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# Studies on Antimicrobial Activity and Certain Chemical Parameters of Freeze-Dried Wild Plums (*Prunus* Spp.)

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**Abstract:** The freeze-drying was applied to increase the shelf life and to preserve the quality of the wild plum (*Prunus* spp.), an economically important fruit and it has almost the same nutritional quality when compared with the fresh ones. Certain chemical and physical properties of the wild plums were investigated like vitamin C content, pH, titratable acidity, total sugar content, soluble solids, fat and ash content, anthocyanins etc. and it was found that no significant changes were observed when compared with fresh. When its antimicrobial property was tested on various food borne pathogens; the rehydrated extract inhibited important species like *Klebsiella pneumoniae* and *Escherichia coli* 0157: H7-933. The inhibitory substance was characterized as benzoic acid. While the concentrated strong acid, weak acid and strong base treatment (1 N) significantly effected the anthocyanin pigments, a treatment of 0.1 N showed no effect. The freeze-dried and the freeze-dried and rehydrated group of wild plums were found to be the most preferred ones by the taste panelists ($p<0.01$).

**Key words:** Wild plum, freeze-drying, antimicrobial activity

## INTRODUCTION

The wild plum (*Prunus* spp.) is one of nature's most unique fruits, reported to be growing extensively in some areas of the world such as at the edges of Oregon, California and Nevada's northern high desert at altitudes between 4000 and 7000 feet in US, in the deep forests-semi arid transition zone of Balkan peninsula, Black Sea and Central-East Anatolia in Turkey, tolerating great extremes of heat, cold, alkaline soils and drought. In its native state, the wild plum grows as a large bush reaching five to six feet tall (Özbek, 1978). Wild plums are used mainly in the industry for its juice and its kernels for extracting oil with an oxidative stability (Picuric-Jovanovic *et al*., 1999).

The wild plum has an economic importance in Turkey as it is mainly processed as jams or compote, especially in the rural areas of Black Sea region, therefore the production rate is around 8000 tonnes and the annual export rate is slightly above 4000 tonnes/year, depending on the climatic conditions. In last years, together with other berries, dried wild plums are also in use in beverage industry to provide a different aroma.

Wild plums are reported to be at the size of cherries or golf balls. The colour of the skin is diverse; it can be deep vivid red, glowing orange, bluish crimson, bright red, bright yellow or dark yellow. All are smooth skinned, hard-pitted drupes, with yellow juicy “meat”, rich of pectic substances within a round, oval, conical, or heart-like shape. Wild plums have a high anthocyanin content are rich in K, Ca, Mg, P, S and Na ions. The fruit has a distinctive tart flavor that makes it an attractive to several insects (Wesche-Ebeling *et al*., 1996; Leskey and Prokopy, 2000; Leskey and Prokopy, 2001; Calisir *et al*., 2005).

The major problem arising in the food industry for fruits is mainly reported as the long term storage. Different methods have been in use to attempt an extension in shelf life, but most of them are including chemical preservation which may cause toxic and adverse effects, and sometimes they may be inadequate. Such inadequate conditions during storage leads to spoilage, nutrient loss and structural defects in fruits. The freeze drying process offers numerous advantages for the protection of natural and additive-free products. Since the water is removed-the taste, colour, texture and nutritional content of the food remains and a dried product with a characteristic aroma is obtained in freeze-drying process. The end product’s weight is reduced by over 90%. The rehydration process allows the fruit to be ready in short times having its nutritional quality, almost equal to the fresh samples by providing an extended shelf life without additives or preservatives (Iwaniw and Mittal, 1987; George and Datta, 2002; Cui *et al*., 2003; Martinez-Romero *et al*., 2003; Valero *et al*., 2003). The innovative action in drying of the wild plums is mainly centered on shortening of drying time and reduction of thermal damage to produce a high quality dried fruit (Di Matteo *et al*., 2002). In addition, the browning reaction by the catalytic action of the Polyphenoloxidases (PPO) in plums are inhibited by the application of freeze-drying (Weemaes *et al*., 1998).

This study aims to investigate the effect of freeze-drying on some important nutritional and antimicrobial characteristics of the wild plum species, mainly as

ingredient. In general, the freeze dried wild plum has a chance to serve as a preservative and flavour agent or additive for the food industry due to the experimental findings presented in this study, also supported by statistical and sensory evidence.

## MATERIALS AND METHODS

**Fruit samples:** A 30 kg weight of wild plums were harvested during early summer (2004) in Elmalik region of Bolu, Turkey. Wild plums were immediately transported to the laboratory for experimental analysis in sealed plastic bags. The defective ones were eliminated as they were damaged or bruised prior to the experiments and the fruit was manually destoned under running tap water for preparation of quick freezing at -80°C. Three different samples were studied from the fresh, freeze-dried and rehydrated plums.

**Freeze-drying:** The freeze-drying process was carried out by a freeze-drier (Heto, type FD 6) for 24 h (shelf temperature: 30°C and condenser temperature: -45°C) and a pressure of 0.10 hectopascals (hPa) (Litvin *et al*., 1998), after a deep-freezing process at -80°C at once; for a period of 24 h. No additives or preservatives were added to the fruits before or after freeze-drying and the lyophilized wild plums were kept in sealed vials for further analysis.

**Vitamin C content:** The ascorbic acid content of fresh (1 mg/mL) and rehydrated (1 mg/mL) wild plums was analyzed without subsampling by 2,6-dichloroindophenol titrimetric method in triplicates (Anonymous, 2000a) and the results were expressed as milligrams of ascorbic acid/100 g sample ($p<0.01$).

**pH detection:** The pH values of both fresh(1 mg/mL) and freeze-dried (1 mg/mL) samples were measured without subsampling by a pH meter (Jenway Scientific model-3505), supported by a solid electrode in triplicates ($p<0.01$).

**Titratable acidity:** In order to measure the titratable acidity, fresh wild plum samples (1 mg/mL), in triplicates, were first blended to homogenicity (Waring Commercial Blender Model 32BL80, USA) for 5 min at the highest degree of speed [setting blender to 2, revolution number: 200 rpm (revolution/minute)]. Subsequently 1 mL of fruit juice was diluted to 5 mL with distilled water and a 2.5 mL sample from this solution for subsampling was titrated with 0.1 N NaOH to a pH of 8.1. The freeze-dried group was subjected to titratable acidity determination via rehydrating a corresponding amount of 1 mg wild plum by 1 mL water, centrifuged and the micro-filtered juice was subjected to 5 mL dilution with distilled water and a 2.5 mL sample from this solution as subsampling; was titrated with 0.1 N NaOH to a pH of 8.1. The results were expressed as malic acid (%) ($p<0.01$).

**Total sugar content:** Total sugar content of the fresh (1 mg/mL) and the freeze-dried (1 mg/mL) wild plums were analyzed in triplicates by HPLC (LKB-Bromma model 2150, equipped with a differential refractometer-KNAUER and Shimadzu C-R-4 Chromatopack monitor). The samples were homogenized (Waring Commercial Blender Model 32BL80, USA) for 5 min at the highest degree of speed (setting blender to 2, revolution number: 200 rpm) and then centrifuged at 5000 rpm for 15 min and subjected to filtration from 0.45 μ pore-size filter (Millipore). The column used in this experiment was a Phenomenex Rezex Cal Monosaccharide C-18 silica gel column with a size of 300 x 7.8 micron, having an attenuation parameter of 2, flow rate = 0.45 μL/min, oven temperature = 55°C with a mobile phase of methanol ($p<0.01$) (Yurdugul, 2002).

**Firmness:** Freeze-dried and fresh wild plums were randomly selected for firmness measurements, by a fruit hardness tester in triplicates (Everwell, Model FT011, Japan). The values were obtained from the same four points on the circumference of each fruit and results were stated in average values in kg ($p<0.01$).

**Water content:** The water content of the fruits were measured according to the following modified method (URL 1):

The plums are washed, dried and cut into two parts. The core and seeds of the plums were removed and the remaining slices were weighed by a precise balance. The slices were spreaded in a single layer on a wire cooking rack, supported on a cooking sheet. The sheet was placed into an oven set to 102°C. When the slices were wrinkled and dried in time, the sheet was removed, and allowed to cool, then the dried pieces were carefully placed on the precise balance and weighed again.

The dry weight was divided to wet and the result was multiplied by 100. The number was subtracted from 100 to get the percent water that was present in the original wild plum.

**Rehydration:** The freeze-dried fruits were rehydrated as 1:10 ratio by sterile water at room temperature (25°C) under normal atmospheric pressure (1 atm).

**Rehydration capacity:** To determine rehydration capacity, uniform dried fruit pieces as cube, were filtered through a filter paper (Whatman No: 2) under a slight vacuum for a minute and weighed. The rehydration capacity can be expressed as the weight ratio between the rehydrated sample and the sample before rehydration; expressed in grams (Prothon *et al*., 2001).

**Soluble solid content:** The soluble solid content was measured for fresh (1 mg/100 mL) and the rehydrated (1 mg/mL) wild plum juice by a refractometer (Carl Zeiss Jena, DDR 818408) in triplicates after homogenization of the samples (Waring Commercial Blender Model 32BL80, USA) for 5 min at the highest degree of speed (setting blender to 2, revolution number: 200 rpm/min), and the results were expressed as °Brix by weight ($p<0.01$).

**Fat content:** The fat content of the fresh and freeze-dried wild plums were determined by the slightly modified Bligh-Dyer Method (Bligh and Dyer, 1959) in triplicates, which allows the homogenization of the sample with a mixture of $CHC1_3$ and $CH_3OH$, forming a miscible system with the water already present in the sample, by a 50 g comminuted wild plum sample, for both fresh and rehydrated; blended with 100 mL methanol and 50 mL chloroform mixture following subsequent blendings of 50 mL of chloroform, filtering, pressing cake to remove solvents, rinsing, funneling, pressing cake with 15-20 mL of Chloroform: Methanol (1:1). After rinsing the extract with 5-10 mL of Chloroform:Methanol (1:1), allowing contents to separate overnight, chloroform layer was slowly drawn off by recording volume. Three aluminium weighing dishes are preweighed to 0.001 g and 10 mL of chloroform solution sample is added onto each of the three preweighed dishes and the chloroform is evaporated to dryness in a Speed Vac (for approximately 1 h) until lipid residue remains. The dishes containing residues are placed in oven set at 103-105°C for 1 h and cooled to room temperature in a desiccator for 15 min. The dry samples and dishes are weighed to nearest 0.001 g ($p<0.01$).

**Ash content:** The ash content of the fresh and freeze-dried wild plums was detected by the gravimetric procedure in triplicates (Anonymous, 2000b). 1.0 g of wild plum sample was weighed into a porcelain crucible at constant weight. The fruit was first dried in an oven at 100°C for 1 h. The crucible was then placed on a hotplate and the dried sample was charred until no smoke was generated. After cooling the crucible in a desiccator, it was reweighed and % ash was calculated as:

% ash = weight of residue x 100 / weight of sample ($p<0.01$)

**Anthocyanin content:** The total anthocyanin content of both the freeze-dried and fresh samples were monitored using the pH-differential spectrophotometric method in triplicates (URL 2), which was performed by the difference of absorbance, at pH 1.0 and pH 4.5. Difference in absorbances between the two samples was calculated using the following equation in order to quantify the anthocyanin content ($p<0.01$):

$$\text{Absorbance} = (A_{510nm}\ \text{pH 1.0} - A_{700nm}\ \text{pH 1.0}) - (A_{510nm}\ \text{pH 4.5} - A_{700nm}\ \text{pH 4.5})$$

The %w/w of total anthocyanins in the sample were calculated as:

$$\%\,w/w = \frac{A}{wt} \times MW \times DF \times \frac{V}{\varepsilon \times L} \times 100\%$$

where: A = Absorbance, ε = Cyd-3-glu (based on cyanidin-3-glucoside, the most common anthocyanin for berries) molar absorbance (26.900), MW = anthocyanin molecular weight (449.2), DF = dilution factor (1 for wild plums in this study), V = final volume (1 mL), Wt = sample weight (mg), L = cell pathlength (1 cm).

**Effect of acid and base treatment on the freeze-dried and fresh wild plums:** Solutions of 0.1 and 1 N NaOH, 0.1 and 1 N HCl and 0.1 and 1 N benzoic acid were added, inspired and adapted from the modified acid treatment methods of Di Matteo *et al.* (2002) without methanol onto fresh and rehydrated samples of wild plum juice in triplicates (1 mg/mL) and the color change was monitored by a spectrophotometer (Pharmacia) at 530 nm ($p<0.01$). The aim of carrying out such kind of an experiment is mainly to investigate the behaviour and compatibility of wild plum in acidic or basic food items.

**Antimicrobial activity:** The antimicrobial activity of freeze-dried wild plums were tested against 18 well-known pathogenic; randomly chosen strains in triplicates including: *Klebsiella pneumoniae*, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeroginosa* ATCC 27853, *Salmonella enteritidis*, *Acinoto iworfii* ATCC 19002, *Klebsiella oxytoca* ATCC 19086, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Bacillus coagulans*, *Enterobacter faecium* ATCC 6057, *Enterobacter faecium* ATCC 2922, ß-*hemolytic Streptococci spp.*, *Escherichia coli*, *Escherichia coli* ATCC 35218, *Streptococcus thermophilus*, *Escherichia coli* 0157: H7-931, *Escherichia coli* 0157: H7-933, *Candida albicans* ATCC 14053 (Table 1). Strains were obtained from The Military Medical Academy of Gülhane, Ankara, Turkey. 1 mg of freeze-dried wild plum were dissolved in 1 mL sterile distilled water and a 10 µL aliquot was dropped onto the sterile filter paper which was placed on the plate containing nutrient agar prior to the experiment (MERCK) and incubated for 48 h at 37°C ($p<0.01$) (Bonner and Mitruka, 1976).

**The characterization assay for the components of the wild plum:** For this purpose, in triplicates, 1 mL/mg freeze-dried wild plum was rehydrated and a subsequent centrifugation was performed at 5000 rpm following a micro filtration process through an 0,45 µ micro-filter. The rehydrated wild plum solution was then

Table 1: The activity spectrum of the freeze-dried wild plums against several important food borne pathogens. *(+: <2 mm,++: 2-4 mm, +++: >4 mm inhibition zone diameter; (-) sign: no inhibition zones)

| Microorganism | Activity test (+) or (-)* |
|---|---|
| *Klebsiella pneumoniae* | ++ |
| *Klebsiella pneumoniae* ATCC 13883 | - |
| *Pseudomonas aeroginosa* ATCC 27853 | - |
| *Salmonella enteritidis* | + |
| *Acinoto iworfii* ATCC 19002 | ++ |
| *Klebsiella oxytoca* ATCC 19086 | + |
| *Staphylococcus aureus* ATCC 25923 | + |
| *Bacillus subtilis* | + |
| *Bacillus coagulans* | + |
| *Enterobacter faecium* ATCC 6057 | ++ |
| *Enterobacter faecium* ATCC 2922 | - |
| *ß-hemolytic Streptococci spp.* | - |
| *Escherichia coli* | + |
| *Escherichia coli* ATCC 35218 | - |
| *Streptococcus thermophilus* | - |
| *Escherichia coli* 0157: H7-931 | + |
| *Escherichia coli* 0157: H7-933 | ++ |
| *Candida albicans* ATCC 14053 | - |

eluted through Sephadex gel chromatography (G-200) (Sigma Chemical Co.) 5 g of Sephadex G-200 (SIGMA) was suspended in 50 mM phosphate buffer solution (Sigma Chemical Co., pH = 7.4) and left for overnight swelling. The gel suspension was loaded onto a column which has a dimension of 50 cm X 1.5 cm. 0.5 mL samples were loaded into this column, eluted with phosphate buffer (Sigma Chemical Co., pH = 7.4). 5 mL fractions were collected by using a fraction collector (LKB-Bromma) (Arinç, 1985; Plummer, 1987). The collected fractions were then checked for antimicrobial activity in the lawn cultures of the eighteen pathogenic strains by applying the sterile filter paper disc method was applied to monitor the inhibitory effect of samples (10 µL) as zones on the lawn culture, incubated at 37°C for 48 h ($p<0.01$).

The characterization assay for the components of the wild plum were carried out by high-pressure liquid chromatography (LKB-Bromma model 2150 equipped with a KNAUER differential refractometer, Shimadzu C-R-4 Chromatopack monitor and Phenomenex Rezex Cal Monosaccharide column, 300 X 7.8 µ; under 55°C with a mobile phase of 0.01% acetonitrile-methanol solution).

**Taste panel:** Twenty previously trained panelists rated the freeze-dried and the fresh wild plums according to texture, taste, color, odor, appearance, pungency, flavor, juiciness and hardness criteria (Table 2 and 3), with a 10-point scale per replication. 10 represented the most-liked score, 6-8 for the marketable level and 1 corresponded to the least liked score. Than the scores were statistically evaluated and categorized from 'a'-the best to the worst 'd'. ($p<0.01$).

**Statistical evaluation:** The statistical evaluation was performed by multivariate two-way Anova software (MS Office) program ($p<0.01$).

## RESULTS AND DISCUSSION

Most of the nutritional characteristics of the fresh wild plums were recovered when freeze-drying was applied ($p<0.01$ for all analysis). The fresh plums were found to contain 17.0±0.03 mg of vitamin C in 100 g fruit while the vitamin C in the freeze-dried samples were found to be 15.9±0.06 mg/100 g which shows that freeze-drying does not decrease the vitamin C content. Both of these values are in the range of good vitamin C source for the fruits, but other certain common high vitamin C containing fruits like tropical guava (*Psidium guajava*, 183 mg/100 g), green kiwifruit (*Actinidia deliciosa*, 98 mg/100 g), melon (*Cucumis melo*; 42 mg/100 g) and rosehip (*Rosa pomifera* cv 'Karpatia'; 1500 mg/ 100 g) (Gitelson *et al*., 2001) are regarded as the excellent and very good vitamin C sources when compared with the wild plums.

The pH value of the rehydrated freeze-dried samples was measured as 3.8±0.02, more or less preserving the acidic characteristic of the fresh fruit, having a pH around 3.6±0.01. The total sugar content of freeze-dried wild plums was found to be 50±0.002%, indicating also the preservation of the 53±0.041% sugar concentration in fresh wild plums.

Although the freeze-drying allows almost 90% water loss from the fruit, which was calculated by weighing and comparing the freeze-dried samples with the fresh ones, it provides a concentrated and stable product without microbial spoilage. The water content of the fresh wild plums were found to be 93.5%, indicating a high amount of water, which allowed the fresh fruits prone to microbial attack, therefore the freeze-drying process allows no water activity, meaning no microbial spoilage.

Firmness is among one of the main concerns of this study, especially to the freeze-dried food item, as it provides the main texture criteria in the mouth, significantly altering the attractivity of the consumer. Firmness values, obtained from different outer and inner parts of the skin of the freeze-dried wild plum fruit indicated 0.59±0.01, 0.62±0.02, 0.65±0.01 kg/f, compared to the values of the fresh wild plum which was obtained as 0.35±0.01, 0.40±0.01 and 0.43±0.01 kg/f. Due to the water removal, the firmness was increased.

The soluble solid content of the both fresh and lyophilized wild plums is 6.0±0.03, expressed as °Brix, showing us that sucrose and other solids, measurable by a refractometer comprise a limited portion of the wild plum juice, giving it a 'sour' taste. This much amount of soluble solid content, which is an important factor determining eating quality of fruit is an expected and

Table 2: Statistical Data for Sensory Evaluation of Freeze-Dried Wild Plums ('a' indicates the best qualified,'d' indicates the least) ($p<0.01$)

| Applications | Hardness | Texture | Taste | Color | Odor | Appearance | Flavor |
|---|---|---|---|---|---|---|---|
| Freeze-dried | b | c | a | a | a | a | ab |
| Control(fresh) | a | cb | ab | ab | ab | ab | b |

Table 3: Statistical data for Sensory Evaluation of Rehydrated Wild Plum('a' indicates the best qualified,'d' indicates the least qualified) ($p<0.01$)

| Applications | Juiciness | Foaminess | Taste | Color | Odor | Appearance | Flavor |
|---|---|---|---|---|---|---|---|
| Rehydrated | ab | cd | a | a | a | a | a |
| Control (fresh squeezed) | a | d | b | ab | ab | a | ab |

feasible result, as most of the fruits has a soluble solid content at around 0-15 interval. The fat content was calculated as 0.1±0.01% in freeze-dried wild plums, that was almost the same as the fresh wild plums. Fat here might be mostly unsaturated fatty acids, which are dominant in such fruits like apple and rosehip (Cefarelli *et al*., 2005). The fat content shows us that the wild plum, as fresh or freeze-dried has beneficial properties with its very low fat content and it can be recommended to high cholesterol-preventing diets. The ash content of the fresh and the lyophilized wild plums was calculated as 0.61±0.047 g for 100 g and 1.12±0.023 g for 100 g respectively, as the slight difference may be due to the great reduction of moisture content due to drying. Although freeze-dried samples removed a high quantity of water, no great difference was observed in ash content, therefore it may imply that several minerals present in the fresh fruit like $Ca^{2+}$, $Mg^{2+}$ are mostly retained (Calisir *et al*., 2005). Titratable acidity was found to be 0.86±0.02 (%) for fresh and 0.62±0.013 (%) for the freeze dried wild plums, measured for testing for adulteration with water.

The antimicrobial activity of the freeze-dried wild plums were studied because there is a folk belief around the area of the harvest (Elmalik region, Bolu, TURKEY) that this particular species of wild plums are effective in treatment of diarrhea. It has been observed that a 1 mg/mL amount of the rehydrated wild plums has a potent efficacy on certain important food borne pathogens (Table 1), including *Klebsiella pneumoniae, Acinoto iworfii* ATCC 19002, *Enterobacter faecium* ATCC 6057 and *Escherichia coli* 0157:H7-933, indicated by clear zone formation around sterile filter paper discs ($p<0.01$) as + sign indicates <2 mm, ++ sign indicates 2-4 mm, +++ sign indicates >4 mm inhibition zone diameter; whereas (-) sign informs that no inhibition zones are present. In order to characterize the antimicrobial substances in the wild plums, Sephadex G-200 gel chromatography and a HPLC assay was applied; subsequently the activity of the fractions were assayed on nutrient agar containing petri plates to observe the inhibitory effect. It was found that; among the peaks obtained in HPLC, benzoic acid showed good inhibitory action against *Klebsiella pneumoniae, Acinoto iworfii* ATCC 19002, *Enterobacter faecium* ATCC 6057 and *Escherichia coli* 0157:H7-933; confirming the results obtained above. Benzoic acid was reported to be an inhibitor, present naturally in certain fruits including cranberries, prunes, raspberries, cloves, cinnamon, plums and other fruits and vegetables and it was also used as a food preservative (Wiley, 1994).

When the taste panel results were considered (Table 2 and 3), there was not so much significant difference between the fresh and the freeze-dried wild plums in all criteria assessed by the panelists, but in general the freeze-dried and the freeze-dried and then rehydrated samples were preferred by the taste panel instead of the fresh (the control) ($p<0.01$). When the hardness values of the freeze-dried wild plums were considered, it was harder than the fresh fruit so the fresh one was preferred by the panelists. In order to show the statistical difference between groups, a lettering system was used after the evaluation of the panelists ranging between 1(the least preferred) to 10 (the most preferred), as 'a' which indicates the best score; then b, c and d comes; in a descending order to the worst score 'd'. Also there were scores between these letters e.g. 'ab' which means a slightly worse score than 'a'; but slightly better than 'b'.

It was expressed by the letter 'a' for the fresh in the Table 2 which means it was evaluated as the best score and 'b' expressed the statistical difference for the freeze-dried which indicated a lower score than the fresh one by the panelists. The control (fresh) also indicated a good texture while it was on the tongue than the freeze-dried, scored slightly well by the panelists as 'cb' shown by the statistical expression. This was mostly dependent on the crispy nature of the freeze-dried wild plums which were scored as 'c', lower than the score of the fresh (control) wild plums as 'cb'. As since the freeze-drying concentrated the wild plums by allowing water removal in the freeze-drying; rated by the panelists; it has a good taste, a good color (more reddish) and a good odor when compared to the fresh one. That's why in the taste, color and odor category of rating it was slightly expressed better as 'a' while the fresh wild plum (the

control) got slightly a low score of 'ab' in Table 2. The appearance of the freeze-dried plums were rated as better with the indication by letter 'a' than the fresh fruits which were scored as 'ab', as red color dominates due to the concentration of the wild plums increasing the attraction of the freeze-dried ones. The flavor of the freeze-dried wild plums was found to be much more acceptable when compared with the fresh wild plum as since, statistically a high score was given to the freeze-dried group indicated as 'ab' when compared with the lower score of 'b' of the fresh wild plums.

When the freeze-dried fruits were rehydrated (1 mg/mL) some criteria (i.e.juiciness, foaminess, color, odor, flavor) were slightly better rated than the control (the fresh fruit). The juiciness score was 'ab' which means a slightly better score than 'b' stating that the rehydrated ones were much more juicy than the fresh squeezed wild plum; the foaminess was evaluated as 'cd' which means a slightly better score than 'd' claiming that the rehydrated one was less foamy than the fresh squeezed one (the control). The color, odor and the flavor was rated as 'a' for the rehydrated ones, which was slightly rated as better than the fresh squeezed wild plum juice which was scored slightly low, as corresponding to 'ab' in statistical means. The panelists rated the appearance as 'a'; meaning the best, for both the rehydrated and the fresh squeezed wild plum juice statistically. The taste was rated as 'a' for the rehydrated ones when compared with the lower score 'b' of the fresh squeezed. This was typically due to the delicious and dark red juice formed, during the rehydration process.

The acid and base tests were done in order to observe the behaviour of the wild plums in acidic or basic food material as an ingredient and the compliance with certain food additives like benzoic acid. Effect of pH changes on anthocyanins for the rehydrated freeze-dried wild plums are given in (Fig. 1, 2 and 3). The expected color change was mainly due to the degradation of anthocyanins by strong acid and base when it was used in the concentrated (freeze-dried) form. When pH was decreased the anthocyanins in the rehydrated solution were degraded.

Anthocyanin content of the wild plum fruits, as a functional food item, were studied in order to benefit them in food industry, as it was regarded an appealing characteristic to consumers due to its antioxidative, free-radical scavenging, anti-cancer activity and cardiac protective benefits. A relatively novel and easy method was used to detect the anthocyanin content in the fresh and freeze-dried wild plums. According to the method proposed by National Science Foundation (21), at pH 1.0, anthocyanins exist in the colored oxonium or flavylium form and at pH 4.5 they are predominantly in the colorless carbinol form. An aliquot of an aqueous anthocyanin solution was adjusted to pH 1.0 and another aliquot to pH 4.5. The difference in absorbance was proportional to the anthocyanin content thus, the

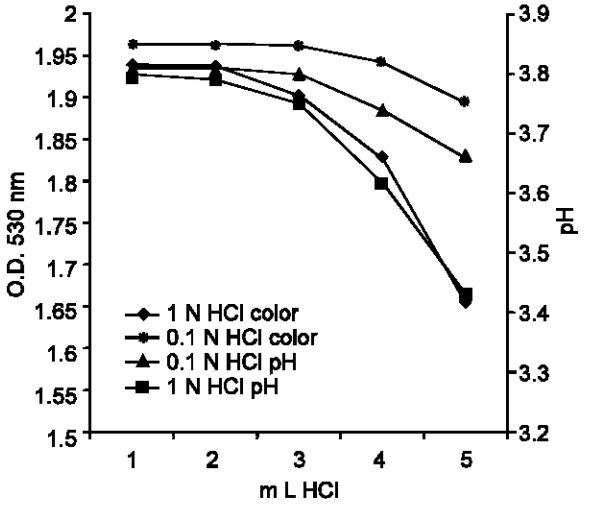


Fig. 1: Effect of strong acid on the color intensity and the pH of the rehydrated wild plum

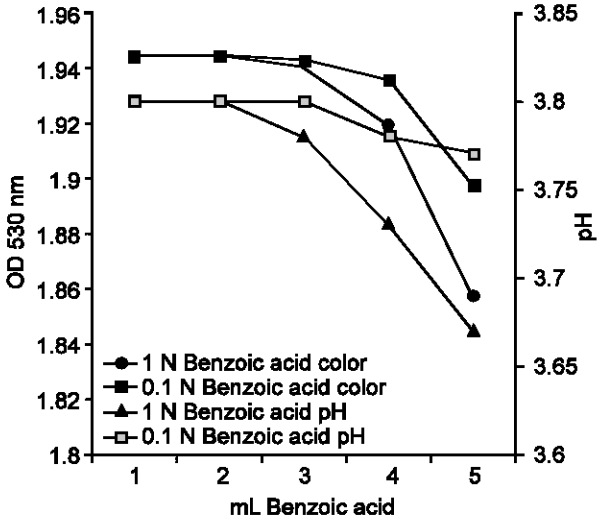


Fig. 2: Effect of weak acid on the color intensity and the pH of the rehydrated wild plum

determination of anthocyanin content was based on Lambert-Beer's Law. Since most fruits contain a mixture of the major anthocyanins and since each anthocyanin can have slightly different extinction coefficients, results may vary depending on the standard chosen. Even though delphinidin-3-glucoside is the major anthocyanin in bilberry, the total anthocyanin content was calculated as cyanidin-3-glucoside because of its historical usage for similar assays and its wide commercial availability, therefore in this study the pH-differential spectrophotometry method was used to detect the anthocyanin content of the wild plums (Gitelson *et al*., 2001; Kim *et al*., 2003).

Consequently, for the anthocyanin content, an average amount of 3.6±0.023 mg anthocyanin as dry matter was

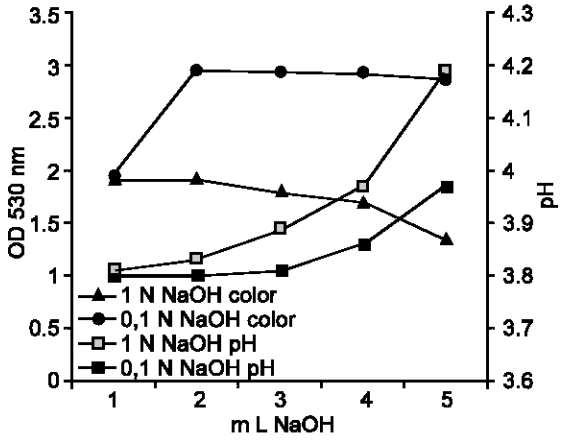


Fig. 3: Effect of strong base on the color intensity and the pH of the rehydrated wild plum

Table 4: Comparison of some chemical and physical characteristics of freeze-dried and fresh wild plums ($p<0.01$)

| Characteristics\ Fresh or freeze-dried | Fresh wild plum | Freeze-dried wild plum |
|---|---|---|
| Vitamin C (mg/100g) | 17.0±0.03 | 15.9±0.06 |
| pH | 3.6±0.01 | 3.8±0.02 |
| Total sugar content (%) | 53±0.041 | 50±0.002 |
| Water loss (%) | 93.5 | 90.0 |
| Firmness(average) (kg/f) | 0.393 | 0.62 |
| Soluble solid (Brix) | 6.0±0.03 | 6.0±0.03 |
| Fat (mg/100g) | 0.1±0.01 | 0.1±0.01 |
| Ash content (mg/100g) | 0.61±0.047 | 1.12±0.023 |
| Titratable acidity (%) | 0.86±0.02 | 0.62±0.013 |
| Anthocyanin content (mg/g) | 3.9±0.008 | 3.6±0.023 |

present in one gram of freeze-dried wild plum, compared with an approximate value of 3.9±0.008 mg in one gram of fresh wild plum. All of the chemical and physical findings were tabulated in Table 4. 70% (w/w) anthocyanin content was detected in the freeze-dried, indicating the retention of most of the pigments in the lyophilization process, but upon strong acid and base addition, a decrease depending of the strength of acid was observed ($p<0.01$).

Freeze-drying allowed the wild plums not only the protection of its nutritional quality, but also a potential to be used as a food ingredient in various commodities like juices etc. due to acid/base stability and the antimicrobial properties which is supported by all of the analytical findings presented above. A similar study was conducted on the alpine strawberries and high quality fruits were obtained (Yurdugül, 2008). The retention of most of the anthocyanin pigments in the lyophilization process was observed, although they are prone to degradation when subjected to strong and concentrated acid or base. The rehydrated wild plum was effective against important food borne pathogens, including *Klebsiella pneumoniae*, *Acinoto iworfii* ATCC 19002, *Enterobacter faecium* ATCC 6057 and *Escherichia coli* 0157: H7-933.

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