

Research Article

Investigation of Some Biochemical Parameters in Fresh and Frozen *Allium ampeloprasum* and *Asphodelus aestivus* L

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ABSTRACT: In this study, analysis of the vitamins A, E, C and β -carotene, lycopene, ghrelin, oxidized and reduced glutathione (GSSG, GSH), 4-hydroxynonenal (HNE) and malondialdehyde (MDA) in fresh and frozen leek (*Allium ampeloprasum*) and wild leek (*Asphodelus aestivus* L.) were analyzed by High-Performance Liquid Chromatography (HPLC). It was found that the amounts of vitamins A, E, C and β -carotene in fresh leek were $(3.10 \pm 0.15; 33.00 \pm 2.30; 52.32 \pm 2.55$ and 10.10 ± 0.90 $\mu\text{g/g}$), ghrelin, GSH, GSSG, HNE and MDA were $38.00 \pm 2.40; 110.40 \pm 8.60; 22.60 \pm 1.00; 6.50 \pm 0.20$ and 0.96 ± 0.08 $\mu\text{g/g}$, respectively. On the other hand, in wild leek the amounts of vitamin A, vitamin E, vitamin C, β -carotene and lycopene were found to be $12.60 \pm 0.90; 88.40 \pm 6.50; 645.00 \pm 32.00; 57.90 \pm 4.00$ and 2.40 ± 0.15 $\mu\text{g/g}$, respectively, ghrelin, GSH, GSSG, HNE and MDA were found to be $69.40 \pm 4.80; 313.80 \pm 28.90; 42.30 \pm 1.70; 4.51 \pm 0.24$ and 0.64 ± 0.05 $\mu\text{g/g}$. These findings suggest that, wild leek is a better food source than the leek. On the other hand, freezing of leek and wild leek, have statistically no effect on the parameters examined ($p > 0.05$).

Keywords: *Asphodelus aestivus* L., *Allium ampeloprasum*, Vitamin, Ghrelin, Glutathione, Stress markers

INTRODUCTION

Leek is (*Allium ampeloprasum*) belongs to the Alliaceae family, includes garlic and onion also. Leek resembles large, flat-leaved plant and do not form bulbs or produce cloves, as opposed to onion and garlic [1]. Leek is known as a rich source of secondary metabolites, including organosulfur compounds. Which have been reported to have anticancer, antimutagenic, antiplatelet, antihyperglycemic, antidiabetic and antioxidant effects? The leek is reported to clean the kidneys and intestines, effectively clearing the blood in the body and the sand and stones in the kidneys [2]. *Asphodelus aestivus* L. is known as wild leek or çiriş and is a member of the *Asphodelaceae* family the genus *Asphodelus*. The leaves of the *Asphodelus aestivus* L. are similar to the leaves of the leek; however, it is rather small compared to leek [3]. Unless otherwise indicated, the term "wild leek" throughout this study is used for *Asphodelus aestivus* L. It has been reported that wild leek can be used in the treatment of diseases such as hemorrhoids, rheumatism, menstruation, diuretics, ringworm, milk increase, eczema, acne, stomach irritation and bone fractures [4]. It is also important for the defense system; as wild leek increases white blood

cells. Studies have shown that wild leek has antimicrobial and antioxidant effects as well [5].

Vitamins, some peptides and carotenoids are essential nutrients that are necessary for various biochemical functions and must be taken with the diet. Especially green plants are the main sources of these nutrients. The lack of vitamins in the diet causes various diseases like scurvy, beriberi, rickets and anemia [6].

Freezing is widely used method in the storage of vegetables and food stuff that prevents physical, chemical, and microbiological spoilage of food by turning the water in the food into ice crystals and ensures that the food remains durable for a long time [7]. Since wild leek is a seasonal vegetable, it should be frozen to consume in all seasons.

The study is based on a comparative investigation of the vitamins (A, E, C), carotenoids (β -carotene and lycopene), functional peptides (glutathione, ghrelin) and oxidative stress markers (GSSG, MDA, and HNE) in fresh and frozen leek and wild leek.

MATERIAL AND METHODS

Materials

In this study, fresh leek and wild leek obtained from the public market (Elaziğ), were used. After the samples were diagnosed at Firat University Biology Department, necessary analyzes were made. The samples were homogenized in a blender and used for the analysis. Fresh samples were analyzed as soon as possible (3 days), on the other hand, frozen samples kept at -20°C and analyzed after 15 days. Chemicals and equipment used by Bakar *et al*, (2019) [8], were also used in this study.

Determination of 4-hydroxynonenal, vitamin A, vitamin E, β -carotene and lycopene

To determine the amount of parameters, 2.0 g of homogenized leek or wild leek samples were taken. Then 6.0 mL of $\text{C}_2\text{H}_5\text{OH}$ was added and sonicated for 10.0 minutes. The suspension was vortexed and then centrifuged for five minutes at 6000 rpm. 1.0 mL n-hexane was added to the supernatant. Vitamins A, E, β -carotene, lycopene and HNE (4-hydroxynonenal) were extracted to the n-hexane phase. This extraction process was replicated twice. Then n-hexane phases were collected and dried under nitrogen stream until dryness. The residue obtained was dissolved in 1.0 mL CH_3OH and taken to HPLC vials then analysed. Supelcosil LC-18 column (25.0 cm x 4.6 mm x 5.0 μm) and CH_3OH : ACN: H_2O (63:33: 4.0) mixture were used as mobile phase at a flow rate of 1.0 mL/min [9-11].

Determination vitamin C, ghrelin, glutathione and malondialdehyde

2.0 Gram of homogenized leek or wild leek samples was taken. Then 6.0 mL of water was added and sonicated for 10.0 minutes. After sonication 1.0 mL of 0.50 M, HClO_4 was added to each homogenate to precipitate proteins and vortexed then centrifuged for five minutes at 6000 rpm. The supernatant was filtered and taken into 1.0 mL of HPLC vials. These parameters were determined according to modified method of Ibrahim, *et al*, (2017) [11] by HPLC (Exsil column 100-5 ODS 25 cm, 4.6 mm ID, 5 μm), flow rate of 1.0 mL/minute).

Statistical Analysis

Statistical analysis was carried out on four different samples in parallel. A pc computer was used for variance analysis and LSD multiple comparison test was performed at $p < 0.05$ level.

DISCUSSION AND CONCLUSION

Plant-derived vitamins are an important source for the healthy life. They are essential for metabolism because of the redox chemistry and they act as enzymatic cofactors not only in animals but also in plants (Asensi-Fabado and Munne-Bosch, 2010). In this study, the levels of some biochemical

parameters in fresh and frozen leek and wild leek were investigated and all results are given in **Figure 1 and 2**.

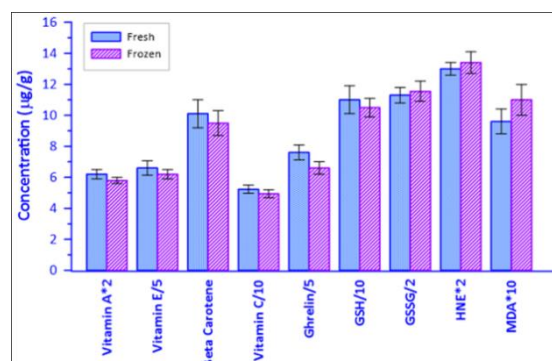


Figure 1. Vitamins A, E, C, β -carotene, ghrelin, GSH, GSSG, HNE and MDA content in fresh and frozen leek (for clarity and to bring into scale; vitamin E and ghrelin values divided by 5, GSSG values divided by 2, GSH and vitamin C values divided by 10, vitamin A and HNE values multiplied by 2, MDA values multiplied by 10).

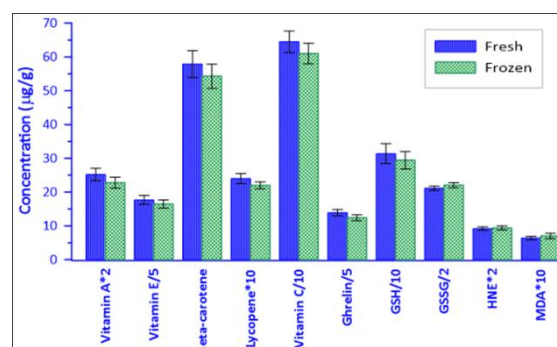


Figure 2. Vitamins A, E, C, β -carotene, lycopene, ghrelin, GSH, GSSG, HNE and MDA content in fresh and frozen wild leek (for clarity and to bring into scale; vitamin E and ghrelin values divided by 5, GSSG values divided by 2, GSH and vitamin C values divided by 10, vitamin A and HNE values multiplied by 2, lycopene and MDA values multiplied by 10).

Vitamin A has an important function such as normal functioning of the visual system, cellular growth with development and maintenance of epithelial cellular integrity and immune function [12]. While the amount of vitamin A in fresh and frozen leek samples were found to be 3.10 ± 0.15 and 2.90 ± 0.10 $\mu\text{g/g}$, respectively. The same figures for wild leek were 12.60 ± 0.90 and 11.40 ± 0.80 $\mu\text{g/g}$ dw, respectively (Figure 1 and 2). In a study conducted by Unal *et al*, (2016) [5], reported that the amount of vitamin A in *Asphodelus aestivus* L. ranged from 4.6 ± 0.5 to 9.7 ± 1.7 mg/kg. While vitamin A in leek was found lower than the literature value of 5.0 $\mu\text{g/g}$ (Kiple and Ornelas, 2000), wild leek has higher vitamin A content.

Vitamin E plays a crucial role in protecting molecules and tissues from free radicals. As seen in **Figures 1 and 2**, the amount of vitamin E in fresh leek and wild leek found to be 33.00 ± 2.30 and 88.40 ± 6.50 $\mu\text{g/g}$, respectively while, the amounts of vitamin E in frozen leek and wild leek found to be 31.00 ± 1.50 and 82.20 ± 5.80 $\mu\text{g/g}$, respectively (**Figure 1 and 2**).

The amount of vitamin E in leek has been reported to be 10 $\mu\text{g/g}$ [13]. Santos *et al.*, (2012) [14] reported the amount of vitamin E in Swiss chard, watercress, spinach, spearmint, garden cress were 1.2, 2.5, 2.9, 1.4 and 1.9 mg/100 g fw, respectively.

β -carotene is a precursor of vitamin A that is found in plants, fruits, and vegetables which is a red-orange pigment of plants and vegetables. While the amount of β -carotene in fresh leek was found to be 10.10 ± 0.90 $\mu\text{g/g}$, 57.90 ± 34.00 $\mu\text{g/g}$ in wild leek respectively. Whereas the amount of β -carotene in frozen leek was 9.50 ± 0.80 and 54.30 ± 3.60 $\mu\text{g/g}$ in wild leek (**Figure 1 and 2**). The amount of β -carotene in leek and *Mentha pulegium* L was determined as 10.0 and 1.24 ± 0.14 $\mu\text{g/g}$ respectively [13,15]. In the study conducted by İsmail and Fun (2003) [16], the amount of β -carotene in lettuce was reported as 2006 $\mu\text{g}/100$ g fw.

The harmful effects of free radicals are reduced or eliminated by some substances such as lycopene [17]. While the lycopene was not found to be in the leek, on the other hand it was found to be 2.40 ± 0.15 and 2.20 ± 0.10 $\mu\text{g/g}$ in fresh and frozen wild leek, respectively (**Figure 2**). It has been reported that the amounts of lycopene in different vegetables and fruits varies between 0.02 with 1902 $\mu\text{g/g}$ [18].

Vitamin C is a powerful antioxidant that plays an important role in many physiological processes and is necessary for the repair of tissues [19]. While vitamin C in fresh and frozen leek were found to be 52.32 ± 2.55 and 49.40 ± 2.50 in the wild leek 645.00 ± 32.00 and 610 ± 30.40 $\mu\text{g/g}$ (**Figure 1 and 2**). Balan *et al.*, (2016) [20] reported that the amounts of vitamin C in fresh spinach, broccoli, green beans and peas were 35.37, 34.45, 15.20 and 21.20 mg/100 g fw, respectively. It has been reported that the amount of vitamin C in lettuce, watercress, spinach, peppermint, garden cress is 0.1, 59.6, 14.4, 0.5, 10.1 mg/100 g fw, respectively (Santos *et al.*, 2012)[14].

Ghrelin has affect the brain to regulate the modulation of glucose metabolism, also improves cardiovascular functions. In addition, its antioxidant effect has been demonstrated by the increase in the total antioxidant capacity of blood in ghrelin treated rats [21].

As seen in Figures 1 and 2, the amount of ghrelin in fresh and frozen leek was found to be 38.00 ± 2.40 and 33.00 ± 2.00 $\mu\text{g/g}$ while, in fresh and frozen wild leek were 69.40 ± 4.80 and 62.00 ± 4.400 $\mu\text{g/g}$. Ibrahim *et al.*, (2017) [11] reported that the ghrelin content in fruits of *Crataegus laevigata* ranged from 19.96 to 79.96 $\mu\text{g/g}$.

Glutathione, which is necessary for the immune system of the cells, has a peptide structure is the most important intracellular antioxidant molecule, also involve in the transport of amino acids in metabolism and reduction of sulfhydryl groups in proteins [22].

While the amount of GSH in fresh and frozen leek was found to be 110.40 ± 8.60 and 105.00 ± 6.00 $\mu\text{g/g}$, the amount of GSSG was found to be 22.60 ± 1.00 and 23.10 ± 1.30 $\mu\text{g/g}$ respectively. Likewise, the amounts of GSH in fresh and frozen wild leek were found to be 313.80 ± 28.90 and 294.50 ± 26.20 $\mu\text{g/g}$, the amount of GSSG was found to be 42.30 ± 1.70 and 44.10 ± 1.50 $\mu\text{g/g}$. (**Figure 1 and 2**). Demirkol *et al.*, (2004) [23] reported that GSH amounts in spinach, broccoli, parsley and green beans were 313 ± 33 , 4 ± 1 ; 17 ± 9 and 230 ± 2.0 nM/g fw) respectively.

The amount of GSH in the leaves of *Mentha pulegium* L. was found to be 185.71 ± 10.61 $\mu\text{g/g}$, while the GSSG was reported to be 280.48 ± 24.58 $\mu\text{g/g}$ (Çötelı *et al.*, 2013) [15]. Leek also contains significant amount of lutein, β -carotene, vitamin C and vitamin E [24]. In the study conducted by Ünal and Kaptan (2016) [5], the antioxidant capacity of the çiriş (wild leek) was found to be high. According to our findings, antioxidant compounds such as vitamins A, E, β -carotene, lycopene, ghrelin and GSH in wild leek were found higher than wild leek. As a result, it can be said that the results of the two studies support each other. According to the findings, the nutritional value of the wild leek was determined to be higher than the leek ($p < 0.05$). This may be explained by the seasonal factors, harvest time, environmental factors, and growing conditions.

4-Hydroxynonenal (HNE), the main secondary oxidation product of lipid peroxidation, is believed to be the second predictor of oxidative stress [25]. As seen in Figures 1 and 2, the amount of HNE in fresh and frozen leek was found to be 6.50 ± 0.20 and 6.70 ± 0.35 $\mu\text{g/g}$ in fresh and frozen wild leek as 4.51 ± 0.24 and 4.70 ± 0.30 $\mu\text{g/g}$ (**Figure 1 and 2**).

Free radicals cause lipid peroxidation by affecting the unsaturated fatty acids in the membranes. As a result of lipid peroxidation, MDA occurs, which is a stress biomarker (Gaweł *et al.*, 2004) [26]. In a fresh and frozen leek the MDA levels

were found to be 0.96 ± 0.08 and 1.10 ± 0.10 $\mu\text{g/g}$, respectively. The similar results observed for the wild leek also which were 0.64 ± 0.05 and 0.70 ± 0.08 $\mu\text{g/g}$, respectively ($p < 0.05$) (**Figure 1 and 2**)

Another stress biomarker was GSH/GSSG ratio. The stress is inversely proportional to the ratio of GSH/GSSG and is proportional to the amount of MDA and HNE. GSH/GSSG ratio was found 4.89 and 4.55 in fresh and frozen leek. The similar values were found as 7.42 and 6.68 for fresh and frozen wild leek. From the obtained results of HNE, MDA and GSH/GSSG values of leek and wild leek, it can be said that the leek is under more stress than wild leek which grows under natural conditions. The excessive stress in leek may be due to environmental conditions and chemicals used such as fertilizers and pesticides used.

Although all values measured in both leek and wild leek are negatively affected as a result of the freezing process, the changes that occur are statistically insignificant ($p > 0.05$).

Since the chemical and biochemical reactions do not stop completely in frozen fruits and vegetables, losses occur in the nutritional properties of the product. These losses arise from enzyme activity at low temperature as in other quality losses as well (Demiray and Tülek, 2010) [7].

CONCLUSION

Wild leek is richer than the leek in terms of the nutritional values examined ($p < 0.05$). A negative change in all parameters was observed as a result of the freezing process and these changes are statistically insignificant ($p > 0.05$). As a result, it has been observed that storing vegetables by freezing does not have a negative effect on nutritional value. According to the findings obtained (GSH/GSSG, MDA, and HNE), it can be said that wild leek is under less stress than the leek. It can be said that vegetables grown in natural conditions are better choice to consume in terms of nutritional value.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethical approval: The study was approved by the Institutional Ethics Committee of Firat University.

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