Effect of Extraction and Processing Conditions on the Water-Soluble Vitamins of Barberry Fruits

S. Berenji Ardestani, M. A. Sahari*, and M. Barzegar

ABSTRACT

The bioactive compounds of barberry such as water-soluble vitamins are used in medical and food industries. In this study, the effect of different extraction conditions and various process conditions were studied on water-soluble vitamins profile. The extraction conditions included varieties (species) are of fruits (B. integerrima: A, B. vulgaris: P), solvents (Water: W, Ethanol: E), light (Presence: L, Absence: T), pH (3 and 1.5), and temperature (25 and 50°C). The process conditions included heating (95 and 80°C), chilling (ref 1, 2 months), freezing (con), microwave (mic) and gamma irradiation (γ at doses of 0.5, 1, 2.5, 5, 7.5, 10 kGy). The highest and lowest amounts of water-soluble vitamins in various extracts of different extraction conditions were respectively as follows: vitamin C in AWL1.550 (highest) and PEL350 (lowest); B5 in PWL1.550 and AEL1.525, AWL1.550; B6 in AWT350, and AEL1.550; B1 in PET350 and PET1.550; folic acid in AET350 and PWT350; biotin in AEL350 and AEL1.550; B2 in AET350 and PWL350 extracts. The highest and lowest amounts of water-soluble vitamins in various process conditions were respectively as follows: vitamin C (A con 460 (highest), A ref1 146.87 (lowest), P con 242.96 (highest), P mic 21.52 (lowest)), B5 (A γ0.5 2919.18, A 95 1312.42, P γ10 3110.88, P ref2 1051.52), B6 (A con 36.30, A γ7.5 21.04, P γ0 12.70, P γ0 8.73), B1 (A con 2113.00, A ref2 965.09, P con 2298.15, P γ0 217.76), folic acid (A γ10 1700.38, A γ10 947.11, P con 104.78, P γ2.5 6.79).

Keywords: B group vitamins, Bioactive compounds, HPLC, Vitamin C.

INTRODUCTION

Vitamins are one of the main groups of organic compounds, which are found in low amounts in natural foods. They have a vital in enzymatic reactions of carbohydrates, lipids and proteins metabolism [4]. Vitamins are divided in two main groups of water- and fat-soluble vitamins. Water-soluble vitamins include B group and C vitamins. Each B group vitamin has an especial structure and performance in human body [10]. Insufficient intake of vitamins is a risk factor in heart diseases, cancers and osteoporosis. Recommended Daily Intake (RDI) is an expression to explain the amounts of vitamins and minerals needed daily to keep the body healthy. There is a growing demand for rapid (to reduce decomposition by light) and special methods to analyze vitamins. The HPLC techniques make it possible to separate and quantify water-soluble vitamins fast and accurately by different preparation and detection methods. Usually the extraction technique such as Solid Phase Extraction (SPE) prior to HPLC analysis is essential to obtain maximum recovery and remove other interfering UV absorption species. Generally, HPLC is an easy and economical procedure. The other benefits of HPLC analysis of vitamins are easy coupling to other techniques, requiring a small amount of sample, and its high selectivity [10].

Separation of complex mixtures by chromatography needs a gradient program. In previous studies, different volume ratios of methanol-water were used as mobile phase, but separating nine water-soluble vitamins was very
difficult, due to overlap of vitamin C and folic acid as well as vitamin B<sub>1</sub> and B<sub>6</sub> peaks. When methanol-phosphate buffer (pH 7) 0.1 mol L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> is used as mobile phase, all peaks are separated well without any overlap and tailing. Vitamins B<sub>3</sub> and B<sub>12</sub> are hardly absorbed on the column they will be eluted only by using 30% methanol in the mobile phase. In gradient mode, 40% methanol is used to reduce the time of separation [26].

Heating, chilling, freezing, microwave and gamma irradiation treatments are common processes in food industries. They undoubtedly affect the bioactive compounds of a product. Heating is the most conventional process in food industry, and the changes resulting from this process are either desirable (deactivation of anti-nutritional agents by heating) or undesirable (destruction of vitamins and other nutrients) [31].

In foods chilled at 4-5°C, some factors such as type of fruits and vegetables, temperature, storage duration, moisture and light must be taken into consideration to protect their nutrients. Some losses of vitamins during chilling are attributed to oxygen and light [32].

Freezing will be the best long-term food preservation method if it is performed correctly. Changes in the nutrients of plant tissues by freezing are just extremely small. In defrosting process, significant amounts of mineral and water-soluble vitamins leaches out from the frozen tissue, especially when the product is cooked. Some vitamins such as ascorbic acid, and folacin are lost if the product is kept frozen for a long time. Reactions destroying these vitamins are catalyzed by enzymes, metals and oxygen [31].

Microwave heating affects the taste and nutritional quality of foods less than the conventional heating methods. Microwave blanching provides maximum shelf life, quality of product, color, ascorbic acid and chlorophyll contents to hot water or steam blanching [9].

Food irradiation is a physical treatment for processing that expose packed food to gamma and X rays as well as electron beams. In optimal irradiation conditions, the loss of vitamins up to dose of 1 kGy appears to be non-significant. Researches have shown that irradiation does not make food radioactive, and the low to moderate doses have a little negative effect on vitamins and nutrients. Loss of vitamins and nutrients in irradiation is similar to other processes like heating or canning [7, 31]. In the case of radiolysis of vitamins, various reactions of free radicals are depended on the vitamins environment. Water-soluble vitamins are exposed to radicals resulting from water irradiation. Some water-soluble vitamins may directly react with hydrated electrons or electrons from other radicals in the aqueous environment. The amount of reaction is determined by the electron reduction potential of vitamins and the strength of H bonds. Hydroxyl radicals react with the main components of foods including lipids, proteins, and carbohydrates more than vitamins because their amounts in the diet are very low. So, vitamins are mostly affected by hydro-peroxides as secondary radicals that are formed during interacting with the main components [16].

In this study, we aimed to study the separation and quantification of water-soluble vitamins in two types of Iranian native barberry fruits by HPLC technique for the first time (first time in Iran and other countries). Another objective was to study the effects of common processes in food industry including heating, chilling, freezing, microwave and gamma irradiation on the profiles and concentrations of the water-soluble vitamins of two varieties of barberry fruits (Berberis integerrima and B. vulgaris).

**MATERIALS AND METHODS**

Based on gardeners’ experience and common harvest time in the area, the fresh and ripen fruit were picked. Berberis integerrima and Berberis vulgaris (each 7 kg), were purchased from Qaen city of South Khorasan Province in Iran on November 2012. Fruit were carefully cleaned from any branch and leaves, stone and any foreign matter by hand. Then, the cleaned fruits were packed in plastic bags and were stored in freezer at -80°C until testing. Extracts were prepared from ground dried fruits. In order to protect color and reduce the effects of drying, fruits were placed in the oven at 50°C for 48 hours for B. integerrima and 72 hours for B. vulgaris based on their initial moisture contents [8]. Then, the dried fruits were powdered by means of a grinder.
Chemicals and Instruments

The following chemicals and instruments were used in our experiments in the present study: Ethanol 96° (Parsian Co., Shiraz, Iran), HCl, methanol, K2HPO4 (Merck, Darmstadt, Germany), standards of water-soluble vitamins (Sigma-Aldrich, Deisenhofen, Germany), C18 cartridges 500 (Teknokroma, Barcelona, Spain), freezer -80°C (JaTeb Lab Equipment, Type JD 300 L), oven (Memmert, Germany), grinder (Moulinex, Type DPA 1, CMMF 800W, France), sampler (Nichipet EX 100-1,000 µL, Japan), laboratory scale with 0.0001 g accuracy (Sartorius, Germany), pH meter (Metrohm, 827 pH lab), shaking water bath (Memmert, Germany), shaker (Heidolph-UNIMA, Germany), vacuum pump (Sparmax, Taiwan), paper filter no. 1 (Whatman, England), rotary evaporator (Heidolph Laborotry 4000, Germany), vacuum oven (Memmert, Germany), freeze drier (Scanvac, UK), centrifuge (Sigma, 3-30 K, Germany), HPLC (WATERS, USA) including Empower software, pump (Waters 600, USA), injection valve (Rheodyne 7125i six-way), injection loop 20 µL and UV-Vis detector (Waters model 2487), C18 column (Discovery, 15 cm×4.6 mm ID, 5 µm particles, Supelco, USA), membrane filter 0.45 µm (Teknokroma, Barcelona, Spain), refrigeraor 4°C (Azmayesh, Iran), freezer -18°C (Pars, Iran) microwave oven (Butan, Iran), gamma cell 220 (radiation rate 1,000 Gy s⁻¹, Nordion, Canada), thermometer, vegetable liquid oil including soy beans, cotton seeds, sunflower seeds, canola and palm olein, TBHQ antioxidant max 120 ppm, and β-caroten 5 ppm (Varamin Factory, Iran).

Thirty two types of extracts were prepared using 5 effective variables each at two levels on the extraction yield, including variety of fruits (B. integerrima and B. vulgaris), solvents (water and ethanol), lighting conditions (presence of light and darkness), pH (3 and 1.5) and temperature (50 and 25°C). To prepare the extract at 25°C, 10 g of barberry (B. integerrima or B. vulgaris) powder were mixed with 40 mL of solvent (water or ethanol) in a flask. The flask was sealed completely, and stirred for 24 hours at 150 rpm. The flask was covered completely with aluminum foil in the dark-treated extract [8]. For the extraction at 50°C, the flask was placed in water bath at 50°C for 2 hours and then kept in dark for 2 hours [8]. Contents of the flask were washed with the solvent to a colorless fluid outlet. The outlet was filtered liquid by using a Buchner funnel and Whitman No. 1 filter paper under vacuum. Approximate initial pH value was about 3 and a few drops of HCl 0.1% were used to adjust the pH at 1.5.

Solutions at concentration of 1,000 ppm of each vitamin were prepared from the extracts of both varieties of barberry fruits. These solutions were centrifuged for 2 min at 12,000 rpm. Solid phase extraction method was used for the extraction of water-soluble vitamins, and removing undesirable interferences. In order to activate the stationary phase, it was washed with 10 mL methanol and 10 mL water (pH was adjusted at the 4.2). The acidic water was prepared by adding drops of 0.005M HCl to it and stirring until pH= 4.2. Then, 10 mL of the centrifuged solution was loaded onto the cartridge. The sample was eluted from cartridges by 5 mL acidified water and 10 mL methanol. The solution leaving the cartridge was collected in a round bottom flask and was dried by vacuum rotary evaporator at 35°C. The residue was dissolved in mobile phase (buffer solution) and then filtered, using a polypropylene flat membrane filter. HPLC separation was performed by injecting 20 µL of the final sample [14]. The mobile phase contained solution of K2HPO4, 50 mM (A) and methanol (B) with a determined gradient. Working conditions were as follows: Mobile phase flow rate 1 mL min⁻¹, pressure less than 1,500 Psi, UV-Vis detector, wavelength 220 nm, and at ambient temperature [4].

Process Conditions

After the first step of study, the remaining samples were put in polyethylene bags and kept in the freezer at -18°C (Control of processed sample= con). The results of fresh fruits in the first step were used as control of frozen sample for statistical analysis. For evaluation of the freezing conditions, the fruits were kept in freezer for 1 year [3]. Heating was done at 95°C for 3 minutes by holding the container of barberry fruit in liquid oil including soy bean, cotton seeds, sunflower seeds, canola and palm olein, TBHQ antioxidant
max 120 ppm and β-caroten 5 ppm) bath at 130±1°C (Tiwari and Cummins, 2011) [37] and at 80°C for 5 minutes by holding the container of barberry fruit in water bath at 95±1°C (Juhasz et al., 2012) [17] and monitoring the temperature of barberry inside both vessels until reaching the desired temperatures.

The samples were treated with microwave in domestic oven at a power of 600W for 60 seconds. The whole fresh barberry fruit before any other treatments, extraction and using solvents was treated by microwave and then water soluble vitamins were extracted by chemical solvents. Fruits were put in a glass plate at the height of 0.5 cm, and the plate was put inside the microwave oven [29].

Barberry fruits were put in glass jars with plastic caps and the glass jars were irradiated by Gamma cell-220 irradiator (Nordion, Canada) at doses of 0, 0.5, 1, 2.5, 5, 7.5 and 10 kGy [2] with dose rate of 3.63 Gy s⁻¹ at ambient atmosphere in Iran Atomic Energy Organization in Tehran. Dosimetry was done chemically, by means of Ferric dosimeter. Afterwards, radiation samples were kept in freezer at -18ºC till analysis.

Then the extracts of processed barberry fruits were prepared, and their water-soluble vitamins were analyzed by HPLC as mentioned above.

All experiments were performed in triplicate and the results were analyzed based on Completely Randomized Designs (CRD) using factorial analysis of variance by SAS 9.1 software (Diliorio and Hardy, 1995). Mean values were compared using Least Significant Difference (LSD) test and considered significantly different at P< 0.05. [12].

The samples were labeled using 3 letters and 2 digits. For example, in Table 1, in AEL1.525 the first character is barberry variety (B. integerrima or Abi= A, B. vulgaris or Poloie= P), the second character is extraction solvent (Ethanol= E, Water= W), the third character is light conditions (Presence: L or Absence: T), the forth character is pH (3 and 1.5), and the fifth character is temperature (25 and 50°C).

The results were expressed in mg 100 g⁻¹ extract because the extracted solutions were freeze-dried completely; then, needed concentrations were made from freeze-dried powder.

**RESULTS AND DISCUSSION**

**Profile and Concentration Determination of Water-soluble Vitamins in Fresh Fruits**

The results of the composition of water-soluble vitamins (mg 100 g⁻¹ extract) in 32 types of extracts of barberry fruits prepared in different conditions are shown in Table 1 and Figure 1 is the related chromatogram. The extracts were prepared by using 5 effective parameters in the yield of extraction at two levels as follows; variety of fruits (B. integerrima and B. vulgaris), solvents (water, ethanol), presence and absence of light, pH (1.5 and 3) and temperature (25 and 50°C). It should be noted that there were not any reports in the literature about the profile and concentration of water-soluble vitamins (with the exception of vitamin C) in barberry fruits before the present study.

As shown in Table 1, in B. integerrima the maximum and minimum extractions of vitamin C were observed in AWL1.550 (1029.57±2.93 mg) and AEL3.25 (254.36±2.25 mg) in 100 g extract.
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<th>Sample</th>
<th>Vitamin B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Biotin</th>
<th>Folic acid</th>
<th>Vitamin B&lt;sub&gt;4&lt;/sub&gt;</th>
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</table>

* Words A, B ... above the numbers show statistical grouping which the same letters in each column has no significant differences (P< 0.05). Numbers in Table represent mean value of 3 repetition±SD. ND= Not Detected. b Variety: A= Berberis integrifolia (Abi), P= Berberis vulgaris (Poloci)/Solvent: W= water, E= Ethanol/Light: L= Presence of Light, T= Absence of light/Temp= Temperature (25 and 50°C).
extract, respectively. The maximum amounts of B₁, B₂ and folic acid in AET350 (2058±110.65, 659.49±213.30 and 2271.59±36.88), B₅ in AWL325 (1472.75±146.10), B₆ in AWT350 (675.34±1.66) and biotin in AEL350 (3641.17±228.59) mg were extracted from 100 g extracts. The minimum amounts of B₂, B₆ and biotin in AEL1.550 (80.38±6.22, 112.67±1.23 and N.D.), B₁ and folic acid in AWT1.525 (546.82±11.97 and 132.51±14.96), and B₅ in AWL1.550 (11.65±0.00) mg were extracted from 100 g of extracts. In B. vulgaris, the most amounts of extraction of C (580.53±7.72), B₁ (2873.42±219.45), B₅ (279.97±35.06), B₆ (495.66±6.11) folic acid (101.86±7.97) and biotin (2725.35±931.40) mg 100 g⁻¹ extracts were observed in PET350. The least amounts of B₅ in PWL350 (1078.40±11.82), B₆ and biotin in PWL325 (162.47±0.09 and 177.08±8.27), C in PEL350 (183.70±0.53), B₁ in PET1.550 (259.90±40.43), B₂ in PWL350 (9.92±5.41), B₅ in PWT1.550 (250.69±23.04) and folic acid in PWT350 (22.37±0.00) mg were obtained from 100 g of extracts. In a study, the water-soluble vitamins of two members of berry family were analyzed. Vitamins were found in blueberries and Saskatoon berry containing vitamin C, thiamine, riboflavin, pantothenic acid, pyridoxine and folate. A small amount of biotin was also found in Saskatoon berry but not in blueberries, and a little amount of niacin was observed in blueberries but not in Saskatoon berry [21]. These results confirm the present study’s findings about the composition of water-soluble vitamins of barberry fruits. The order of eluting water-soluble vitamins from the column on the basis of ascending retention time in the extracts of barberry fruits was C, B₅, B₆, B₁, folic acid, biotin and B₂ (Figure 2). This is in agreement with the report of Supelco [4] and the results of Ekinci (2005) [14] using the same chromatographic conditions. Water-soluble vitamins are very hydrophilic. They can be eluted from a reverse phase column by a little amount of aqueous methanol or acetonitrile. B₂ and B₁₂ are relatively hydrophobic while other water-soluble vitamins are quiet hydrophilic. The mobile phase, which can elute B₂ and B₁₂, elutes the other vitamins together at the same time from the column. Then, the consumption of two mobile phases with different organic composition is recommended to have a desirable separation [4]. The dissociation constants (pKₐ) for water-soluble vitamins are as follows: C (4.19), B₅ (4.82), B₆ (5), B₁ (4.80), folic acid (5.35), biotin (6.22), and B₂ (10.58) [25]. The greater pKₐ displays the less dissociation and ionic species concentrations and, as a result, more affinity

Figure 2. Chromatogram of water-soluble vitamins in irradiated barberry fruits extract: C (1), B₅ (2), B₆ (3), B₁ (4), folic acid (5), biotin (6) and B₂ (7).
to non-polar stationary phase; therefore, they will be observed with longer retention time at the end of the chromatogram as in the present study. Also, B$_5$, B$_6$, C, and B$_3$, with smaller $pK_a$ and less affinity to non-polar stationary phase, are displayed as initial peaks at the beginning of the chromatograms, similar to the present study results.

In addition, hydrophobic interactions play a great role in the separation of water-soluble vitamins. The retention of B$_1$, B$_6$, C, and B$_3$ with polar or ionized groups on the reverse phase column is small, and for B$_2$ and B$_12$ with great hydrophobic groups, it is vice versa. Hydrogen bonds also contribute to separation of water-soluble vitamins. B$_1$, B$_6$, B$_3$, and C include hydrogen donating groups (-OH, -COOH, -NH$_2$) that can form hydrogen bonds [25].

In analysis of water-soluble vitamins by normal phase HPLC, highly polar compounds may be absorbed intensively to the column, so that their elution is too hard and sometimes impossible. Therefore, the reverse phase HPLC has many advantages in this field. The polar solutes in the aqueous dispersion of vitamins do not absorb to the lipophilic stationary phase. In this way, retention volumes are repeatable, the column shelf life will be increased, and the time of sample preparation will be decreased. In this system, ascorbic acid, even in non-ionic status, is highly polar, which has little affinity to lipophilic stationary phase, so, will be eluted at the beginning of the chromatogram [36]. The elution of WSV from C$_{18}$ Bondapak column was done by descending polarity. The retention times of B$_2$, B$_{12}$, niacin amide, and pyridoxine notably decreased by increasing the methanol portion of elution solvent, while the retention times of more polar vitamins were not affected by the composition of the solvent. Similar to the mentioned results, in the present study, in both cultivars of barberry, vitamin B$_2$ had the most retention time because of its more hydrophobicity and affinity to the C$_{18}$ column, and vitamin C with the hydrophilic nature was observed at the beginning of the chromatogram as the first peak. In addition, the maximum extraction of more polar vitamins as C, B$_3$ and B$_6$ at the beginning of chromatogram were observed in water (as a solvent), and other vitamins with less hydrophilic nature, such as B$_1$, folic acid, biotin and B$_3$, were mostly extracted in ethanol (as a solvent).

Biotin is generally found to bind with protein and free forms. Enzymatic hydrolysis with papain or acid hydrolysis along with heat treatment is usually used to break these bonds. Decrease of biotin concentration observed after acid hydrolysis can be attributed to its decomposition [20]. In this study, the most extraction of biotin was at pH= 3, and the least amounts were observed at pH= 1.5 in B. integerimma, which can be attributed to the acidic hydrolysis of biotin at lower pH.

The analysis of variance for the single and mutual effects of five factors (barberry varieties, extracting solvent, present or absence of light, temperature and pH) on the extraction of water-soluble vitamins is represented in Table 2.

The results showed that fruit variety (species) had significant effect in the extraction of all vitamins, with the exception of B$_6$. Generally, most of the vitamins were extracted from B. integerimma rather than B. vulgaris. The results showed that fruit variety (species) had significant effect in the extraction of all vitamins, with the exception of B$_6$. Generally, most of the vitamins were extracted from B. integerimma rather than B. vulgaris.

Plant tissues are important sources of water-soluble vitamins. The vitamin content of a certain species is considerably dependent on variety, growth conditions, maturation, preservation after harvesting, and processing [31]. Water-soluble vitamins easily degrade upon exposure to heat, oxygen, and light. Other effective parameters in their decomposition are water activity, pH and the trace amounts of metals such as iron or cupper [27]. The vitamin C content of cranberry is dependent on variety and growth conditions [13].

Solvent had a significant effect on the extraction of all water-soluble vitamins in this study. The vitamins such as C, B$_3$ and B$_6$, which are more hydrophilic, showed their maximum extraction in water as a solvent. Supelco Application Note (Anonymous, 2000) also mentioned similar reports about the vitamins, hydrophilic structure and elution [4].

Light factor had significant effect on the extraction of vitamins C, B$_1$, B$_2$, and biotin but not any meaningful effect on B$_6$, B$_3$ and folic
acid in both cultivars of barberry fruits. Other researchers found that light affected vitamins C, B₃, and folic acid, but it was ineffective for B₁, B₅, B₇, and biotin [35].

In this study, pH affected the extraction yield of all water-soluble vitamins, with the exception of B₅ in both barberry fruits.

Ascorbic acid oxidizes in the presence of air under alkaline and neutral conditions (effectiveness of pH) [27]. The optimum pH of vitamins was reported as follows: C and B₉ (5-7), B₁ (3-4.5) and folic acid (6-9); however, it was not critical in B₅, B₂ and biotin [35]. Ascorbic acid and thiamin did not display repeatable amounts at the pH above 7, and folic acid was unstable at pH below 7 [38]. In barberry fruits, similar to results of Will et al. (1977), ascorbic acid showed more extraction at lower pH (1.5) but folic acid mostly extracted at higher pH (3), which means more stability of folic acid at higher pH. In thiamin, the barberry fruits had more extraction at higher pH (3); this finding is not in line with the report of Wills et al. [38].

Temperature affected the extraction yield of all water-soluble vitamins, with the exception of B₆ in both barberry fruits in this study.

Vitamins C, B₁, and folic acid did not show thermal stability when added to foods. B₂ and biotin had thermal stability, and for B₆ and B₃, thermo-resistance was relatively good [35]. Heating decomposed thiamin, but light was ineffective. Riboflavin under alkaline conditions and in the presence of light was converted into very active compounds called “lumiflavin”, which accelerates the destruction of other vitamins. B₃ was stable against oxygen and acidic pH but was unstable at alkaline pH and was very sensitive to visible light (500-520 nm). At the exposure of light, its destruction rate was increased by increasing the pH. Heating in neutral or acidic conditions did not destruct this vitamin [27]. All these confirm most of the results of the present study about effective factors on the extraction of water-soluble vitamins from B. integerrima and B. vulgaris.

Finally, the extracts with the highest vitamin content including AET350 (for B. integerrima) and PET350 (for B. vulgaris) were selected for studying the effects of conventional food processing like freezing, chilling, heating, microwave and gamma irradiation on water-soluble vitamins.

Effects of Different Food Processing on the Profile and Concentration of Water-soluble Vitamins

The amounts of water-soluble vitamins (C, B₃, B₅, B₁, folic acid, biotin and B₂) after processes including heating, chilling, freezing, microwave and gamma irradiation (Figure 2) are presented in Table 3.

According to Table 3, vitamin C content of the samples after different processing significantly decreased in both varieties of barberry fruits compared with the controls, with the exception of B. integerrima in gamma irradiation at the dose of 1 kGy, which did not show meaningful changes.

The contents of vitamin B₃ significantly increased after one year freezing and gamma irradiation at the dose of 10 kGy in both varieties of barberry fruits, and in B. integerrima at the dose of 0.5 kGy of gamma. This vitamin showed meaningful decreases compared with the controls after irradiation at the doses of 1, 2.5, 5 and 7.5 kGy in both cultivars, and after heating (95 and 80°C), chilling (1 and 2 months), microwave treatment and 0.5 kGy of gamma in B. vulgaris.

The amounts of vitamin B₆ decreased significantly after one year freezing in both varieties, and after heating (95 and 80°C), chilling (1 and 2 months) and all doses of gamma irradiation in B. integerrima. This vitamin did not show any significant change compared with the controls after the other processes (except freezing) in B. vulgaris, and after microwave treatment in B. integerrima.

The content of B₁ significantly decreased compared to the controls in both varieties after all processes, with the exception to freezing, which increased in B. integerrima and decreased in B. vulgaris, significantly.

The amounts of folic acid decreased after heating (95 and 80°C), chilling (1 and 2 months), freezing (for 1 year), microwave and gamma irradiation at the doses of 1, 7.5 and 10 kGy. Also, folic acid content increased after gamma
Table 2. The analysis of variance for the single and mutual effects of five factors (barberry varieties, extracting solvent, present or absence of light, temperature and pH) on water-soluble vitamins contents. *

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Vitamin B₂</th>
<th>Biotin</th>
<th>Folic acid</th>
<th>Vitamin B₁</th>
<th>Vitamin B₆</th>
<th>Vitamin B₃</th>
<th>Vitamin C</th>
<th>Degree of freedom</th>
</tr>
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<td>Variety (V)</td>
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<td>0.1418</td>
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<td>pH (P)</td>
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<td>&lt;0.0001</td>
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</table>

* Variety: A= Berberis integerrima (Abi), P= Berberis vulgaris (Poloei); Solvent: W= Water, E= Ethanol; Light: L= Presence of light, T= Absence of light; Temp= Temperature (°C). The P values < 0.05 are significant and P values > 0.05 are none significant.
### Table 3.
The effects of conventional processing in food industry (gamma irradiation, microwave, heating, refrigerating and freezing) on water-soluble vitamins content of studied varieties (B. integrerrima and B. vulgaris).a

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vitamin C</th>
<th>Vitamin B₃</th>
<th>Vitamin B₆</th>
<th>Folic acid</th>
<th>Biotin</th>
<th>Vitamin B₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁₀₀_h</td>
<td>522.44±7.8</td>
<td>1391.12±16.3</td>
<td>361.37±15.5</td>
<td>2058.99±10.6</td>
<td>2271.59±36.7</td>
<td>2978.77±62.0</td>
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<tr>
<td>A₁₀₂</td>
<td>460±3.5</td>
<td>2506.06±28.4</td>
<td>36.20±0.2</td>
<td>2113.41±5.4</td>
<td>1591.36±40.1</td>
<td>2011.84±24.9</td>
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<tr>
<td>A₁₀,5</td>
<td>208.25±4.3</td>
<td>2919.18±102.7</td>
<td>24.04±0.6</td>
<td>1860.42±30.4</td>
<td>1700.38±10.3</td>
<td>579.92±6.8</td>
</tr>
<tr>
<td>A₁</td>
<td>453.11±10.1</td>
<td>1824.08±65.5</td>
<td>25.18±0.9</td>
<td>1787.86±175.2</td>
<td>230.23±56.1</td>
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<tr>
<td>A₁₂</td>
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<td>1577.18±136.6</td>
<td>24.40±0.7</td>
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<td>31.04±0.5</td>
<td>1917.39±10.8</td>
<td>1346.7±77.4</td>
<td>589.96±29.1</td>
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<td>495.66±6.1</td>
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a Words A, B ... show statistical grouping which the same letters in each column has no significant differences (P<0.05). The analysis of each sample was repeated 3 time. ND= Not Detected. a con: frozen sample (control of processed sample), γ: Gamma irradiated samples, mi= Microwave treatment, 95 and 80°C= Heating treatment at 95 and 80°C, Ref l and 2= Refrigerating treatments for 1 and 2 months, A= Berberis integrerrima (Abi), P= Berberis vulgaris (Poloei).
irradiation at the dose of 0.5 kGy, but it did not show any significant change after gamma irradiation at the doses of 2.5 and 5 kGy in B. integerrima. There was significant decrease in this vitamin content after heating (95 and 80°C), chilling (1 and 2 months), and gamma irradiation at the doses of 5, 7.5 and 10 kGy; however, it had no meaningful change after other treatments in B. vulgaris.

The biotin content of B. integerrima decreased after all treatments, with the exception of microwave, but increased in B. vulgaris after freezing (for 1 year) and did not show any significant change after the other treatments. The amounts of B2 decreased significantly after some treatments as follows: after heating (95 and 80°C), chilling (1 and 2 months), gamma irradiation at the doses of 1, 2.5, 5, 7.5, and 10 in both varieties, after freezing in B. integerrima, and after microwave treatment in B. vulgaris compared to the controls. It increased after gamma irradiation at the dose of 0.5 in B. integerrima, and after freezing in B. vulgaris. The content of this vitamin did not display any significant change after microwave treatment in B. integerrima, and after gamma irradiation at the dose of 0.5 in B. vulgaris.

As a result of heating, vitamin C showed various reflections in previous researches including increase because of ascorbic acid oxidase deactivation [22] or decrease in different ways such as thermal decomposition to diketogluconic acid, oxidation, hydrolysis, polymerization to inactive products [28], and leaching to blanching water [5]. There are two main reasons for the decrease in the amounts vitamin C in fruit upon chilling: (1) Oxidative reactions of enzymes like cytochrome oxidase, ascorbic acid oxidase and peroxidase, and (2) Anaerobic reactions [6, 39]. Ascorbic acid begins to decompose immediately after harvesting; however, chilling can decrease the rate of decomposition [30]. Vitamin C losses increase by increasing the frozen storage duration [32]; losses can be generally 10-80%, with the average of 50%, which is mainly attributed to the decomposition of this vitamin [30]. In microwave treatment, vitamin C was the most sensitive water-soluble vitamin [27]. The vitamin C content of berry fruits decreased after treatment with microwave, but less than conventional heating methods [13]. Vitamin C is the most sensitive water-soluble vitamin to irradiation [19]. Changes of vitamin C during irradiation are more likely due to metabolic changes rather than chemical changes in the plant tissues [18]. In some fruits such as strawberries, the hydro-ascorbic acid content increases immediately after irradiation; therefore, despite oxidation of ascorbic acid, the decrease in the content of vitamin C (ascorbic acid-hydro-ascorbic acid) is indicated only about 5%. Thermal changes affect vitamin C during irradiation. Vitamin C losses were reduced after some doses of gamma irradiation, which can be attributed to reduction in microbial population, inhibition of the activity of polyphenol oxidase enzyme, and delay in physiological metabolism. The stability of ascorbate in fruits is related to their high acid content and the location of vitamin C in the plant tissues. For example, in some fruit in which ascorbate is located in the vacuole, due to its low pH and bonding to phenolic antioxidants, it is more stable. However, in the majority of plants, ascorbate is placed in the cytosol and other cellular components such as chloroplasts that are not protected by phenolic and low pH of vacuoles [39]. The contents of vitamin C in B. integerrima and B. vulgaris were reduced after all processing. These results are in agreement with the reports of Perez-Coneza et al. [28] and Arancibia-Avila et al. [5] about heating, Barba et al. [6], Zhang et al. [39] and Rickman et al. [30] about chilling, Severi et al. [32]; Rickman et al. [30] about freezing, Okmen and Bayindirli [27] and Dorofejeva et al. [13] about microwave treatment, and Kim and Yook [19] about irradiation.

Vitamin B3, leaches in water during blanching so that its amount decreases by 55-65% in spinach. Free panthetonic acid leaches in water more easily than its stable complex form [11]. This vitamin is stable against heat, light, and air at pH 5-7 and its stability is related to the pH of the medium [23]. In the present study, the B3 contents of both varieties of barberry fruits decreased by about 12-47% after heat treatment, and probably the acidic medium (pH 3) was effective as well. During the freezing process, free panthetonic acid reduced significantly but no significant change was observed in the amount of the total panthetonic acid of spinach leaves [11].
Vitamin B_{5} did not show any changes in irradiation at the doses ≥ 10 kGy in many foods [16]. Freezing increased the vitamin B_{5} of B. integerrima (80\%) and B. vulgaris (32\%) fruits in this research.

Vitamin B_{6} is stable to heat. This vitamin is found in three chemical structures including pyridoxine, pyridoxal and pyridoxamine. Pyridoxal and pyridoxamine are more sensitive to heat, light, and oxygen than pyridoxine (the main primary vitamer in plants). This vitamin has shown decreases of about 10-61\% in plant foods because of bleaching in water. Also, it had decreases of about 45-58\% in animal foods because of thermal degradation [23]. Heating reduced the B_{6} content of the seeds of African breadfruits [1]. Also, in the present study, heating decreased the vitamin B_{6} of both cultivars of barberry fruits by about 10-35\%. Freezing without blanching has reduced the vitamin B_{6} content of vegetables [33]. During the microwave treatment, pyridoxine decreases were less than the conventional heating like autoclaving or boiling in water, because they cause bleaching and chemical degradation of vitamin [9]. The sensitivity of B_{6} to irradiation is less than B_{1} and is similar to B_{2} [18]. The results of the present study about the effect of freezing and microwave treatment on B_{6} contents of both barberry varieties are similar to those of Siong [33], and Chandrasekaran et al. [9]. Vitamin B_{6} was reduced at the most doses of gamma in both B. integerrima and B. vulgaris.

The main reason of thiamin (B_{1}) reduction (sometimes up to 25\%) during blanching heat treatment is its leaching in water. Some chemical decompositions are effective as well. In foods, some components like proteins protect thiamine but its mechanism is not clear [33]. Storage at 4-6°C for 1 and 3 weeks caused decrease in thiamine up to 13 and 46\% in spinach, respectively. Also, green bean lost 23\% of its thiamin content after 3 weeks storage at 4°C [30]. After freezing of bean (Phaseolus vulgaris L.), its thiamin content decreased by up to 72-80\% [34] (Slupsky, 2012). The reduction of B vitamins like B_{1} during frozen storage will increase if the products are not blanched [33]. Thiamin is the most sensitive vitamin of B group to microwave treatment [27]. Oxidative degradation is responsible for the dose-dependent loss of vitamin B_{1} (the most sensitive vitamin of B group to irradiation). During the irradiation of B_{1}, reduction of its absorption spectra shows the destruction of its pyrimidine ring. Loss of the amino group is observed, which corresponds to the radiation dose of the produced ammonia. It is believed that the ammonia source is the sixth amino group of pyrimidine of thiamine, and is less likely to be supplied from the nitrogem of pyrimidine ring or thiazole [16]. Gamma irradiation greatly decreases the content of B_{1} (thiamine) in sorghum. When the products are irradiated in the presence of oxygen and moisture, reductions can be attributed to the formation of the primary very active free radicals from the products of water radiolysis or the secondary reaction products such as hydroperoxides, which probably attack the vitamins. The amounts of B\_1 losses can be reduced with some plans such as temperature decreasing at higher doses or elimination of oxygen from the ambient medium. Thiamine is more sensitive to temperature than irradiation [18]. The thiamin contents of both barberry cultivars decreased after all processing treatments, with the exception of a little increase (about 2\%) after freezing in B. integerrima. Therefore, the results of the present study are confirmed by the results of above mentioned studies.

Folic acid is sensitive to heat in acidic medium, light, and air. Folate decreases during heating because the heating breaks and leaches it into the water. Presence of reducing agents such as ascorbic acid may protect folate during thermal processing [23, 28]. Conventional cooking reduces folic acid by up to 50\%. Folate stability depends on the kind of food and the processing method. For instance, in meat products, folic acid will not be affected at sterilization temperature; and will decompose at near 190°C by losing glutamic acid after removal of the amid group. Folic acid has shown decreases during chilling; which is attributed to its metabolism at 4°C. This vitamin has good stability in frozen vegetables at -60°C. Sample texture plays an important role in folic acid protection [15]. Microwave cooking is preferred to conventional methods to preserve folate [23]. Folic acid is sensitive to gamma irradiation in fresh and moist textures, and shows decreases at the doses ≥ 2 kGy [24]. The results found in the present study about folic acid in both barberry varieties were in agreement with the above results, with the exception of the
Water-Soluble Vitamins of Barberry Fruits

Insufficient intake of vitamins is a risk factor in heart diseases, cancers, and osteoporosis [10]. In this study, the effect of conditions of extraction on the profile and concentration of water-soluble vitamins was studied for the first time. The results showed that in the descending order, solvent, variety, pH, temperature, and light have great effects on the extraction of water-soluble vitamins. The effects of thermal and non-thermal processes on the profile and concentration of water-soluble vitamins were also investigated. Contents of the studied vitamins were decreased after some treatments; however, they were increased after some other processes. Also, contents of some water-soluble vitamins did not change after conventional food processing.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Agriculture, Tarbiat Modares University, Iran.

REFERENCES


فلیک اسید در PET350 و PET1.550؛ بیوتین در AEL350 و PWL350؛ بیوتین در AET350 و بیوتین در AEL1.550. بیشترین و کمترین مقادیر ویتامین‌های محلول در آب در شرایط مختلف فراوری، ویتامین، ویتامین (کمترین) C (A\textsubscript{con} 460/87 (کمترین)، A\textsubscript{ref1} 87/146 (کمترین)، A\textsubscript{ref2} 100/88 (کمترین)، A\textsubscript{95} 2919/18 A\textsubscript{0.5} (B\textsubscript{3} (کمترین))؛ بیشترین، بیوتین در PWL350 تیستیتی و کوتزیتی ویتامین در آب در شرایط مختلف فراوری، بیترتیب، ویتامین (کمترین) B\textsubscript{5} (کمترین)، P\textsubscript{con} 96/242 (کمترین)، P\textsubscript{mic} 52/21 (کمترین))؛ بیشترین، بیوتین در AET350 و بیوتین در AEL1.550

B\textsubscript{1} (A\gamma 0.5 395/1700، A\gamma 10 11/947، P\textsubscript{con} 78/104، P\textsubscript{95} 76/12) تیستیتی B. vulgaris (پس از فراوری یافت شد)، بیوتین (کمترین) P\textsubscript{mic} 58/2267، A\gamma 10 63/1404، P\textsubscript{con} 72/324، P\textsubscript{95} 73/8) تیستیتی

B\textsubscript{2} (A\gamma 0.5 43/586، A\gamma 10 34/274، P\textsubscript{con} 19/297، P\textsubscript{95} 79/6) تیستیتی

B\textsubscript{5} (A\textsubscript{con} 00/2113، A\textsubscript{ref2} 09/965، P\textsubscript{con} 15/2298، P\textsubscript{95} 76/8) تیستیتی