



Evaluation of microbiological safety and antioxidant activity of stored seedless barberry fruit from the main production regions of South Khorasan province, Iran

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ABSTRACT

Purpose: The microbial load and nutritional value of products have always been important issues in human nutrition. This research aimed to identify and determine the microbial levels and total antioxidant contents of dry, seedless barberry fruits collected from different regions of South Khorasan Province, which is the main production area in Iran.

Research Method: Fruit samples were prepared from four regions, including the Birjand region, Zirkoh region, Darmian region, and Qaen region. Chemical traits and microbial analysis were evaluated. The microbial load was determined and compared with the maximum limit (ML) standards. **Findings:** Microbiological analyses of fruits from the Birjand region, Zirkoh region, Darmian region and Qaen region revealed that, fortunately, *E. coli* bacteria were not found in any of the studied regions. However, the highest amounts of total aerobic bacteria ($4.60 \log_{10} \text{CFU.g}^{-1}$) and yeast/mold bacteria ($4.17 \log_{10} \text{CFU.g}^{-1}$) were obtained from fruits prepared from the Darmian region, which was higher than the standard defined by the Food and Drug Organization of Iran. The highest coliform level was related to the fruits of the Darmian region ($1.69 \log_{10} \text{CFU.g}^{-1}$) and the Qaen region ($1.69 \log_{10} \text{CFU.g}^{-1}$), which were lower than those of the MLs. Considering the standards defined with the MLs in Iran, the microbial load regulations in Iran exhibit a higher level of stringency than those in other nations do. Additionally, chemical analyses revealed that the highest amount of total soluble solids and the lowest pH of fruit juice were from the fruits of the Birjand region. The highest amount of titratable acidity and the highest antioxidant activity were related to the fruits of the Qaen region. **Research limitations:** No limitations were found. **Originality/Value:** The nutritional value of barberry fruit is the highest in the Qaen region, followed by the Birjand region. Although the pre- and postharvest stages can affect the microbial load of products, storage conditions during the drying period of seedless barberry fruits play a crucial role in determining the microbial load.

INTRODUCTION

Seedless barberry (*Berberis vulgaris* L.) is an important medicinal and small fruit of the *Berberidaceae* family that is native to Iran (Behrad et al., 2023). Iran is the main seedless barberry fruit producer in the world. Its annual production is more than 22,322 tons of dried berries on approximately 19,220 hectares in Iran. More than 85% of seedless barberry fruits are produced in South Khorasan Province, which is located in East Iran (Javadzadeh, 2013). Most of the consumed seedless barberry is in the form of dried berries. The medicinal properties of barberry fruit, such as anticancer, antimicrobial (Och & Nowak, 2021), reducing morphine addiction and dependence (Sobhani et al., 2021), rehabilitating, hypoglycemic, and antidiabetes (Shidfar et al., 2012), and reducing LDL and total cholesterol (Ardestani et al., 2013), have been investigated.

Fresh and processed fruits, particularly small fruits such as barberries with high nutritional value, play important roles in the growth and health of the human body (Mathur et al., 2014); these products are rich and excellent sources of natural antioxidants that help prevent various diseases, such as cancer and cardiovascular diseases, in our body. Food insecurity, malnutrition, and lifestyle diseases such as obesity, hypertension, carcinogenesis, and diabetes are among the most important global issues that have increased the demand for healthy foods, especially fruits and vegetables (Gogo et al., 2017). It is approximated that up to one-third of the population in developed countries contracts foodborne diseases annually, and this is likely to be even more widespread in developing countries. Presently, individuals are cognizant that adhering to a nutritious diet comprising salads, vegetables, and fruits is crucial for enhancing their quality of life. Nevertheless, it is imperative to acknowledge that the intake of raw fruits and vegetables has the potential to lead to bacterial and infectious ailments owing to the presence of microorganisms such as *Escherichia coli* and *Salmonella* (Mritunjay & Kumar, 2015). Raw fruits and vegetables may be contaminated with fungi, molds, or various pathogenic microorganisms, such as *Salmonella* spp. and *Escherichia coli* (Alp & Bulantekin, 2021). The postharvest loss of fresh fruits and vegetables in developed countries is approximately 20% due to spoilage. Fruits and vegetables can sometimes be associated with harmful bacteria and viruses known as pathogens. In most developing countries, there is little information about food safety, and there are no strict measures in the production cycle of products (Abdel-Moneim et al., 2014). Factors related to the safety of fruits and vegetables include natural pesticides, contaminants such as chemical waste, heavy metals, and microbial contamination of fresh produce, which is highly susceptible to fungi. Contamination with pathogenic or corrosive microorganisms as a result of contact with soil, wastewater and dust during the pre- and postharvest stages is significant for fruits and vegetables, as it may cause various diseases, such as cramps, diarrhea, and even death (Alp & Bulantekin, 2021).

Fresh, seedless barberry fruit has a high water content (75–80%), and the majority of the produced fruit is dried via different methods to reduce the moisture content and microbial load and extend its storage life (Alavi and Mazlounzadeh, 2012; Moradinezhad et al., 2024). To maintain visual quality and nutritional value and minimize organoleptic changes during the drying process, conditions are kept as mild as possible, which enables many microorganisms to function under these conditions (Morgan et al., 2006; Jayaraman and Das Gupta, 2020). Although microbial growth is partially prevented or delayed in dried products due to severe water loss, when sufficient numbers of pathogenic microorganisms are present after the drying process, it may pose a risk to the consumer (Bourdoux et al., 2016). Although microbial growth is inhibited in the desiccated state, vegetative cells and spores can survive for months (Beuchat et al., 2013). In addition, when dried products are exposed to water during food preparation and absorb water, the growth of viable microorganisms may be enhanced (Bourdoux et al., 2016;

Saad et al., 2021). This leads to rapid spoilage of products and increases the risk to consumer health. However, microbial contaminants occur during both the pre- and postharvest stages (Zhao et al., 2021; El-Araby et al., 2023; Adewoyin, 2023). However, storage conditions are also crucial. One of the main issues in terms of storage conditions during product drying is controlling the temperature and humidity of the storage location (Mongi et al., 2023; Firdous et al., 2023). If optimal storage conditions are provided, drying the products may be the only way to reduce the existing microbiota (Moradinezhad et al., 2019).

The literature shows that little information is available about the microbial load of dried, seedless barberry fruit, as mentioned in a recent study (Bideli et al., 2022). In addition, no information has been found regarding the microbial load and antioxidant activity of seedless barberry fruits cultivated and dried from significant producing regions in Iran. The purpose of this research was therefore to determine the microbial load and chemical properties of four major regions of seedless barberry cultivation in Iran.

MATERIALS AND METHODS

In the present study, the microbial load and several biochemical traits of four major barberry regions in South Khorasan Province, Iran, were compared. Dried, seedless barberry fruit samples were prepared from four regions, including the Birjand region, Zirkoh region, Darmian region, and Qaen region. Fruit were dried traditionally in a shaded house during autumn and winter. The date of barberry collection from different regions ranged from the 1st to the 20th of May 2022. They were then transferred to the Horticulture Laboratory, Faculty of Agriculture, University of Birjand, South Khorasan Province, Iran. The methods used for fruit picking, storage conditions and the monthly average temperature and relative humidity of the shade houses used in all the studied regions are presented in Tables 1 and 2.

Sample preparation

Two kilos of dried, seedless barberry fruit were prepared from each region. Four replicates were prepared from each region, and 500 grams of barberry were used for each replicate. A total of 8 kilos of dried barberry were purchased from prominent producers in the four regions. These regions were considered fully representative. For analysis, berries were sorted, and berries with abnormal color and visible contamination (such as leaves, twigs, and soil) were excluded.

Table 1. The type of fruit picking, height of branches mass, and storage conditions in the four studied regions of South Khorasan province, Iran.

Regions	Fruit picking	Height of fruit branches mass (cm)	Storage conditions
Birjand	With branches	30	One-row metal drying rack, with ventilation
Zirkoh	With branches	40	Double-row wooden drying rack, with ventilation
Darmian	With branches	10	On the cement floor, no ventilation
Qaen	With branches	40	One-row metal drying rack, with ventilation

Table 2. Monthly average of temperature and relative humidity (RH) of shade-house in different regions during 2022-2023.

Regions/Months	November 2022	December 2022	January 2023	February 2023	March 2023	April 2023
Temperature (°C)						
Birjand	10	9.3	8.2	6.8	15.9	17.3
Zirkoh	7.6	6.8	5.2	5	10.2	13.3
Darmian	11	10.1	8.6	7.3	17.4	18.5
Qaen	9.8	8.4	6.2	5.7	13.5	16.9
Relative humidity (RH%)						
Birjand	48	52	67	41	32	28
Zirkoh	59	63	58	49	40	33
Darmian	45	49	60	37	28	25
Qaen	52	68	65	52	43	30

Microbiological analyses

The microbiological analyses included total aerobic bacteria (TAB), coliforms, *E. coli* and molds/yeasts from dried, seedless barberry fruit, which were carried out at the Microbiology Laboratory of the Research Institute of Food Science and Technology (RIFST), Mashhad, Iran. Dried fruit samples weighing 25 g were subjected to homogenization for 120 seconds in 1:5 dilutions of 1% sterile peptone water via a stomacher apparatus (HG400 pro stomacher). The use of filtered stomacher bags aimed to eradicate soil particles from the homogenates, and tenfold dilution were subsequently prepared in peptone-buffered water for plating as necessary. Total aerobic bacteria (TAB) were quantified through spread-plating on plate count agar (Merck) and incubated at 30°C for 48 hours; coliforms and *E. coli* on Coli-ID (Biomérieux) were quantified via double layer inclusion and incubation at 37°C for 48 hours; and molds and yeasts on glucose yeast extract agar with a 10 mg/ml solution of oxytetracycline chlorhydrate were assessed via pour plating and incubation at 25°C for five days.

Chemical properties

The chemical properties determined were titratable acidity (TA), total soluble solids (TSS), juice acidity (pH), and total antioxidant activity (TAA).

The method of Talebzadeh et al. (2022) was used to prepare dried barberry extracts. First, 20 grams of dried fruit was weighed and turned into powder through a mortar. Then, 20 ml of water was added, and the mixture was placed in a shaker (orbital shaker made by IKA, Germany - KS260 digital) for 25 minutes at a speed of 50 rpm. The solution was then passed through filter paper. This prepared extract was used to determine the biochemical characteristics of dried barberry fruit.

To evaluate the total soluble solids, a few drops of the prepared extract were poured onto the prism of an optical refractometer (RF 10, Brix, 0–32%, Extech Co., USA). The TSS data are presented in °Brix (Hosseini et al., 2021).

The titratable acidity of the barberries was determined via the titration method with 0.1 standard NaOH and with a desktop pH meter (AZ 86502 model, made in Taiwan). For this purpose, 10 ml of the extract was diluted with 100 ml of distilled water. The solution was then titrated with 0.1% NaOH until it reached pH 8.23. Finally, the results were calculated as a percentage via formula (1) (Saebi et al., 2023).

$$TA (\%) = \frac{(\text{ml base titrant}) \times (\text{N of base in } \frac{\text{mol}}{\text{L}}) \times \text{equivalent weight of acid}}{\text{sample volume in ml}} \quad (1)$$

The pH of the fruit extract was measured via a digital desktop pH meter (AZ 86502 model, made in Taiwan) with a measurement accuracy of 0.01.

The ability of the extracts to scavenge the DPPH radical was determined via the method of Blois (1985), with slight modifications. Fifty microliters of fruit extract was added to 2 mL of methanol, and then the DPPH compound (24 µg/mL) was added. The mixture was kept in the dark at room temperature for 60 min, and the absorbance was measured via a spectrophotometer (model Novaspec II; Pharmacia LKB, Uppsala, Sweden) at 517 nm. The percentage of DPPH radical scavenging activity was calculated via the following equation (2):

$$\text{DPPH radical scavenging activity (\%)} = (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \times 100 \quad (2)$$

where A_{blank} is the absorbance of the blank (containing all the reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Statistical analysis

Analyses were performed via JMP Statistical Discovery Pro v13.2.1, and Excel Ver. 2019 software was used to draw graphs. Additionally, the comparison of the means was performed via the least significant difference (LSD) test at the 5% probability level. The statistical plan used for this experiment was a completely randomized design (CRD) with four replications.

RESULTS AND DISCUSSION

Microbiological analysis

Table 3 presents the mean levels of total aerobic bacteria (TAB), coliform bacteria, *E. coli* and mold/yeast in dried, seedless barberry fruits selected from four regions.

The mean TAB in the samples obtained from the Darmian region was $4.60 \pm 0.37 \log_{10} \text{CFU.g}^{-1}$, which was the highest TAB level. Although the lowest TAB level was related to the samples from the Birjand region ($2.30 \pm 0.12 \log_{10} \text{CFU.g}^{-1}$), it was not significantly different from the samples from the Qaen region ($2.47 \pm 0.21 \log_{10} \text{CFU.g}^{-1}$) and Zirkoh region ($2.47 \pm 0.24 \log_{10} \text{CFU.g}^{-1}$) ($p < 0.05$). The results revealed no *E. coli* bacteria in any of the samples selected from the four regions. According to the data presented in Table 3, the mean level of coliform bacteria in the samples significantly varied from $0.00 \log_{10} \text{CFU.g}^{-1}$ to $1.69 \log_{10} \text{CFU.g}^{-1}$. The highest overall level of the forms was $1.69 \log_{10} \text{CFU.g}^{-1}$, which was related to the samples prepared from the Darmian region ($1.69 \pm 0.11 \log_{10} \text{CFU.g}^{-1}$) and the Qaen region ($1.69 \pm 0.12 \log_{10} \text{CFU.g}^{-1}$). The lowest total form was observed in the samples from the Zirkoh region (0.00), followed by the samples from the Birjand region ($1.32 \pm 0.10 \log_{10} \text{CFU.g}^{-1}$), with the lowest level of the overall form. The mean amount of yeast mold in dry, seedless barberry samples from the four regions significantly varied between $1.77 \log_{10} \text{CFU.g}^{-1}$ and $4.17 \log_{10} \text{CFU.g}^{-1}$. The highest amount was obtained from the samples prepared from the Darmian region ($4.17 \pm 0.28 \log_{10} \text{CFU.g}^{-1}$). Additionally, the lowest overall level of forms was related to the Birjand region ($1.77 \pm 0.13 \log_{10} \text{CFU.g}^{-1}$), followed by the Zirkoh region ($2.30 \pm 0.20 \log_{10} \text{CFU.g}^{-1}$).

Table 3. Total aerobic bacteria, *E. coli*, coliforms, and molds/yeasts isolated from dried seedless barberry fruit selected from different regions of South Khorasan province, Iran.

Regions	Total aerobic bacteria	<i>E. coli</i>	Coliform	Yeast/Mold
Birjand	$2.30^b \pm 0.12$	ND	$1.32^b \pm 0.10$	$1.77^a \pm 0.13$
Zirkoh	$2.47^b \pm 0.24$	ND	ND ^c	$2.30^b \pm 0.20$
Darmian	$4.60^a \pm 0.37$	ND	$1.69^a \pm 0.11$	$4.17^a \pm 0.28$
Qaen	$2.47^b \pm 0.21$	ND	$1.69^a \pm 0.12$	$2.60^b \pm 0.19$

Means $\pm$ SEs (n=4) followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.05$) according to the LSD test. Quantitative ($\log_{10} \text{CFU.g}^{-1}$). ND: not detected. CFU: colony-forming unit.

Plate counting of aerobic mesophilic microorganisms in products is one of the key microbiological tests used to evaluate the quality of products (Aycicek et al., 2006). The number of aerobic mesophilic organisms in products indicates their exposure to pollution and favorable conditions for the proliferation of microorganisms (Alp & Bulantekin, 2021). In other words, the determination of this microbiological parameter helps us to understand whether cleaning, disinfection, and temperature control during processing, transportation and storage of products are sufficient and valuable (Lambert, 1995). According to Iranian standards, the ML of total aerobic bacteria is less than $4.30 \log_{10}$ CFU.g⁻¹ (standard number 5752-1). Therefore, according to Iran's food standards, the food standards of the Birjand region, Zirkoh region and Qaen region are defined as being lower than the ML. However, the results obtained from the samples from the Darmian region were not satisfactory, and the total number of aerobic bacteria was greater than that in the ML. Some researchers have reported that the expiration date of products is generally considered inappropriate when the TAB population reaches $7 \log_{10}$ CFU.g⁻¹ (Gómez-López et al., 2008). In the present study, the TAB level in all dried, seedless barberry fruit samples from different regions was less than $7 \log_{10}$ CFU.g⁻¹. The total number of aerobic bacteria detected in the present study ranged from $2 \log_{10}$ CFU.g⁻¹ to $5 \log_{10}$ CFU.g⁻¹. Similarly, Artimová et al. (2023), in their study on leafy vegetables and berries, reported that the range of the microbial load varied between $5 \log_{10}$ CFU.g⁻¹ and $8 \log_{10}$ CFU.g⁻¹. Additionally, the total number of aerobic bacteria has been reported to be $2 \log_{10}$ CFU.g⁻¹ to $5 \log_{10}$ CFU.g⁻¹ (Quansah et al., 2019; Kuźniar et al., 2020). Macori et al. (2018) reported slightly lower values (mean $3.89 \log_{10}$ CFU.g⁻¹) in berry fruit samples obtained directly from 50 growers and reported the highest microbial load in currants and blueberries. Nizam et al. (2019) investigated the microbiological quality and sensory evaluation of semidried mango for fruit salad. They reported that increasing the percentage of active water in semidried mango fruit slices increased the activity of anaerobic bacteria. The total number of microorganisms in mango slices ranged from $3 \log_{10}$ CFU.g⁻¹ to $6.5 \log_{10}$ CFU.g⁻¹, which is consistent with the results of the present study (from $2.30 \log_{10}$ CFU.g⁻¹ to $4.60 \log_{10}$ CFU.g⁻¹).

Escherichia coli (*E. coli*) are a bacterium that is commonly found in the intestines of warm-blooded organisms (Allocati et al., 2013). Most *E. coli* strains are harmless, but some can cause severe food poisoning. Shiga toxin-producing *E. coli* (STEC) is a bacterium that can cause severe foodborne disease (Smith et al., 2014; WHO, 2018). In most cases, the illness is self-limiting, but it may lead to a life-threatening disease, including hemolytic uremic syndrome (HUS), especially in young children and elderly individuals (Smith et al., 2014). The STEC is sensitive to heat. When preparing food at home, the basic principles of food hygiene, such as "cooking thoroughly", must be observed (WHO, 2018). One of the factors that prevent the growth of *Escherichia coli* is the pH of the product. Owing to the presence of acids such as citric acid, malic acid, ascorbic acid, etc., fruits have a pH less than neutral or acidic. It has been reported that *E. coli* bacteria can survive in highly acidic environments and resist and tolerate them (Lu et al., 2011). Brown (1991) reported that *E. coli* secretes carboxylase enzymes preferentially at very acidic pH values. The role of these enzymes is to increase the external pH value and thus induce acid tolerance under certain conditions (Rowbury, 1997). However, Ndjongoue-Yossa et al. (2022) focused on removing *E. coli* via pH changes. They reported that a slightly acidic pH (5.5) has antibacterial effects and high inactivation efficiency of *E. coli* at this pH. The results of the present study revealed that *E. coli* bacteria were not present in the samples from all four studied regions. Therefore, this was highly likely due to the lower pH range (<3.5) of the fruit samples, which did not allow the growth and proliferation of *E. coli* bacteria in the dried, seedless barberry. Similarly, Hyun et al. (2019), who evaluated the microbial load of dried persimmons during 70 days of storage, reported that *E. coli* was not present in the samples. They reported that *Escherichia coli* cannot be detected when the value

is less than $0.48 \log_{10} \text{CFU.g}^{-1}$. Another noteworthy point in the findings of Hyun et al. (2019) was that the different storage temperatures (-20 , 5 , 12 , and 25°C) did not significantly affect the microbial load value of the samples. Under conditions of low acidic pH, undigested weak acids have the ability to freely diffuse through the cytoplasmic membrane. Upon entry into the cytoplasm, these weak acids undergo degradation, thereby increasing the internal pH of *E. coli* (Li et al., 2015; Beales, 2004). Consequently, the capacity to maintain the intracellular pH at a nearly neutral level (pH homeostasis) becomes compromised, resulting in cellular death. As explicated by Wiggins (1975), alterations in pH within the cell can instigate chemical modifications in crucial compounds such as DNA or adenosine triphosphate. Furthermore, oxidative and reductive reactions may transpire within the cellular framework (Gilliland & Speck, 1967), culminating in cellular disintegration and death, hence impeding the growth and persistence of *E. coli* bacteria.

Coliform bacteria are widely found in the natural environment, and the feces of animals and humans do not necessarily cause disease. However, its high levels can indicate the presence of other, more dangerous pathogens. This makes the coliform bacteria test a useful "indicator" for detecting bacterial contamination in products. A simple coliform count test can be a good indication of the safety and hygiene of a product. The results of the present research revealed that all the studied regions presented coliform values less than $2 \log_{10} \text{CFU.g}^{-1}$. According to the National Standard Organization of Iran under numbers 11166 and 9263, the acceptable ML of total forms in products is less than $2 \log_{10} \text{CFU.g}^{-1}$. Therefore, on the basis of the Iranian standard, dried, seedless barberry fruits prepared from different regions have acceptable coliform limits. The mean coliform count in this study was lower than the value reported by Tango et al. (2018). They reported mean coliform counts ranging from $2.2 \log_{10} \text{CFU.g}^{-1}$ to $7.9 \log_{10} \text{CFU.g}^{-1}$ on apple, mandarin, and cherry tomato fruits. Our results are also acceptable regarding hygiene criteria for vegetables, fruits and products set by the European Union (Tango et al., 2018). In a study on dried persimmon fruit (Hyun et al., 2019), the mean number of total forms during 70 days of storage was $1.92 \log_{10} \text{CFU.g}^{-1}$, which was greater than the coliform concentration recorded in barberries from different regions in our research. In general, dried fruits and vegetables have lower coliform counts than fresh fruits do (Alp & Bulantekin, 2021).

Like bacteria, yeasts are unicellular organisms that are typically larger in size than bacteria. Most yeasts encounter growth inhibition beyond 100°F (Silva et al., 2019). A substantial water supply is usually imperative for the growth of common yeasts; nevertheless, numerous yeasts can thrive in environments with elevated sugar or salt concentrations. In general, yeasts require less water than bacteria do but more water than molds do (Babič et al., 2016). Sugars and acids are good food sources for yeasts. Growth is abundant in food substances containing carbohydrates (sugars or starch) and varying amounts of acid (Stratford, et al., 2019). The growth of most yeast is favored by an acid reaction. Yeasts grow well at a pH of $4-4.5$ (Bărbulescu et al., 2022). Their growth on food or other nutrient sources results in a mass of loosely entrained filaments collectively called mycelia. Individual filaments are called hyphae (Padmavathi et al., 2020). According to standard No. 10899-3 (Iranian National Standard Organization) (ISIRI, 2013), the acceptable amount of mold/yeast in products is $3 \log_{10} \text{CFU.g}^{-1}$. According to this standard, the barberry samples prepared from the Birjand, Qaen, and Zirkoh regions are below the ML. However, the samples from the Darmian region presented more yeast mold ($4.17 \log_{10} \text{CFU.g}^{-1}$) than the ML samples did. However, the results obtained from the counting of coliform bacteria were in accordance with the guidelines of Gilbert et al. (2000), who reported that a count below $5 \log_{10} \text{CFU.g}^{-1}$ for coliform bacteria in products is acceptable. However, considering that there is little information about the ML of the microbial load on dried fruits, it is challenging to evaluate and compare the results. Therefore, one can infer that the microbial load regulations in Iran are more stringent than those in other nations.

Victor et al. (2017), in a study on dried fruits and vegetables, reported that the amount of yeast in vegetables is very high compared with that in fruits; for example, the number of molds and yeasts counted in dried spinach leaves is $6.74 \log_{10} \text{CFU.g}^{-1}$. Victor et al. (2017) reported that fruits are more acidic than other fresh produce and that the combination of low pH and low temperature during storage also inhibits yeast/mold growth. The acidic environment during refrigeration (5°C) is the inhibitory factor for the growth of mold and yeasts. The role of yeasts in the spoilage of fresh vegetables has not been well studied, although yeasts have been implicated in the spoilage of fermented vegetable products and the development of soft rot (Fleet, 2003). Ragaert et al. (2006) noted that some volatiles (rotting odor) associated with molds and yeasts were detected when populations were $5.0 \log_{10} \text{CFU.g}^{-1}$ or greater. In a study on dried mango, the yeast and mold counts ranged from $4.18 \log_{10} \text{CFU.g}^{-1}$ to $6.90 \log_{10} \text{CFU.g}^{-1}$ (Nizam et al., 2019).

Microbial contamination in vegetables is attributed to sources such as soil, manure, and water and poor handling and storage after harvest (Halablab et al., 2011). Notably, in dried products, fertilizers, harvesting, drying and storage have a direct effect on increasing or decreasing the microbial load (Kadam et al., 2009; El-Dengawy et al., 2018; Mongi, 2023). In the present research, the highest amounts of mold and yeast in the samples were observed in the Darmian region. The drying and storage facilities in this region were unfavorable. Therefore, the barberry fruit dried on the ground, which probably caused an increase in the microbial load in the samples from this region.

Biochemical properties

An evaluation of the biochemical properties of dried, seedless barberry fruit revealed significant differences in the biochemical traits of the fruit selected from the four regions.

A mean comparison (Table 4) revealed that the Birjand region had the highest content of TSS among the other studied regions ($6.33 \pm 0.22^{\circ}\text{Brix}$), followed by the Zirkoh region ($5.00 \pm 0.21^{\circ}\text{Brix}$), the Darmian region ($4.00 \pm 0.19^{\circ}\text{Brix}$) and the Qaen region ($3.31 \pm 0.10^{\circ}\text{Brix}$). Additionally, the highest content of TA was obtained from the barberry of the Qaen region ($3.10 \pm 0.11\%$), and the lowest value was related to the barberry of the Darmian region ($2.12 \pm 0.15\%$). The pH analysis revealed that the Darmian region (3.44 ± 0.24) had the highest pH value among the studied regions. However, there was no statistically significant difference in the pH of dried barberry between the Darmian and Qaen regions (3.28 ± 0.20). The lowest pH value was also obtained from the samples from the Birjand (3.05 ± 0.29) and Zirkoh (3.19 ± 0.27) regions.

The highest total antioxidant activity (TAA) in dried seedless barberry was obtained from the samples prepared from the Qaen region ($71.03 \pm 5.88 \text{ mg.g}^{-1}$), and the lowest amount with a significant difference was related to the barberry from the Darmian region ($30.02 \pm 3.09 \text{ mg.g}^{-1}$) (Table 4).

Table 4. Total soluble solids (TSS), titratable acidity (TA), pH, and total antioxidant activity (TAA) of dried seedless barberry fruit selected from different regions of South Khorasan Province, Iran.

Regions	TSS ($^{\circ}\text{Brix}$)	TA (%)	pH	TAA
Birjand	$6.33^{\text{a}} \pm 0.22$	$2.54^{\text{b}} \pm 0.11$	$3.05^{\text{c}} \pm 0.29$	$61.19^{\text{b}} \pm 4.41$
Zirkoh	$5.00^{\text{ab}} \pm 0.21$	$2.51^{\text{b}} \pm 0.17$	$3.19^{\text{bc}} \pm 0.27$	$62.16^{\text{b}} \pm 3.92$
Darmian	$4.00^{\text{b}} \pm 0.19$	$2.12^{\text{c}} \pm 0.15$	$3.44^{\text{a}} \pm 0.24$	$30.02^{\text{c}} \pm 3.09$
Qaen	$3.31^{\text{b}} \pm 0.10$	$3.10^{\text{a}} \pm 0.11$	$3.28^{\text{ab}} \pm 0.20$	$71.03^{\text{a}} \pm 5.88$

Means $\pm$ SEs (n=4) followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.05$) according to the LSD test.

The TSS content of the fruit is usually obtained from assessing the °Brix of the fruit. At the beginning of the ripening process, the sugar/acid ratio is low because of the low sugar content and high fruit acid content, which makes the fruit taste sour. During the ripening process, the fruit acids are degraded, the sugar content increases, and the sugar/acid ratio increases. The results of the present research revealed that the TSS contents in different studied regions differed from each other. The highest amount of TSS was observed in barberries from the Birjand region, and the lowest amount was obtained from samples from the Qaen region. The concentration of soluble solids can vary due to soil composition, weather conditions, fruit yield, and ripeness. In regions of southern Ukraine, superior sweet cherry varieties present soluble solids contents ranging from 12.1--19.9 °Brix, whereas in the central part of the country, this measure ranges from 11.3--12.8 °Brix (Slavin and Lloyd, 2012; Bublyk et al., 2014; Ivanova et al., 2021). Climatic factors have been shown to have a decisive effect on TSS accumulation compared with other factors (such as cultivar) (Sansavini & Lugli, 2008; Serdyuk et al., 2020). As mentioned, weather has a direct effect on TSS accumulation; however, more precisely, among weather factors, the difference between day and night temperatures has a positive effect on the accumulation of dry solids and the sugar content in fruits. However, the amount of rainfall and daily temperature do not significantly affect the accumulation of TSS in fruit (Sheiko et al., 2019; Caprio & Quamme, 2006). The range of data obtained from the TSS of dried, seedless barberry was consistent with those of Balandari et al. (2023) and Moradinezhad et al. (2018).

Titrateable acidity indicates the amount of organic acids present in a fruit (Etienne et al., 2013). Titrateable acidity represents the organic acids that affect overall fruit quality and flavor. The berry family is rich in organic acids and has useful benefits (Ardestani et al., 2015). The most important organic acids in berries are malic, citric, tartaric, oxalic and fumaric acids (Koyuncu, 2004). In the present research, significant differences in the amount of TA in seedless barberries were detected between the studied regions. The highest amount of TA was obtained from the Qaen region, and the lowest amount was obtained from the Darmian region. Many factors influence the amount of TA in fruits. The literature shows that the plant source–sink ratio, mineral fertilization, water supply, and temperature are the agro-environmental factors that have the greatest impact on fruit acidity (Anthon & Barrett, 2012; Famiani et al., 2015). Orchard management practices such as fruit thinning, plant pruning, or defoliation affect the source–sink ratio of the plant, which usually results in altered sugar supply and fruit growth. Water stress tends to increase the organic acid content and TA content in ripe fruits through a simple dilution/dehydration effect (Lez-Altozano & Castel, 1999). An increase in temperature during fruit growth or storage decreases fruit TA (Wang and Camp, 2000; Gautier et al., 2005). Changes in organic acid metabolism in response to temperature are likely due to the effects of temperature on the reaction rates of glycolysis and the TCA cycle (Araujo et al., 2012). Therefore, the differences in the TA and pH of dried, seedless barberries in the different studied regions are likely due to differences in climate and agricultural operations. In line with the results of the present research, Moradinezhad et al. (2018) reported that barberries prepared from two different regions presented different pH and TA values.

If the fruit juice is acidic, the concentration of hydrogen ions increases, which increases the acidity and decreases the pH of the solution. This increase in acidity and decrease in the pH of fruit juice are related primarily to the presence of organic acids such as citric acid, malic acid and tartaric acid. Other studies have shown that fruits exhibit a decrease in pH during ten days of storage (Suriati et al., 2020; Gao et al., 2018). Carbohydrates in fruits undergo conversion during storage, producing organic acids that help lower the pH (Dorostkar et al., 2022; Saki et al., 2019). In an experiment, Vwioko et al. (2013) investigated the effects of preservatives on the pH and microbial load of *Annona muricata* water. They reported that preservatives that

reduce the pH of fruit juice (garlic and ginger) significantly reduce the microbial load of fruit juice. They identified pH as an important microbial growth factor (Ukwo et al., 2010). Additionally, the results of this research revealed that, compared with those in other regions, the microbial load of barberry fruits in the Birjand region and Zirkoh region was lower. Our results are consistent with the findings of other researchers (Ekanem and Ekanem, 2019; Fleet, 2003).

In addition, the terms “antioxidants,” as well as the concepts of these compounds, are different in the food and biomedical sciences, and the antioxidant indices obtained via chemical assays often cannot be generalized to studies in vivo (López-Alarcón and Denicola, 2013; Yang et al., 2018). Fruits have been proven to be excellent sources of natural antioxidants (Carlsen et al., 2010; Kusano and Ferrari, 2008). These compounds protect cells from the damaging effects of reactive oxygen species (ROS) and neutralize the mechanisms of active free radicals such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals or disrupt their destructive reactions. These events are related to the chemical composition of antioxidants (Rekha et al., 2012; Wang and Jiao, 2000; Lee et al., 2009). These plant compounds have several biological functions, such as anti-inflammatory, anticancer, antidegenerative, antiaging, and antidiabetes properties, among others (Jideani et al., 2021; Lu et al., 2021; Pérez-Lamela et al., 2021). One of the most important properties of antioxidant compounds (such as phenolic compounds) is their antimicrobial and antibacterial properties (Suriyaprom et al., 2022). The mechanisms of these compounds in bacterial cells are diverse. Antioxidants can cause deterioration of the cell wall, destabilize the cytoplasmic membrane, deactivate the enzymes that exist inside the cell and are responsible for the metabolic processes of the cell, and, in turn, through interactions with nucleic acids, inhibit reproduction processes. And transcription (Henie et al., 2009; Cendrowski et al., 2020). These actions may damage the structure and function of pathogenic microorganisms. Phenolic compounds cause protein denaturation in microbial cells (Cowan, 1999). Previous reports suggest that phenolic compounds are likely to have toxic effects at the membrane surface. Phenols affect membrane function by changing the ratio of proteins to lipids in the membrane and causing potassium ion leakage and cell death (Negi, 2012). Additionally, flavonoids, another class of antioxidant compounds, damage bacteria and cause the accumulation of bacterial cells, which may lead to disruption of bacterial growth (Tarahovsky et al., 2014). In this context, the results of the present study revealed that the lowest amount of antioxidant activity in dried barberry was obtained from the Darmian region. Additionally, considering the microbial load data, the low antioxidant activity in the Darmian region likely caused the increase in the barberry microbial load in this region. A comparison of the microbial load and antioxidant activity results revealed that a lack of compliance with health protocols likely increased contamination of the shadehouse environment of barberry fruits. On the other hand, the antioxidant compounds of fruits have been used to fight against pathogenic microorganisms and reduce their effects. This probably caused the reduction in the antioxidant activity of fruits in the Darmian region. Our results are consistent with those of other researchers (Cendrowski et al., 2020; Aslam et al., 2023).

Correlation coefficients of traits

Significant strong correlations revealed among investigated variables in this experiment. The results showed positive significant correlations between TAB and yeast/mold ($r = 0.96$), TAB and pH ($r = 0.85$). Also negative significant correlations between TAB and TA ($r = -0.71$), and TAB and TAA ($r = -0.95$) were observed. There was also a positive significant correlation between yeast/mold and pH ($r = 0.96$), and a negative significant correlation between yeast/mold and TAA ($r = -0.84$). Some of chemical traits had strong correlations too. TA had a

positive significant correlation with TAA ($r = 0.88$), and TSS showed a negative significant correlation with pH ($r = 0.80$).

In line with the Pokhrel et al. (2022), the results of correlations revealed that fruit from Darmian region, which had lower TAA and higher pH compared to other regions showed the higher TAB and yeast/mold. Zitouni et al. (2020) reported significant strong correlations among pH, TSS and TAA of strawberry. Higher levels of microbial inactivation at lower pH values could be due to the changes within the cell membrane allowing the diffusion of acids from the environment to the cytoplasm, which decreases the internal pH of the cell. Therefore, bacterial inactivation can be enhanced in acidic fruit with lower pH as we observed in seedless barberry fruit selected from all regions than Darmian.

CONCLUSION

This study investigated the effects of different regions on the microbiological analysis and chemical characteristics, especially the antioxidant activity, of barberry fruits. The results briefly revealed high amounts of total aerobic bacteria, mold and yeast in barberry fruits selected from the Darmian region shadehouse. However, other regions were at an acceptable level regarding the microbial load, which shows that the health protocols in these regions (the Birjand, Zirkoh, and Qaen regions) have been carried out effectively. Notably, this comparison was made with the standards of the Food and Drug Organization of Iran. However, according to other standards, the levels of total aerobic bacteria, mold and yeast may be at acceptable levels. The fruits of the Birjand region had the highest TSS content and the most acidic pH value of the fruit juice, which suggests that the fruits of this region have better taste quality than those of other regions. Additionally, fruits prepared from the Qaen region presented the highest amount of TA and total antioxidant activity. This finding highlights the high nutritional value of the fruit of the Qaen region compared with that of other regions, which is highly likely due to the higher altitude of the Qaen region compared with other regions. Therefore, to reduce the risk of microbial contamination and maintain the nutritional value of dried, seedless barberry fruit, it is necessary to provide particular standards for shadehouses.

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Conflict of interest

The authors declare no competing interests.

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