that acetic acid as a secondary metabolite has a positive role in increasing phenolic compounds and stimulates the biosynthesis of these compounds.Probably acidic solutions havereleased antioxidants, and after that, the content of polyphenols has increased [29].Alolga et al. (2021), regarding the use of pre-treatments before drying the garlicsamples using ultrasound and salt, stated that salt causes the osmotic pressure gradient, loosening, and disintegration of the cell structure.Moreover, the creation of small holes in the garlic tissue has increased the diffusion of moisture and the drying speed and reduced the drying time. As a result, it has caused the preservation of bioactive compounds and improved mass transfer [1].

4.2. Total flavonoid content(TFC)

The resulting equation from Querstein's standard curve is y=5.8045x+0.0211 with R^2=0.998.According to the results of the variance analysis of the samples (Table 1), there was no significant difference ($p<0.05$) between the garlic samples without pre-treatment and the sample pre-treated with ultrasound. However, between these two samples and others,there was a significant difference ($p<0.05$).The highest amount of total flavonoid was related to the samples pre-treated with ultrasound along with pure apple vinegar, and the lowest amount was related to the control sample, which showed that apple vinegar contains beneficial flavonoid compounds (according to the tests performed on the consumed apple vinegar),which entered the tissue of the product and caused an increase in the total flavonoid content, which the use of ultrasound treatment also caused to some extent.

Osae et al. (2019), in their research on the pretreatment of sugar solution and ultrasound, stated that higher mass transfer and lower temperature are the most effective ways to preserve flavonoid compounds [30].Alolga et al. (2021) also stated in their studies that the diffusion of moisture, increased drying speed, reduced drying time, and the use of some pre-treatments caused the preservation and

improvement of bioactive compounds in the dried product.The studies of Feng et al. (2019) agree with the above results [1, 31].

4.3. Antioxidant activities

The antioxidant activity of various samples by the DPPH method in Table 1 shows that control samples and samples containing ultrasound pretreatment with vinegar had the lowest and highest antioxidant capacities, respectively. The analysis of the data showed a significant difference ($p \leq 0.05$) among all the treatments.Because of the short drying time, effective compounds, such as the phenolic group, which has antioxidant activities, were preserved. The use of ultrasound pretreatment has also had a positive effect on increasing antioxidant activities.

Research has shown that when using ultrasound, the speed of drying increases, which in turn reduces the duration of the process. Therefore, the antioxidant compounds present in the raw sample may remain higher than when ultrasound is not used.On the other hand, this technique increases bound antioxidants such as phenolics and ascorbic acid contents, which in turn causes an increase in antioxidant activity. The use of ultrasound can increase the mass transfer rate of compounds based on the cavitation created in the material. The polymer structure of the cell wall is destroyed, and as a result, the release of bioactive compounds from plant materials increases. Osae et al. (2019), during the studies conducted on ginger in the application of non-thermal pretreatments such as sucrose, stated that the use of these pretreatments increased the formation of free radicals and caused the polymerization of phenolic compounds, which led to an increase in antioxidant activity [24]. Feng et al. (2020) also attributed a significant decrease in antioxidant compounds to the low number of bioactive compounds in their studies [8]. In their research, Tao et al. (2018) considered the increase in antioxidant activities to be related to the production of bioactive compounds that make up the antioxidant profile of garlic [22].

Table 1. Antioxidant activities, TPC, and TFC of garlic slices under different pretreatment conditions

samples	TPC (mg GAE/gdw)	TFC (mg QE/gdw)	Antioxidant activities (%)
C	2/24±0/05^{a}	5/60±0/23^{a}	24/69±3/62^{a}
US	2/95±0/02^{b}	5/87±0/09^{a}	34/88±0/27^{b}
PAV	4/62±0/04^{c}	7/27±0/28^{b}	54/56±3/65^{c}
US+PAV	5/02±0/01^{d}	7/96±0/28^{c}	63/53±0/37^{d}

Means with different letters within a column indicate significant differences ($p<0.05$).
C: Control, US: Ultrasound, PAV: Pure Apple Vinegar and US+PAV: Ultrasound+Pure Apple Vinegar

4.4. Fourier-transform infrared spectroscopy (FT-IR)

According to Figure 1, the peaks in all samples were at 3434, 2926, 2383, 1645, 1418, 1127, and 1032 cm^{-1}. The most substantial peak is related to the wave number 3434cm^{-1} to the stretching of O-H bonds, which indicates the presence of polyhydroxy compounds such as flavonoids and saponins [32]. The results of the content of phenol, flavonoid, and antioxidant capacity in this research were consistent with the above results; according to the clauses presented, the compounds contain an OH group. The next peak at 2926 cm^{-1} is related to the stretching of C-H bonds in alkanes and aromatic compounds. The wave number 2383 cm^{-1} is related to the stretching of S-H bonds [33]. The signal at 1645 cm^{-1} is related to the stretching of C=C bonds of the allyl group and the signal at 1418 cm^{-1} is related to CH_2 bending in

the lipid group, and the wave number 1126 cm^{-1} belongs to the C-N group and is in amino acids. The wave number 1032 cm^{-1} corresponds to the S=O group in the sulfonyl group [1].

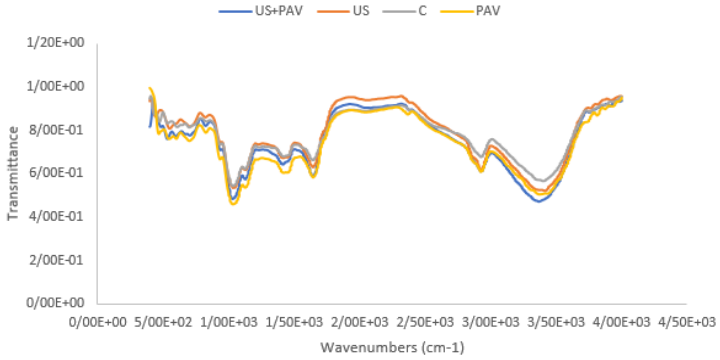

Fig. 1. Effect of different pretreatments on FTIR spectra

C: Control, US: Ultrasound, PAV: pure Apple Vinegar and US+PAV: Ultrasound+ Pure Apple Vinegar

4.5. Hydroxymethylfurfural content (HMF)

As a result of heat and the reaction between amino acids and reducing six-carbon sugars, during the non-enzymatic or Maillard browning reaction and sometimes during caramelization, a harmful compound such as HMF is formed, which changes the color, taste, and nutritional properties of food.According to the results of the analysis of the samples (B) and the standard curve of HMF (A) in Figure (2), no peak was observed in the eighth minute for the samples, which indicated that this substance was not formed during the process.In all the reviewed articles about garlic, there was no report of the formation of this substance.

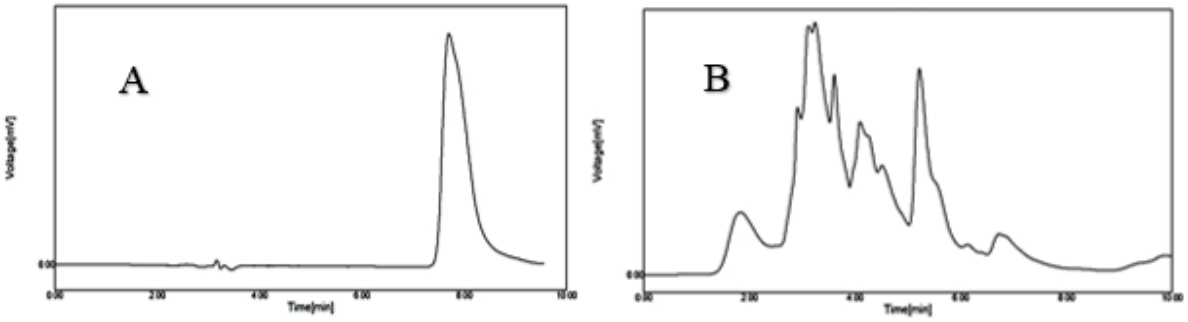

Fig. 2.(A): HMF standard curve and (B): Curve of different pretreatments for production HMF

4.6. Antibacterial activities

According to the obtained results, the ethanol extract of the samples at a concentration of 50 µg/ml has antibacterial properties (Table 2).The results of the analysis of the variance of the data indicated a significant difference ($p \leq 0.05$) among the samples in terms of antibacterial properties.Listeria monocytogenes is the most resistant bacteria, and Staphylococcus aureus is the most sensitive among them to effective substances in the antibacterial properties of dry garlic samples.Among the dried garlic samples, the control sample had the lowest, and the sample pre-treated with

ultrasound and pure apple vinegar had the highest antibacterial properties.Sulfur compounds and saponins in garlic have the most antibacterial properties, the intensity of which varies according to the type of microorganism, the type of garlic, the content of effective compounds in garlic, the method of processing, and the type of extracted extract.Allicin is a powerful antibacterial sulfur-containing compound in garlic, which in low concentrations has a high inhibitory role in antifungi, gram-negative bacteria, gram-positive bacteria, protozoa, and viruses [34].During research conducted on the investigation of the antimicrobial property of dried garlic powder products, two factors, temperature and time, were introduced as practical factors against the antibacterial property, and high temperature and longer processing time destroyed the practical factors in the antibacterial property [35].

Table 2. The diameter of inhibition zones/mm of the investigated in dried garlic samples

samples	*Listeria monocytogenes*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Escherichia coli*
C	0/0±0/0^{a}	10/21±0/4^{a}	10/20±0/01^{a}	8/59±0/17^{a}
US	0/0±0/0^{a}	10/88±0/5^{ab}	11/97±0/37^{b}	11/75±0/02^{d}
PAV	11/18±0/0^{b}	11/53±0/3^{bc}	12/06±0/24^{b}	10/17±0/08^{b}
US+PAV	11/47±0/33^{b}	11/67±0/1^{c}	12/32±0/41^{b}	10/38±0/01^{c}

Means with different letters within a column indicate significant differences ($p<0.05$).
C: Control, US: Ultrasound, PAV: Pure Apple Vinegar and US+PAV: Ultrasound+Pure Apple Vinegar

4- Conclusions

With a general look at the results obtained from this research, it can be concluded that using various pre-treatments is one of the most valuable ways to achievehighfunctional properties in Foodstuffs.The results showed that pure apple vinegar has high functional properties due to the increase in the content of phenolic and flavonoid compounds and antioxidant activities, and it caused an increase in the content of these compounds in the treated garlic slices.Likewise, the findings showed that if ultrasound pretreatment is used, the results have a significant effect on the content of bioactive compounds and antibacterial properties.Since the effect of food pre-treated with ultrasound on the human body has not been fully investigated, the use of this method has not yet been industrialized and requires further studies.

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بررسی تاثیر پیش‌تیمار فراصوت و سرکه سیب خالص بر روی خصوصیات فراسودمندی و ضدباکتریایی برش‌های سیر خشک شده تحت هوای داغ

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چکیده

خشک کردن یکی از راه‌های موثر در جهت افزایش ماندگاری و حفظ ترکیبات مفید مواد غذایی است که با به کارگیری روش‌ها و مواد مختلف می‌توان این خصوصیات را بهبود بخشید. استفاده از مواد فراسودمند به عنوان پیش‌تیمار می‌تواند یکی از راه‌های موثر در افزایش خصوصیات فراسودمندی و ضدباکتریایی باشد. این پژوهش با هدف به کارگیری همزمان پیش‌تیمار فراصوت و سرکه سیب خالص قبل از فرآیند خشک کردن برش‌های سیر صورت گرفت. برش‌های سیر تحت پیش‌تیمارهای سرکه سیب خالص و فراصوت به مدت ده دقیقه در دمای ۳۵ درجه سانتی‌گراد قرار گرفت و با استفاده از خشک‌کن جابه‌جایی هوای داغ در دمای۵۰ درجه سانتی‌گراد تا رطوبت نهایی ٦٪ بر پایه مرطوب، خشک شد. خصوصیات شیمیایی شامل محتوی ترکیبات فنلی و فلاوونوئیدی کل، تعیین گروه‌های عاملی، خصوصیات آنتی‌اکسیدانی و ضدباکتریایی نمونه‌ها مورد ارزیابی قرار گرفت. نتایج نشان داد که به کارگیری همزمان پیش‌تیمار فراصوت و سرکه سیب خالص تاثیر معناداری در میزان ترکیبات فراسودمند و خواص ضدباکتریایی برش‌های سیر خشک شده داشت. نمونه‌های پیش تیمار شده با سرکه سیب خالص در مقایسه با پیش‌تیمار فراصوت تاثیر بیشتری در افزایش خواص فراسودمندیداشت. با توجه به نتایج حاصله کاربرد پیش‌تیمارهای فراصوت و سرکه سیب خالص، سبب بهبود کیفیت، خصوصیات فراسودمندی و ضدباکتریایی نمونه‌های سیر خشک برش خورده شد.