Original article

# An evaluation of the retention of quality characteristics in fresh and freeze-dried alpine strawberries

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**Summary** Alpine strawberries (*Fragaria vesca*) are used in fruit juice, marmalade, or jam production and as a result have economical importance in food sector. The fresh alpine strawberries have a tendency to lose their quality in a few days as a result of high water loss and spoilage. In this paper, the results of a study on the effects of freeze drying process on the characteristics of the alpine strawberries, such as firmness, sugar content, pH, colour, weight loss, dissolved solids, anthocyanin and vitamin C content with reference to the fresh, are reported. Freeze-drying indicated no difference in the characteristics of the alpine strawberries when compared with the fresh. It is found that a slight acid or base addition onto the rehydrated alpine strawberry juice preserved the stability of pigments and the colour. In addition, the rehydrated alpine strawberry juice exhibited an antimicrobial activity towards an important foodborne pathogen, *Enterobacter faecium* ATCC 6057.

**Keywords** Alpine strawberries, freeze-drying, shelf life.

## Introduction

Alpine strawberries (*Fragaria vesca*) are grown in temperate forests of the Black Sea region of Turkey up to an altitude of 2 000 m. It has excellent organoleptic and antioxidative properties and high vitamin C content. Therefore, it is consumed as fresh or dried fruit, as well as used in jam and marmalade production. Leaves of *F. vesca* have trace amount of alkaloids and also contain volatile compounds such as quercetin, flavonoid and silicium acids which are thought to be important antioxidant agents. Other than vitamin C, the fresh fruits contain vitamin B and provitamin A. The fruits are reported to be rich in etheric oil, aroma esters, phosphorus salt, and sugars (Boschetti *et al*., 1999; Garcia *et al*., 2001; Lara *et al*., 2004).

It is generally accepted that fresh fruits and vegetables are of particular importance for and make positive contributions to a healthy diet. Most fruits contain high levels of antioxidant molecules and an increase in the serum antioxidant capacity in humans has been shown to occur after consumption of strawberries (Guohua *et al*., 1998). Freeze-drying is a valuable technique in food industry to store vegetables and fruits for an extended shelf life. This technique involves the removal of water from the fruit or vegetable, a direct transfer from liquid to gas phase with sublimation method. When compared with other methods such as sun-drying or tray-drying; freeze-drying is quite expensive but it has been claimed to provide advantages for obtaining high quality in fruit or vegetable preservation (Hammami & René, 1997; Cui *et al*., 2003). This study aims to increase the concentration of active substances in different fruits and vegetables including sugars, pigments, and vitamins (Khalloufi & Ratti, 2003; Bernardez *et al*., 2004; Delgado & Rubiolo, 2005) in alpine strawberries through freeze-drying.

This study focuses on the freeze-drying application on alpine strawberries, where nutritional quality of the fresh fruit was aimed to be conserved in lyophilised form. To that end, most of the physical and chemical properties of the lyophilised alpine strawberries were investigated in comparison with the fresh ones. Moreover, the behaviour of the freeze-dried form in certain acid and base solutions, differing in concentration was subjected to further investigation and the antimicrobial properties were screened out for understanding the preservative activity against certain pathogens which might exist in juices or other forms of the rehydrated product.

## Materials and methods

### Preparation of the extract

Twenty kilograms of alpine strawberries were harvested from Akçakoca region, Düzce, Turkey, each in ten baskets exactly weighing 2 kg, and immediately transported to the laboratory to prevent the postharvest

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damage and spoilage. Fruits were washed and dried, and the defective ones were eliminated prior to the treatment. Afterwards, they were kept frozen at −80 °C for a period of 24 h and lyophilised by a freeze-dryer (Heto, FD 6 type, Heto-Holten A/S, Allerod, Denmark) for 24 h, and kept in closed vials for further treatment and measurements. All measurements were performed by sampling ten strawberries from each of the ten baskets.

### Antimicrobial activity

The antimicrobial activity of the thawed alpine strawberry extract (TASE) was tested on sixteen different bacterial strains in triplicates, provided by Gulhane Military Medical Academy, Ankara, Turkey and Middle East Technical University, Food Engineering Department, Ankara, Turkey, including severe foodborne pathogens, as listed: *Staphylococcus aureus*, *Escherichia coli 931* & *Escherichia coli 933* & *Escherichia coli* ATCC 25922 & *Escherichia coli* ATCC 35218, β-hemolytic *streptococci*, *Acinoto iworfi* ATCC 19002, *Enterobacter faecalis* ATCC 6057, *Enterobacter faecium* ATCC 2922 & *Enterobacter faecium* ATCC 6057, *Streptococcus thermophilus, Klebsiella pneumoniae* ATCC 19086 & *Klebsiella pneumoniae* ATCC 13883, *Salmonella enteritidis*, *Bacillus subtilis*, *Bacillus coagulans*. Disc-diffusion method was used by pour-plating cultures as an indicator strain and 10 μL aliquots from TASE as 1 mg $mL^{-1}$ were served for observing the inhibition zones around the sterile filter paper discs, at 37 °C for 24–48 h' incubation period ($P < 0.05$).

### Effect of acids and base on thawed alpine strawberry extract

0.1 mg alpine strawberry was dissolved in 1 mL distilled water. Different concentrations of 0.1 and 1 N HCl, 0.1 and 1 N benzoic acid, 0.1 and 1 N NaOH was added in triplicates. The colour change in the extract was monitored by a spectrophotometer (Pharmacia, model LKB, Pharmacia LKB Biochrom Ltd., Cambridge, UK) at 550 nm, in both fresh and lyophilised TASE ($P < 0.05$).

### Detection of anthocyanin pigment

The total anthocyanin content of both the freeze-dried and fresh samples were measured using the pH-differential spectrophotometry method (NSF, 2004), by the difference of absorbance, at pH 1.0 and pH 4.5 in triplicates. Difference in absorbances between the two samples was calculated using the following equation in order to quantify the anthocyanin content ($P < 0.01$):

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

The %w/w of total anthocyanins in the sample was calculated as ($P < 0.05$):

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

where $A$ is absorbance, ε is Cyd-3-glu (based on cyanidin-3-glucoside, the most common anthocyanin for berries) molar absorbance (26 900), MW is anthocyanin molecular weight (449.2), DF is dilution factor, $V$ is final volume (1 mL), Wt is sample weight (mg) and $L$ is cell pathlength (1 cm).

### Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) of the fresh and freeze-dried samples were recorded at a scanning speed of 2 mm $s^{-1}$, cosine apodisation accumulation of 16, a gain value of 128 and a resolution of 4 $cm^{-1}$ by a Jasco FT/IR Model 430 (JASCO Corporation, Tokyo, Japan). The samples were mixed with spectroscopic KBr (Merck) for pellet formation with pestle and mortar and squeezed under pressure of 10 mbar by a French press unit, prior to FTIR analysis in triplicates ($P < 0.05$).

### Quantification of vitamin C

The quantity of vitamin C in both the fresh and freeze-dried TASE was determined by 2,6-dichloroindophenol titrimetric method (AOAC, 1995) in triplicates and the results were expressed in milligrams of ascorbic acid per 100 g of the sample ($P < 0.05$).

### Detection of total sugar content

The total sugar content of the control and the treated group was measured by HPLC analysis (LKB-Bromma model 2150, LKB-Bromma, Bromma, Sweden, equipped with a differential refractometer – Knauer and Shimadzu C-R-4 Chromatopack monitor) in triplicates. The column used in this experiment was a Phenomenex Rezex Cal Monosaccharide column with a size of 300 × 7.8 μm, having an attenuation parameter of 2, speed of 2 m $s^{-1}$, flow rate of 0.45 μL $min^{-1}$, oven temperature of 55 °C with a mobile phase of methanol and stationary phase of acetonitrile, with respect to the standard of sugars ($P < 0.05$) (Torreggiani *et al.*, 1999; Bernardez *et al.*, 2004).

### Dissolved solid substances

Dissolved solids of the TASE were determined by a refractometer (Carl Zeiss Jena, DDR 818408) in triplicates and the results were given as Brix % by weight ($P < 0.05$).

### Firmness

Firmness of the freeze-dried strawberries was measured by a hardness tester (Everwell model FT 011, Everwell Instruments Ltd., Hong Kong, China) in triplicates and the results were expressed in Newton ($P < 0.05$).

### Colour

The colour of the lyophilised and the control group of strawberries were determined by using Hunter – Lab spectroscopy (Shimadzu) in triplicates. The L, a and b values were monitored by the spectroscopical analysis ($P < 0.05$).

### pH

The pH values were determined for the squeezed alpine strawberry juice in triplicates for the fresh and freeze-dried samples, diluted by distilled water by using a pH meter (Jenway Scientific, model 3505, Jenway Scientific Inc., Dunmow, Essex, UK) ($P < 0.05$).

### Weight determination

Five fruits from the control and the treated group were selected and weighed during storage with a digital balance (Mettler-Toledo, model AX 504, Mettler-Toledo Inc., Columbus, OH, USA) in triplicates to determine weight loss ($P < 0.05$).

### Fat content

The fat content of the alpine strawberries were determined in triplicates by the Bligh-Dyer method, allowing homogenisation of the sample with a mixture of chloroform and methanol, forming a miscible system with the water already present in the sample, by a 50 g of comminuted alpine strawberry sample 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, funnelling, 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 were preweighed to 0.001 g and 10 mL of chloroform solution was added onto each of the three preweighed dishes and the chloroform was evaporated until lipid residue remains. The dishes containing residues were placed in an 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 were weighed to nearest 0.001 g ($P < 0.05$).

### Ash content

The ash content of the fresh and the freeze-dried alpine strawberries were measured in triplicates according to a gravimetric procedure (AOAC, 2000). 1.0 g of alpine strawberry samples was weighed in a porcelain crucible. The fresh and freeze-dried strawberries were first dried in an oven at 100 °C for an hour. The crucible was then placed on a hot plate and the dried sample was charred until no smoke was generated. A muffle furnace was set at 525 °C to ash the sample overnight. After cooling the crucible in a desiccator, it was reweighed and percentage ash was calculated.

### Taste panel

Thirty panellists rated the fresh, freeze-dried and rehydrated forms of alpine strawberries according to texture, taste, colour, odour, appearance, flavour, juiciness and hardness criteria, with a 10-point scale per replication. Ten represented the most-liked score, six to eight for the marketable level and one corresponded to the least liked score ($P < 0.05$).

### Statistical evaluation

The statistical analysis was performed on the fresh and dry weight comparisons by multivariate two-way ANOVA software program ($P < 0.05$) (Sokal & Rohlf, 1994).

## Results and discussion

### Chemical, physical and nutritional analysis

When some chemical and nutritional properties of the strawberries were concerned, the vitamin C content of the alpine strawberries was measured to be 51 mg/100 g in freeze-dried fruits. The total and the reduced sugar content were found to be 7.4 and 2.1%, respectively in the freeze-dried fruits. The fat content of the strawberries was found to be 0.1% by weight, which indicates a nearly non-fat product. A 50 mg/100 g anthocyanin content was observed by freeze-dried fruits, whereas the value measured for the fresh form was 53 mg/100 g.

Certain physical properties of the fresh and freeze-dried alpine strawberries were also in concern, together with the chemical and the nutritional properties. The pH of the rehydrated extract was found to be 3.85. Soluble solid content was expressed in Brix° of 6.1 for the rehydrated alpine strawberries. In addition, it was found that there was no difference for the ash content of the freeze dried and the fresh. A significant difference was found when firmness value was measured as 0.56 N for the freeze-dried, compared with 0.1 N for the fresh fruits.

For the colour characteristics, an average value of 58, 23 and 26 were obtained for the freeze-dried samples as

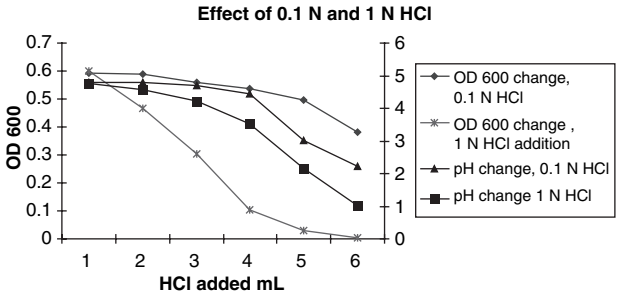


**Figure 1** Effect of strong acid (HCl) with 0.1 and 1 N concentrations on the rehydrated alpine strawberry.

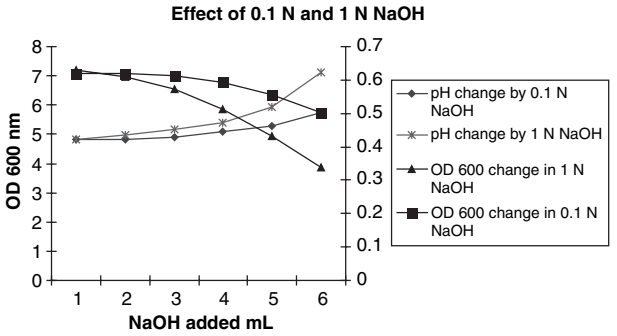


**Figure 2** Effect of strong base (NaOH) with 0.1 and 1 N concentrations on the rehydrated alpine strawberry.

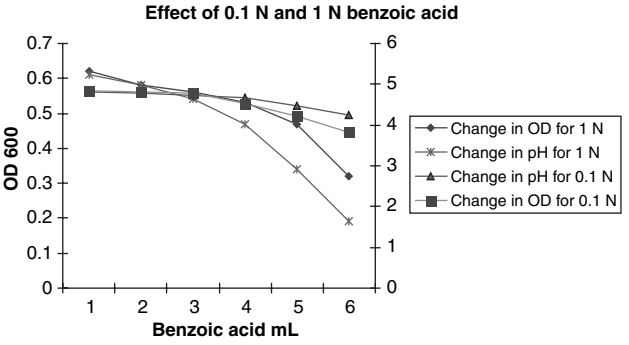


**Figure 3** Effect of weak acid (benzoic acid) with 0.1 and 1 N concentrations on the rehydrated alpine strawberry.

L, a and b values, where no such difference was found for the fresh ones. For all of the treatments, the rehydration is carried out as 1 mg $mL^{-1}$ dilution ($P < 0.05$) (Fig. 1 and Table 1) (Sokal & Rohlf, 1994; Steel *et al*., 1996).

All these values for the chemical, physical and nutritional properties of the fruits indicated that there are no significant differences between the fresh and the freeze-dried (Table 1).

## Effect of the acid and base application

When the freeze-dried alpine strawberries which were dissolved (1 mg $mL^{-1}$) in sterile distilled water were exposed to the addition of strong acid (0.1 and 1 N HCl), a sharp decline in absorbance and pH was observed (Figs 2 and 3). When the amount of strong acid addition was high, the colour of the rehydrated strawberry solution got pale because of the degradation of anthocyanin pigments which gives the native colour of the strawberries. The addition of weak base and weak acid did not show a high amount of pigment degradation when compared with the strong ones. HPLC results demonstrated that malic acid, tartaric acid and citric acid were present in low amounts, but a significant level of ethyl acetate was detected, consistent with the findings of Mochizuki *et al.* (1997). The fat content of the strawberries indicated a 0.1% value, probably because of its etheric oil content. Ascorbic acid retention of the strawberry purees was reported to be as 93.6% in freeze-drying. (Abonyi *et al*., 2002; Cui *et al*., 2003; Delgado & Rubiolo, 2005) ($P < 0.05$). All sensory analysis results were consistent with the analytical results (Tables 2 and 3).

Strawberries are among the most important fruits which contain secondary phenolic metabolites, playing an important role in plant defense mechanisms and human health. The effects of three common postharvest processing treatments (freezing, freeze-drying and air-drying) on the total phenolics content of these

**Table 1** Data concerning the standard deviations for the chemical evaluation of selected ten fresh and freeze-dried alpine strawberries from each of the baskets

| | Vitamin C (mg/100 g) | Sugar (mg/100 g) | Brix° | pH | Firmness (Newton) | Fat (mg/100 g) | Anthocyanin (mg/100 g) | Colour (L) | Colour (a) | Colour (b) |
|---|---|---|---|---|---|---|---|---|---|---|
| Fresh | 50.7 | 7.28 | 6 | 3.81 | 0.1 | 0.09 | 53 | 57 | 24 | 26 |
| Freeze-dried | 51 | 7.4 | 6.1 | 3.85 | 0.56 | 0.1 | 50.22 | 58 | 23 | 26 |
| Standard deviations (fresh) | 0.2023 | 0.0679 | 0.056 | 0.0265 | 0.3111 | 0.005 | 1.9234 | 0.7221 | 0.6785 | 0.004 |
| Standard deviations (freeze-dried) | 0.2128 | 0.0645 | 0.0462 | 0.0268 | 0.3121 | 0.0196 | 1.9086 | 0.7349 | 0.7785 | 0.0158 |
| Standard deviation (among groups) | 0.2121 | 0.0848 | 0.0707 | 0.0282 | 0.3252 | 0.0070 | 1.9657 | 0.7071 | 0.7071 | 0 |

$P < 0.05$ for all application.

**Table 2** Statistical data for sensory evaluation of freeze-dried alpine strawberries

| | Hardness | Pungency | Texture | Taste | Colour | Odour | Appearance | Flavour |
|---|---|---|---|---|---|---|---|---|
| Freeze-dried | ab | e | c | ab | ab | a | a | ab |
| Control | a | d | b | ab | a | a | a | ab |

$P < 0.05$ for all application. Letters indicate the change from the best qualified to the least while rating the fruits in fresh form (control) and the freeze-dried, where 'a' indicates the best qualified and 'd' indicates the least qualified.

**Table 3** Statistical data for sensory evaluation of rehydrated alpine strawberries

| | Juiciness | Pungency | Foaminess | Taste | Colour | Odour | Appearance | Flavour |
|---|---|---|---|---|---|---|---|---|
| Rehydrated | a | de | e | a | ab | a | a | ab |
| Control | a | d | de | a | a | a | a | ab |

Letters indicate the change from the best qualified to the least while rating the fruits in fresh form (control), and the rehydrated, where 'a' indicates the best qualified and 'd' indicates the least qualified.

agricultural products were also investigated in a study conducted by Asami *et al*. and it was reported that, statistically higher levels of total phenolics were consistently found in organically and sustainably grown foods as compared with those produced by conventional agricultural practices. In all samples, freeze-drying preserved higher levels of total phenolics in comparison with air-drying (Asami *et al*., 2003).

The analytes obtained in our study identified included esters, carbonyl compounds, terpenoids, several alcohols and acids in line with the findings of Ducruet *et al*. (2001), Pirker *et al*. (2002), Bernardez *et al*. (2004), Lara *et al*. (2004) and Komes *et al*. (2003) and the FTIR measurement assisted for understanding the nature of these chemicals prior to the HPLC analysis.

Various acids and bases were added in different concentrations in order to test the compatibility of rehydrated alpine strawberry juice in weak or strong acid or base medium. Benzoic acid, a food preservative, was an example of such trial in order to monitor the compatibility of the juice. Strong acid addition changed the anthocyanin content, whereas colour was effected, therefore, weak acid and strong base addition had a least effect in pigment degradation. A potent inhibitory action against *E.faecium ATCC 6057*, was observed, showing that this freeze-dried form could serve as an antimicrobial compound to some extent, by formation of quite good zones around the filter paper discs containing the rehydrated juice.

As a conclusion, removing water by freeze-drying method eliminated the water activity; thus microbial growth was completely excluded, supported by Knudsen *et al*. (2001) and Shishehgarha *et al*. (2002); as providing a perfect quality value. According to the FTIR analysis, most of the phenolic acids and aromatic substances resisted to freeze-drying. Strong acid addition had revealed the change of anthocyanin content, whereas weak acid and strong base treatment indicated less degradation of pigments. There was no nutritional quality difference between fresh and lyophilised alpine strawberries, besides the shelf-life extension of the fruits was provided when the experiments were repeated even after one and two years. These properties may allow the alpine strawberries to be used as natural food colourant and antioxidant in future.

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