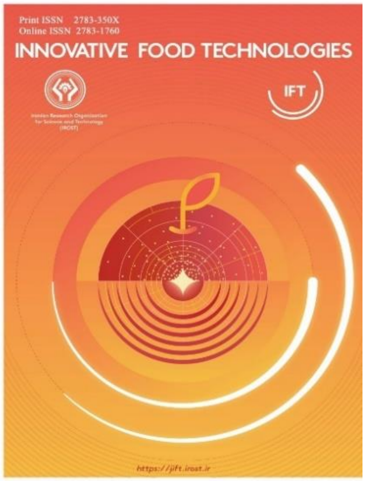

Effect of ultrasonic and infrared treatments on microbial population, physicochemical properties, and total phenols of sprouted wheat powder

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Running title: Physicochemical properties of sprouted wheat powder

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Abstract

Sprouting is a simple technique for improving the nutritional and quality characteristics of cereal grains. The purpose of this work was to examine ultrasonic pretreatment and drying methods (hot-air and infrared) on microbial population (total bacterial counts, molds, and yeasts), physicochemical properties (ash content, moisture, acidity, pH, lightness, redness, and yellowness), and total phenolic content of sprouted wheat powder (SWP). The results confirmed that the sonication decreased the total bacterial count of SWP. In this work, drying sprouted wheat with the hot-air and infrared radiation killed all molds and yeasts in the powders, and no molds or yeasts grew on the microbial plates. Moisture content, redness, yellowness, and phenolic content of SWP dried by the infrared dryer were higher than those by the hot-air dryer. The acidity and lightness of SWP dried by the hot-air dryer were higher than those by the infrared dryer. Applying ultrasonic pretreatment to sprouted wheat increased the amount of phenolic compounds in the powders, but this increase was not significant ($p>0.05$). In general, ultrasonic pretreatment and the use of infrared dryers are promising techniques for production of SWP with high phenolic content and low microbial population.

Keywords: Acidity, Lightness, Mold and yeast, Phenolic content, Total microbial count.

1. Introduction

Wheat (*Triticum aestivum* L.), as one of the most important grains, constitutes the majority of human nutritional needs, and its products occupy a special place in the diets of people all over the world [1]. Currently, the use of sprouted grains in people's diets is increasing due to the publication of scientific studies on the nutritional value and phytochemical content of sprouted grains [2,3]. In addition, the sprouted grains powder or flour is considered an excellent source of nutrients for enhancing and improving the quality and physicochemical properties of food products (include noodles, pasta, chiffon cakes, bread, sausages, etc.) [3-5]. Jokar et al. (2019) used sprouted wheat powder in the production of sausage. Their results confirmed that adding sprouted wheat flour to the formulation improved the moisture retention capacity of sausage samples [3]. The impacts of the addition of sprouted mung bean powder at various concentrations (0, 10, 20, and 30%) on noodles production characteristics were studied by Liu et al. (2018). The results confirmed that the protein content of samples increased during sprouting period [4].

Ultrasonic pretreatment as a non-thermal food processing technique is a better pretreatment for food processing due to its advantages such as saving energy, preserving original freshness and nutrient levels, retaining bioactive compounds, and reducing processing duration [6,7]. Yang, Gao, Yang, and Chen (2015) studied the influence of ultrasound on improving the nutritional characteristics and quality attributes of soybean sprouts. They treated soybean seeds with different ultrasonic powers (0–300 W) and found that sonication increased sprouting rate, sprout length, and gamma-aminobutyric acid content [8]. Tavakoli et al. (2019) studied the effects of sprout-derived extracts of three different wheat cultivars grown in Iran and ultrasound exposure on the oxidative stability of soybean oil. The results confirmed that ultrasound increased the values of polyphenolic and tocopherol compounds, but had a greater effect on the extraction of polyphenolic compounds [9].

Food dehydration is a complex procedure including simultaneous transfer of mass and heat, and in this process, a product undergoes several changes in its physicochemical characteristics, structural, and textural properties. It is important to consider and select an appropriate method for drying sprouted grains [5,10]. Manikantan et al. (2022) studied and modeled thin-layer drying of sprouted wheat in a tray-dryer. Based on the results of this study, the dehydration parameters of sprouted wheat are significantly influenced by the drying condition (dryer temperature and drying time) [11]. Among various food dehydration techniques, infrared drying is one of the most widely used moisture reduction techniques. The main advantages of infrared drying method compared to hot-air drying are higher heat transfer rate, shorter drying time, higher quality dried fruit and vegetable products, high performance, and energy saving in the process [12].

Pretreatment and drying conditions can significantly affect the physicochemical characteristics of sprouted grains. The goal of this work was to estimate the impacts of ultrasonic pretreatment and drying approach on the microbial population (total bacterial count, molds, and yeasts), physicochemical properties (ash content, moisture, acidity, pH, lightness, redness, and yellowness), and total phenolic content of SWP.

2. Materials and Methods

2.1. Germination process

Grains of wheat cultivar Pishgam were harvested in the fields of Hamedan, Iran. Grains were manually cleaned to remove dust and stored in a dry and cool place until use. Samples were washed and soaked in water (1:4 grain to water ratio) for 24 h at 25±1°C [2]. The soaked grains were stored in polyethylene containers covered with clean kitchen towels and allowed to germinate at 25±1°C for 72 h.

2.2. Sonication

Sonication was applied to the germinated grains using an ultrasonic water bath (Backer, vCLEAN1-L2, Iran) of size 15×14×15 cm, water capacity 2 L, 40 kHz, and 100 W. The germinated grains were placed directly in the water bath at 25°C for 5 min.

2.3. Hot-Air drying

After sonication, the sprouted wheat grains were dried in an oven (70±2°C, Fan Azma Gostar, Iran), until they reached a constant weight.

2.4. Infrared drying

In this work, an infrared dryer (L=440 mm, W=200 mm, and H=400 mm) equipped with an infrared radiation source (250 W, near-infrared (NIR), Noor Lamp Company, Iran) was used to dry germinated wheat grains. In this dryer, the distance from the radiation lamp to the sprouted grains was 5 cm.

2.5. Powder making

An industrial electric grinder (Best, 350, 1600 W, 25000 rpm, China) was used to grind the dried sprouted grains (Figure 1). The prepared powder was poured into a polyethylene bag (moisture proof), sealed and stored in a freezer (-18°C).

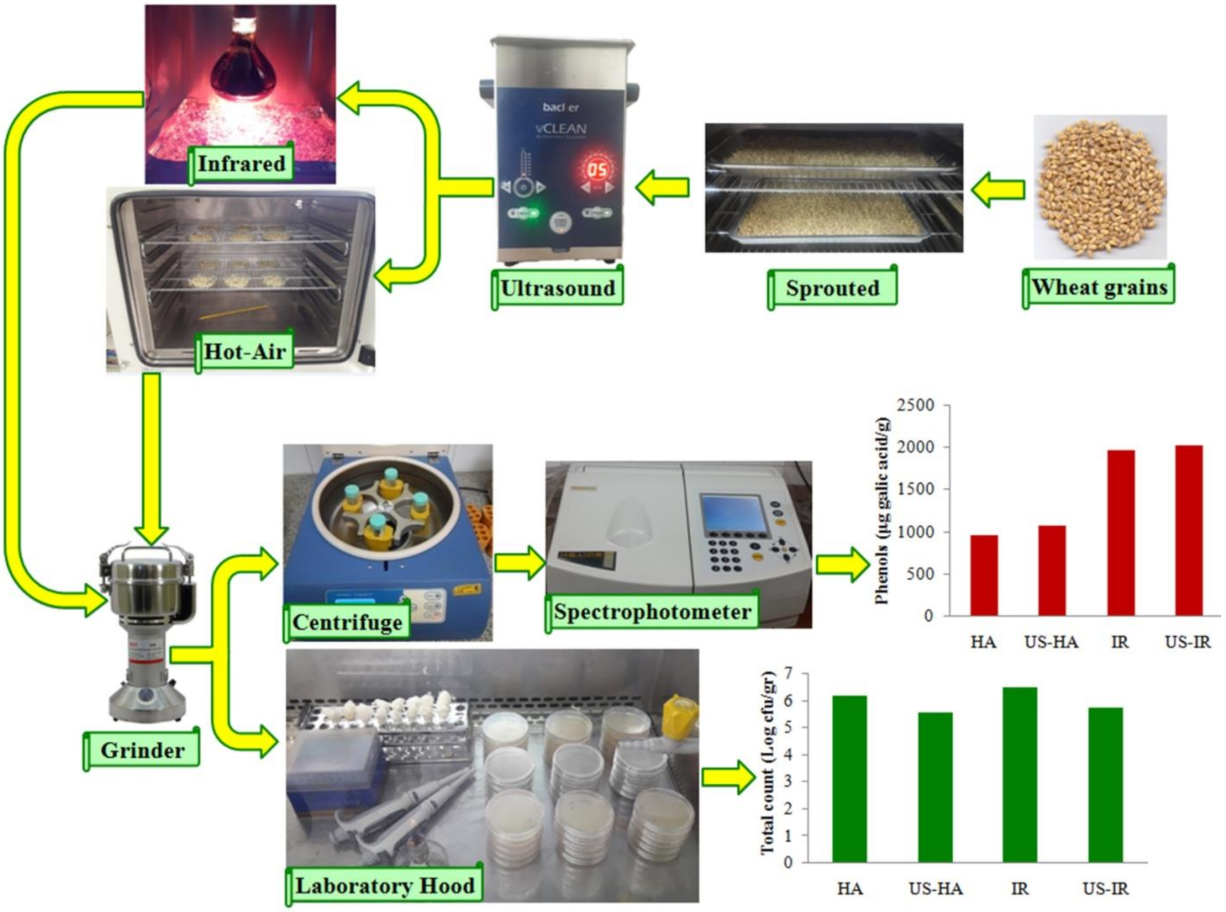


Figure 1- Schematic of ultrasonic pretreatment, drying process, and grinding of sprouted wheat grains

2.6. Microbial population of powders

In this research, Plate Count Agar (PCA, Quelab, Canada) medium was used for the total count of bacteria within SWPs, and Chloramphenicol Agar (YGC, Conda, Spain) medium was used for mold and yeast counts.

2.7. Moisture and ash content

In this study, the moisture content of fresh, soaked, and sprouted wheat grains, and SWPs were measured using an oven at 105°C for 5 h (Fan Azma Gostar, Iran). At this stage, the mass change of the SWP sample was recorded using a laboratory balance (AND, EK-410i, Japan) with a sensitivity of ±0.01 g.

The ash content of fresh wheat grains and SWP was measured using a laboratory electric furnace at 600°C for 8 h (Shimifan, Iran). The ash content (%) of the samples was calculated using Equation 1.

$$\text{Ash (\%)} = \left(\frac{M2 - M3}{M1}\right) \times 100 \tag{1}$$

In this equation, M_1 is the initial weight of the SWP (3 g), M_2 is the weight of the SWP + crucible after the furnace, and M_3 is the weight of the empty crucible. At this stage, the SWP sample weight was recorded using a laboratory balance (Sartorius, TE-124S, Switzerland) with a sensitivity of ±0.0001 g [4].

2.8. Acidity of powders

To measure the acidity of SWP samples, 10 g of the SWP was poured into a conical flask (Erlenmeyer). 50 mL of 67% ethyl alcohol was added, and the mixture was covered, and then stirred with an electric stirrer for 5 min. The above solution was then passed through filter paper. 25 ml of the filtered solution was then placed into an Erlenmeyer flask, and 3 drops of phenolphthalein reagent (3% w/v, Merck, Germany) was added and titrated with 0.1 N NaOH solution (Kian Kaveh Azma, Iran). As soon as a pink color appeared, we stopped the titration process, and the amount of sodium hydroxide solution consumed was recorded. We calculated the acidity of SWP samples using Equation 2.

$$\text{Acidity (\%)} = \frac{2 \times \mathrm{V} \times 1.8}{\mathrm{M}} \tag{2}$$

In this equation, V is the volume of NaOH solution consumed (mL) and M is the weight of the sample (10 grams).

2.9. pH of powders

To measure the pH of samples, 10 g of homogenized SWP was mixed with 100 mL of freshly boiled distilled water and allowed to stand for 20 min. The pH of the aqueous portion was then recorded using a laboratory pH meter (Denver, Ultrabasic, USA). The sample was in contact with the electrode for approximately 20 seconds at room temperature, after which the value displayed on the device was recorded as the pH of the powder.

2.10. Color measurement

Using computer vision techniques, we investigated the effects of sonication and drying approaches on the color index of SWPs. The SWP samples photographs were taken using a scanner (HP Scanjet-300). The SWP photos were converted from RGB to L^*(lightness), a^*(green/red), and b^* (blue/yellow) indexes using Image J software (V.1.42e, USA) and its color space conversion plug-in [13].

2.11. Total phenol content

To prepare the SWP sample extract, 10 mL of 80% methanol was added to 1 g of SWP and mixed with a magnetic stirrer (hot plate stirrer, Alpha, model HS-860, Iran) for 30 min. After this step, the mixture was transferred to the falcon tube**.** The falcon tubes were centrifuged for 5 min at a speed of 4000 rpm using a Centrifuge device (Fartest, Iran). The supernatant of the mixture was then used as the SWP extract.

Total phenol content was calculated as Gallic acid equivalent (GA) according to the Folin-Ciocalteu method. Transfer an aliquot (0.5 mL) of the SWP extract to a laboratory test tube. 0.5 mL of Folin-Ciocalteu reagent (Merck, Germany) is added. After 5 minutes, 2 mL of Na_2CO_3 (20% w/v) (Ghatran Shimi T. Co., Iran) was added and shaken for 30 seconds. After incubation for 15 minutes at room temperature (25±1°C), 10 mL of distilled water was added and the precipitate that formed was removed using centrifugation (Centrifuge, Fartest, Iran) for 5 minutes at 4000 rpm. In the end, the absorbance of the supernatant was recorded at 725 nm by a spectrophotometer (Thermo Spectronic, Helios α Spectrophotometer, England) and compared with the GA standard curve. Results are given in μg GA/g dry matter [14]. To generate the standard curve, distilled water was first used to prepare GA solutions (Merck, Germany) with concentrations of 0.04, 0.02, 0.01, 0.005, and 0 g/100 ml. Then, instead of the SWP extract, we used 0.5 mL of these GA solutions and followed the steps in the previous section. Equation 3 can be used to calculate the total phenols of the SWP extract based on units of g GA/100 mL. Equation 4 was also used to calculate the total phenolic content of SWP based on micrograms of GA per gram of dry matter (μg GA/g), and this equation was used in this study.

$$\text{Total phenol} = 0.0185\text{ABS} - 0.0007\left[\text{gGA/100ml}\right] \quad (3)$$

$$\text{Total phenol} = 0.5 \times \left(0.0185\text{ABS} - 0.0007\right)/100 \times 20 \times 10^{6}\left[\mu\text{gGA/g dry}\right] \quad (4)$$

In these two equations, ABS is the absorbance value of the sample at a wavelength of 725 nm and GA represents Gallic acid [4].

2.12. Statistical analysis

This work was carried out in a factorial experiment based on a fully randomized design with 3 replicates to investigate the impacts of ultrasonic pretreatment and drying methods (hot-air and infrared) on the microbial population (total bacterial count, mold, and yeast), physicochemical properties (ash, moisture, acidity, pH, lightness, redness, and yellowness), and phenolic content of SWP. The mean values of responses were compared using Duncan’s multiple range test at p-value<0.05 (using the SPSS 21 software).

3. Results and Discussion

3.1. Microbial population of powders

Figure 2 shows the effect of ultrasonic and drying techniques on the total count of microorganisms within the SWPs. This figure shows that the application of ultrasonic pretreatment reduced the microbial population of the SWP samples. Due to the longer dehydration period of the sprouted wheat grains in the hot-air dryer, the powder produced from the sprouts dried with this dryer showed a lower microbial load than the infrared dryer. Regarding the total count of microorganisms, there was no significant difference between untreated and hot-air and infrared dried powders (p>0.05).

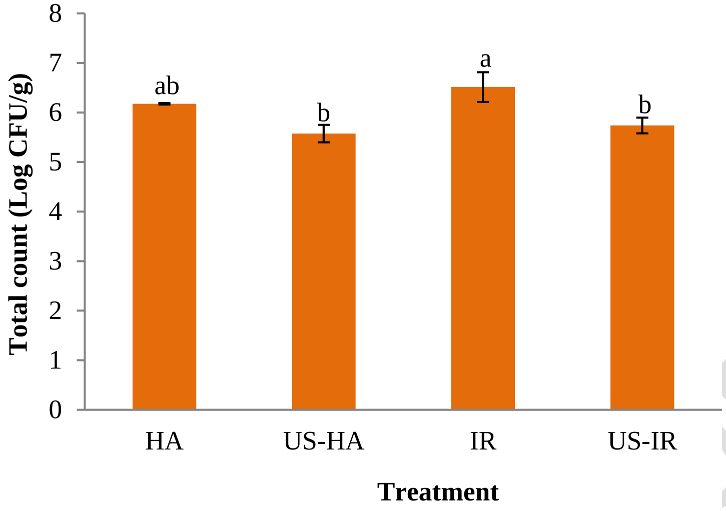


Figure 2- Effects of ultrasonic pretreatment (US), hot air (HA), and infrared (IR) drying processes on the total microbial count of sprouted wheat powder

In our study, SWP that was sonicated and dried in the hot-air dryer had the lowest total bacterial counts (5.57 Log CFU/g). In this work, drying sprouted wheat with hot-air and infrared radiation killed all molds and yeasts in the powders, and no molds or yeasts grew on the microbial plates (Figure 2). In line with the results of our study, Amjadi, Alizadeh, and Roufegarinejad (2018) reported that sonication (cavitation effect) of orange juice significantly reduced microbial contamination [15]. Ding et al. (2015) studied using an infrared dryer to improve the shelf-life of rough and brown rice [16]. The results of this research showed that infrared radiation did not adversely affect the quality of dried rice. This method also reduces processing costs and extends the shelf-life of the products.

3.2. Moisture and ash content

In this study, the moisture content (wet basis) of fresh, soaked, and germinated wheat grains was 11.60%, 42.23%, and 66.65%, respectively. Figure 3(a) shows the influence of ultrasonic pretreatment and dehydration method on the moisture content of SWP samples. Application of ultrasound did not significantly influence the moisture content of SWP samples in both types of dryers ($p>0.05$). The moisture content of the infrared-dried SWP was higher (6.93%).

The wheat grain used in this study had an ash content of 1.53%. Additionally, the average ash content of the produced SWPs was 1.49% (Figure 3(b)). Regarding ash content, there was no significant difference between samples prepared using hot-air, ultrasonic hot-air and infrared methods (p>0.05). Javaheripour et al. (2022) SWP has an ash content of 1.65, which is numerically close to the value determined in our study [17]. Ozturk et al. (2014) also reported the ash content of wheat flour and SWP as 1.51 and 1.80, respectively [18].

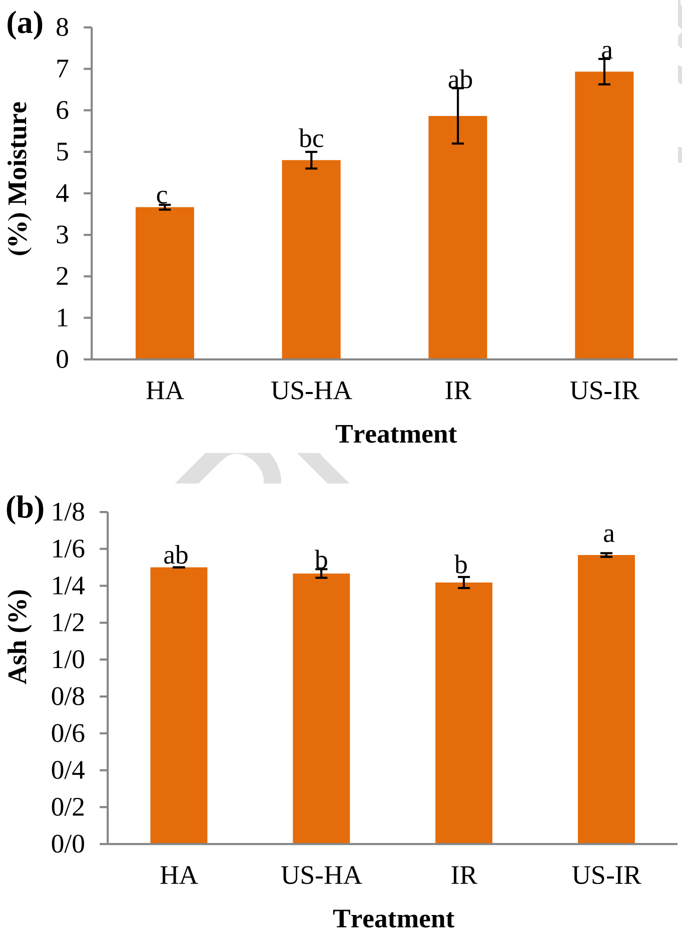


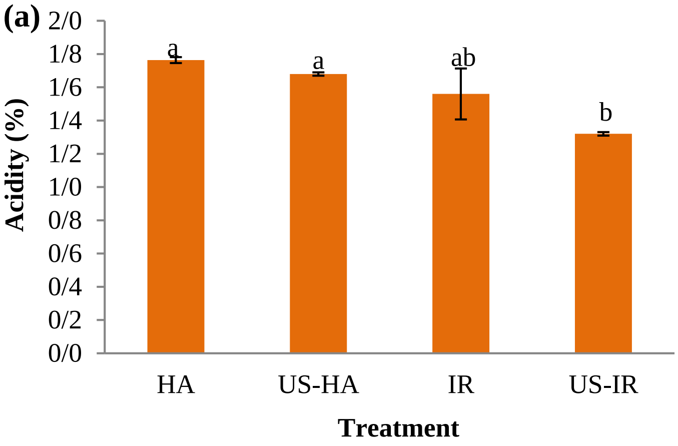
Figure 3- Effects of ultrasonic pretreatment (US), hot air (HA), and infrared (IR) drying processes on the moisture (a) and ash (b) content of sprouted wheat powder

3.3. Acidity and pH of powders

In this study, the acidity of whole wheat flour was 0.22%. Fateh Nikoo et al. (2019) reported acidity of 0.11 and 0.12% for their two varieties of Chamran and Sardari wheat flour, respectively [19]. Germination increases the activity of microorganisms and enzymes, thus increasing SWP acidity. As can be seen from

Figure 4 (a), the acidity of the powders produced by hot-air and infrared dryers is higher than that of whole wheat flour. Applying ultrasonic pretreatment decreased the acidity of the dried samples, but this decline was not statistically significant ($p>0.05$).

In this study, the wheat grains used had a pH of 6.15. Fateh Nikoo et al. (2019) also reported that the pH of two varieties of Chamran and Sardari wheat flours was 6.5 and 6.4, respectively. Germination increases microbial and enzymatic activity, thus decreasing the SWP pH. In this research, the average pH of SWPs ranged from 5.58 to 5.67 (Figure 4 (b)). Ozturk et al. (2014) reported the pH of wheat flour and SWP to be 6.24 and 5.36, respectively [18], which are close to the values obtained in this study. However, Javaheripour et al. (2022) reported that the pH of SWP was 6.21, which is higher than the values obtained in our research [17]. As can be seen in Figure 4 (b), there is no significant difference in pH between the sonicated SWPs and the hot-air and infrared dried samples ($p>0.05$). Amjadai et al (2018) reported that sonication had no significant effect on acidity, pH, and soluble solids content of orange juice samples [15].



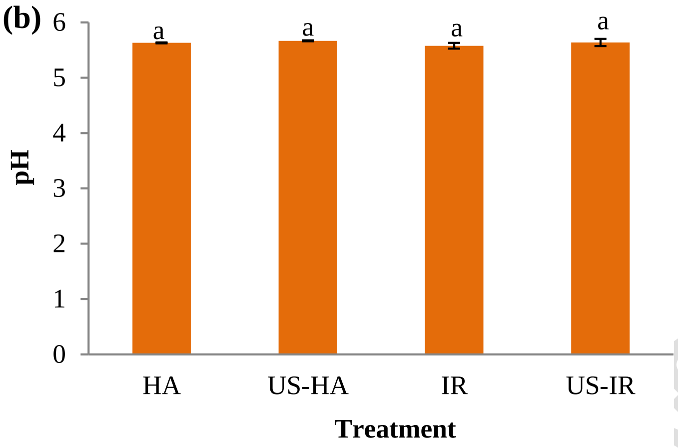


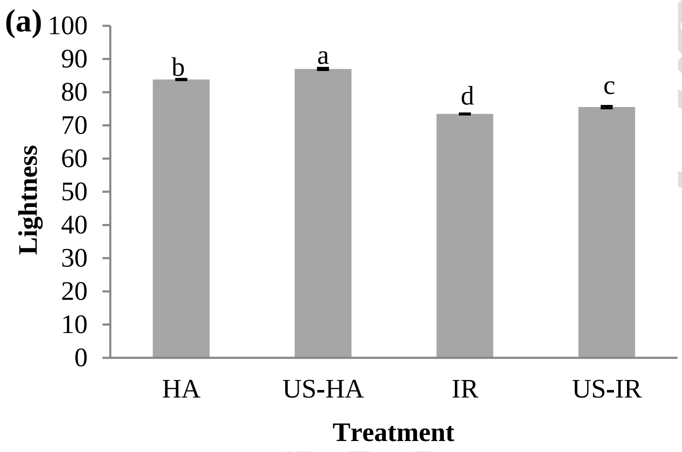
Figure 4- Effects of ultrasonic pretreatment (US), hot air (HA), and infrared (IR) drying processes on the acidity (a) and pH (b) of sprouted wheat powder

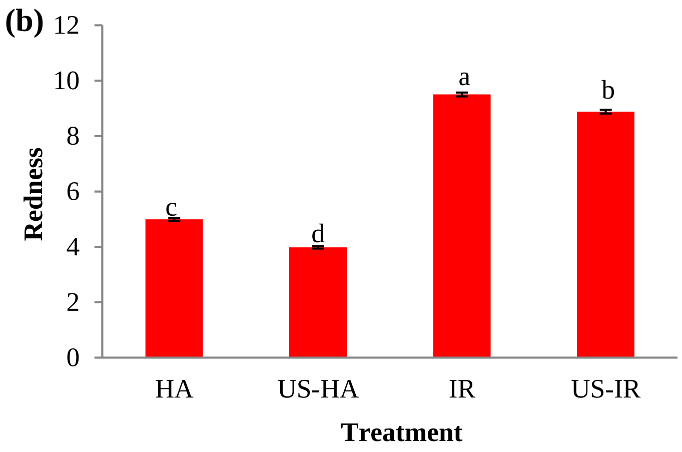
3.4. Color parameters

The lightness, redness, and yellowness indexes of the whole wheat flour used in this research were 89.06, 3.64, and 14.76, respectively (Figure 5). After applying sonication to wheat sprouts and then preparing powders from dried sprouts, it was observed that powders prepared from sonicated samples were lighter. Figure 5 (a) illustrates that there is a significant difference in lightness index among the sonicated SWPs and the hot-air and infrared dried samples ($p<0.05$). The bright powder was the powder prepared from the sonicated and hot-air dried sample ($L^*=86.98$). The infrared dried powders were darker than the hot-air dried samples and the lightness index of these powders was lower. Ozturk et al. (2014) reported that SWP was darker in color than wheat flour, and upon sprouting the lightness index of the powder decreased and the red and yellow indexes of the powder increased [18].

After applying sonication to wheat sprouts and subsequently preparing powder from dried sprouts, it was observed that the powder prepared from untreated samples was redder (higher redness index). Additionally, the infrared dried samples were redder and the redness index of these powders was higher than the hot-air dried samples. Figure 5 (b) also shows that there is a significant difference in the redness index among the sonicated SWPs and the hot-air and infrared dried samples ($p<0.05$). The reddest powder was the powder prepared from untreated sprouts dried in the infrared dryer ($a^*=9.50$).

In addition, Figure 5 (c) demonstrates that there is a significant difference in yellowness index between SWPs prepared by hot-air and infrared dryers, and the samples dried by infrared dryer being yellower ($p<0.05$). The yellowest powder was the powder prepared from sonicated and infrared-dried sprouts ($b^*=23.00$), which was significantly different from the other samples ($p<0.05$).





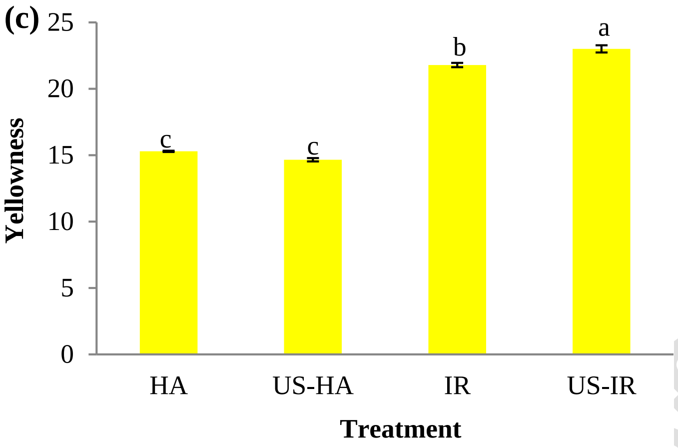


Figure 5- Effects of ultrasonic pretreatment (US), hot air (HA), and infrared (IR) drying processes on the color indexes (lightness (a), redness (b), and yellowness (c)) of sprouted wheat powder

3.5. Total phenols

In this study, the total phenolic content of whole grains was 470.20 µg GA/g (Figure 6). Germination of wheat increases the amount of phenolic compounds, so the powder produced from the sprouts also has a higher amount of phenolic compounds. Figure 6 shows the influence of the ultrasonic pretreatment and dehydration method on the phenolic content of SWP. Since the drying time of the sprouts in the infrared dryer is much shorter than in the hot-air dryer, more phenolic compounds remain in this state and the amount of these compounds in the prepared powder is also higher. Applying ultrasonic pretreatment to sprouted wheat increased the amount of phenolic compounds in the powders, but this increase was not significant ($p>0.05$). The sonicated and infrared dried powder had the highest amount of phenolic compounds (2010.63 µg GA/g) and the untreated hot-air dried powder had the lowest amount of phenolic compounds (951.83 µg GA/g). Consistent with the results of this study, Tavakoli et al. (2019) found total phenol content in extracts of wheat cultivars Tajan, N8019, and Morvarid to be 679, 783, and 545 µg GA/g, respectively. After application of ultrasound, the amounts of these compounds increased to 777, 891, and 593 µg GA/g, respectively [9]. Gan et al. (2017) also reported that hot-air dehydration not only enhanced the total phenols and antioxidant capacity of sprouted mung bean, but also caused them to brown [20].

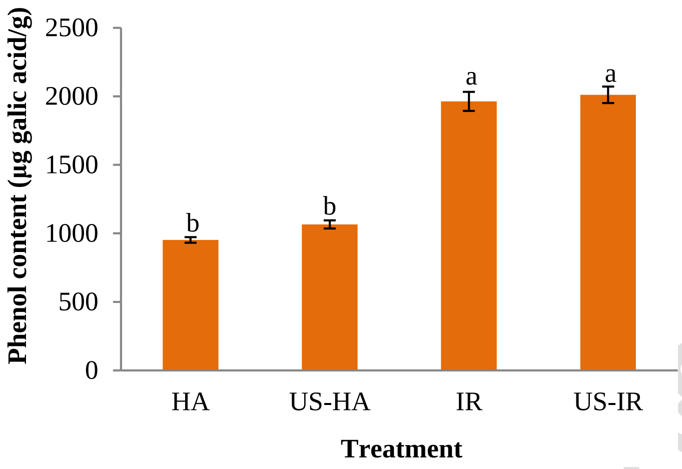


Figure 6- Effects of ultrasonic pretreatment (US), hot air (HA), and infrared (IR) drying processes on the phenolic content of sprouted wheat powder

4. Conclusions

SWP is a valuable natural source for enhancing nutritional value and improving the sensory characteristics of foods. In this research, we investigated the effect of ultrasonic pretreatment (frequency 40 kHz, power 100 W) on microbial population (total bacterial counts, molds, and yeasts), and physicochemical properties of SWPs. We found that powders made from sprouted wheat that had been pretreated with ultrasound and dried with hot-air had the lowest total bacterial counts. Drying the sprouted wheat with the hot-air and infrared dryers destroys any mold and yeast in the powders. Infrared drying increased the moisture and ash content, pH, redness and yellowness indexes, and total phenols, and decreased acidity and lightness index of SWP. Generally, sonicating sprouted wheat and drying in the infrared dryer is recommended to production SWP.

Declarations

Conflict of interest: The authors declare no competing interests.

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تاثیر تیمارهای فراصوت و مادون قرمز بر جمعیت میکروبی، خواص فیزیکوشیمیایی و فنل کل پودر گندم جوانه زده

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چکیده

جوانه زدن یک تکنیک ساده برای بهبود ویژگی های تغذیه ای و کیفی دانه غلات است. هدف از این مطالعه بررسی روش های پیش تیمار و خشک کردن اولترا سونیک (هوای گرم و مادون قرمز) بر روی جمعیت میکروبی (تعداد کل باکتری ها، کپک ها و مخمرها)، خواص فیزیکو شیمیایی (میزان خاکستر، رطوبت، اسیدیته، pH، روشنی، قرمزی و زردی) و میزان فنلی کل پودر گندم جوانه زده. نتایج بدست آمده نشان داد که سونیکاسیون تعداد کل باکتری پودر گندم جوانه زده را کاهش داد. در این مطالعه، خشک کردن جوانه گندم با هوای داغ و تشعشعات مادون قرمز، همه کپک ها و مخمرهای موجود در پودرها را از بین برد و هیچ کپک یا مخمری روی محیط های کشت رشد نکرد. میزان رطوبت، قرمزی، زردی و همچنین مقدار فنل پودر گندم جوانه زده خشک شده توسط خشک کن مادون قرمز بیشتر از خشک کن هوای گرم بود. اسیدیته و سبکی پودر گندم جوانه زده خشک شده توسط خشک کن هوای گرم بیشتر از خشک کن مادون قرمز بود. اعمال پیش تیمار فراصوت بر روی گندم جوانه زده باعث افزایش میزان ترکیبات فنلی در پودر آن شد، اما این افزایش معنی دار نبود ($p>0.05$). به طور کلی، پیش تیمار فراصوت و استفاده از خشک‌کن‌های مادون قرمز، تکنیک‌های امیدوارکننده‌ای برای تولید پودر گندم جوانه زده با محتوای فنلی بالا و جمعیت میکروبی کم هستند.

واژگان کلیدی: اسیدیته، جمعیت میکروبی کل، روشنی، کپک و مخمر، محتوای فنلی.