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Amino acid profile and content of dried apricots containing SO_2 at different concentrations during storage

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RESEARCH ARTICLE

Abstract

The profile and content of amino acid in dried apricots containing various SO_2 concentrations (0, 451, 832, 2,112 and 3,241 mg SO_2/kg) were determined during storage at 4, 20 and 30 °C for 379 days. Major amino acid was aspartic acid (2,872-5,692 g/kg dw), followed by glutamic acid (695-989 g/kg dw), glycine (90-144 g/kg dw), alanine (16-35 g/kg dw) and valine (17-29 g/kg dw). Good correlation (r=0.712) was found between SO_2 concentration and aspartic acid content. SO_2 led to the reduction in the synthesis of glutamic acid, glycine, alanine and valine before storage. The highest stabilities of amino acids during storage were found in the samples containing 451 mg SO_2/kg. Reduction rates for aspartic and glutamic acids at 30 °C were generally slower than those at 20 °C. As a result of this finding, the main reason for the reductions in aspartic and glutamic acids in dried apricots could not be due to Maillard reaction. Thus, the food additive to be used in place of SO_2 to prevent browning in dried apricots must slow down the synthesis of glycine, alanine and valine.

Keywords: dried apricots, SO_2, amino acids, storage

1. Introduction

Apricots contain many health promoting compounds, such as carotenoids, phenolics, minerals and amino acids (Sochor *et al.*, 2011; Türkyılmaz *et al.*, 2013, 2014). Amino acids have beneficial effects on nervous, digestion and immune systems (Sochor *et al.*, 2011). Addition to the health benefits, amino acids, along with sugars and organic acids, are the major contributors to the organoleptic characteristic of fruits (Iwaniac *et al.*, 2016; Sochor *et al.*, 2011). Therefore, to determine the changes in amino acids contents of fruits during processing and storage is very important.

The golden yellow colour is indicator of high quality in dried apricots for consumers. Therefore, commercially produced dried apricots are usually sulphured for both inhibiting the enzymatic browning and providing the protection from non-enzymatic browning reactions during drying and storage. However, due to the sensitivity of some people to SO_2, researchers have studied to develop the SO_2 substitutes. Unfortunately, to date, SO_2 substitute as functional as SO_2 itself could not have been developed. The main prevention mechanism of SO_2 on browning is not well-known. To investigate the changes in amino acid contents of fruits will be useful to understand the mechanism of browning occurred in fruits because amino acids are the substrates of Maillard reaction.

Besides Maillard reaction, transamination catalysed by transaminase has also significant effect on the changes in amino acid contents of plants during postharvest drying (Costantini *et al.*, 2006) and storage (Hansen *et al.,* 2001). Although transamination in plants is a general enzymatic reaction as in animal tissues and bacteria, the role of this reaction in plant tissues has not fully elucidated (Wilson *et al.,* 1954). By transamination, many amino acids can be converted to α-ketoglutarate which plays a vital role in Krebs cycle (Wilson *et al.,* 1954). The presence of a transaminase (aspartate transaminase) was determined in apricots (Pashkoulov, 1993). The stabilities and activities of the transaminase are very different, depending on their source. In the literature, the results from the study by Wilson

et al. (1954) indicated that the stability of α-ketoglutaric acid-aspartic acid system in plants was similar to the stability in animals. Moreover, while α-ketoglutaric acid-glycine system was very active, the valine, lysine, asparagine and methionine systems were moderately active (Wilson *et al.,* 1954). However, their activities in the sulphured dried apricots could also change in the presence of SO_2. Although bisulphite ions may trigger the transamination depending on their concentrations (Hayatsu, 1976), to date, no study has investigated the effect of SO_2 on the amino acid contents of dried apricots during storage. Taking into consideration of data about transaminase in literature, the effects of the enzymes on the amino acids of sulphured dried apricots were discussed in the present study.

The main objective of this study was to determine the effect of various SO_2 concentrations (0, 451, 832, 2,112 and 3,241 mg/kg) on the amino acid contents of dried apricots during storage. For this purpose, the profile and contents of amino acids in dried apricot samples were determined during storage at 4, 20 and 30 °C for 379 days.

2. Materials and methods

Materials

Fresh apricots (*Prunus armenica* L., var. Hacıhaliloğlu) were harvested in Boğaz region in Malatya, Turkey in July 2014. The harvested apricots were sulphured and sun-dried in the village of Yaka in Malatya. The dried apricots containing no SO_2 were evaluated as the control group. The details of sulphuring and sun-drying were given in our previous study (Salur-Can *et al.,* 2017).

Storage

The dried apricots subsamples (20 kg for each storage temperature) were stored at 4 and 20±0.5 °C (Sanyo MIR 153 and 253, Gunma, Japan) and 30±0.1 °C (Memmert BE 400, Schwabach, Germany) for 379 days. Samples were periodically (45 days at 30 °C, 90 days at 20 °C and 180 days at 4 °C) taken from incubators to determine amino acid contents as well as pH, titratable acidity, water activity and moisture content.

Preparation of samples to analysis

Dried apricots were ground through a plate with 4 mm orifices (Tefal Maxi Power 1,800 W, Ecully, France) and were then hand-kneaded to obtain fully homogenous samples. These homogenised samples were used in all analyses.

Compositional analyses

The moisture content was determined by the Method 934.0 of AOAC (2000). pH and the water activity (a_w) were measured with a pH meter (WTW Inolab Level 1, Weilheim, Germany) and a hygrometer (AquaLab 3, Decagon Devices, Pullman, WA, USA), respectively. Titratable acidity of dried apricots was expressed as 'g anhydrous citric acid/100 g sample.' The details of these analyses were given in our previous study (Türkyılmaz *et al.,* 2013).

Determination of SO_2

The modified Monier-Williams distillation method was used for the determination of SO_2 in the samples. The details of the analysis were given in our previous study (Türkyılmaz *et al.,* 2013).

Amino acid analysis

Extraction

The amino acids from homogenised sample were extracted according to the method described by Hermosin *et al.* (2003). 1 g (±0.0001 g) of the sample was mixed with 9 ml HCl (6 *M*). The mixture was homogenised at 15,000 rpm for 3 min in a high-speed homogeniser (Heidolph SilentCrusher M, Schwabach). A 5-g (±0.001 g) of the homogenate was weighed into a glass vial and 375 µl γ-aminobutyric acid solution (1 g γ-aminobutyric acid in 0.1 *N* HCl) was added as an internal standard.

The mixture was then subjected to N_2 (g) for 2 min and each vial was sealed with a chlorobutyl stopper and flip-off aluminium seal. The vials were incubated in the oil bath (Heto, IBN 18, Lillerod, Denmark) at 110 °C for 2.5 h. As soon as the completion of acid hydrolysis through incubation, the samples were filtered through Whatman No. 1 filter paper and 2 ml of the resulting filtrate was then transferred to a tube in rotary evaporator (Heidolph Laborota 4003, Schwabach) to remove residual liquid at 40 °C for 15 min. The concentrated sample was brought to the volume of 10 ml with the borate buffer (Sodium tetraborate-HCl, pH 9.00) and 20 µl diethyl ethoxymethylenemalonate was added. The prepared mixture was heated in a water bath (Memmert WB 14, Schwabach) at 50 °C for 50 min for derivatisation. This sample was filtered through a 0.45 µm polyvinylidene fluoride filter (Sartorious AG, Goettingen, Germany) directly into amber-coloured (2 ml) vials used in the high-performance liquid chromatography (HPLC) sampling unit. The contents of vials were then incubated at 4 °C for two days before being injected into HPLC.

Instrumentation and chromatography

Separation and quantification of amino acids were performed on the HPLC whose system consists of a binary pump, a photodiode array detector, a thermostatted auto-sampler, a degasser and a thermostatted column compartment. The obtained chromatograms were evaluated using the 'ChemStation rev.B.02.01' software program (Agilent Technologies, Wilmington, DE, USA). Amino acids were separated on a C_{18} (5 µm) column (250×4.6 mm) (Water Co., X-Bridge Columns, Wexford, Ireland) with a C_{18} (5 µm) guard column (20×4.6 mm, 5 µm) (Water Co., X-Bridge Columns). The eluents were (A) 100% acetonitrile and (B) acetate buffer (25 m*M*, pH 5.8) with a flow rate of 0.9 ml/min. Separation was performed with gradient elution using a modification of the elution profile described by Hermosin *et al.* (2003). The linear gradient programme for the separation of amino acids was as follows: holding at 1% A for 8 min, from 1 to 16% A in 5 min, from 16 to 18% in 0.5 min, holding at 18% A for 3.5 min, from 18 to 22% in 3 min, from 22 to 32% in 12 min, holding at 32% A for 7 min, and from 32 to 1% in 11 min. The sample injection volume was 50 µl, the column temperature was 16 °C, and the detector was set at 280 nm.

Identification of amino acids was carried out by comparing retention times and absorption spectra of unknown peaks with external reference standards. Quantification of amino acids was carried out using calibration curves of the following external reference standards: aspartic acid (R^2=0.970), glutamic acid (R^2=0.849), glycine (R^2=0.967), alanine (R^2=0.997) and valine (R^2=0.998). The calibration curves for each amino acid standard contained five data points. Quantification of total amino acids by HPLC was calculated based on aspartic acid.

Calculation of kinetic data

The reaction rate constants (k), half-life periods ($t_{1/2}$) and temperature quotient (Q_{10}) were calculated by the following equations:

$$\ln(C_t/C_o) = -kt$$

$$t_{1/2} = -\ln 0.5 \times k^{-1}$$

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)}$$

where, C_o is the initial content of amino acids in samples and C_t is the content of amino acids after t min exposure of the samples to a given temperature (T).

Statistical analyses. Experimental data were analysed using Minitab statistical software, version 15 (Minitab Inc., State College, PA, USA). SO_2 concentration and storage temperature were considered as the main effects. Statistical differences among means were determined by Duncan's multiple range test at 5% significance level.

3. Results and discussion

SO_2 concentration and some compositional properties of the samples

SO_2 concentrations (0, 451, 832, 1,594, 2,112 and 3,241 mg/kg), titratable acidity (0.96-3.03%), pH (3.95-5.37), moisture (20.53-26.04%) and water activity (0.625-0.705) values of dried apricot samples before and after storage are presented in Table 1 (from Salur-Can *et al.*, 2017). The effects of the changes in SO_2 concentration and compositional properties on amino acid profile and contents were discussed in following sections.

Amino acid profile of the samples

The separation of amino acids by HPLC is shown in Figure 1A. As seen from Figure 1B, aspartic acid (2,872-5,692 mg/kg dw) was the major amino acid in all dried apricot samples, followed by glutamic acid (695-989 mg/kg dw), glycine (90-144 mg/kg dw), alanine (16-35 mg/kg dw) and valine (17-29 mg/kg dw). Similarly, the same amino acids were also determined in 11 different apricot cultivars (Bebeco, Canino Tardivo, CV1F, CV2F, Fronne Fresche, Monaco Bello, Monte Ruscello, Skaha, Sun Giants, Vitillo and 265) (Voi *et al.*, 1995). However, the major amino acid in those cultivars was asparagine (2,452-6,895 mg/kg), followed by aspartic acid (84-197 mg/kg), glutamic acid (73-184 mg/kg), alanine (52-195 mg/kg), serine (51-275 mg/kg), threonine (25-52 mg/kg), glutamine (16-89 mg/kg), valine (12-78 mg/kg), glycine (8-33 mg/kg), proline (5-101 mg/kg) and histidine (5-25 mg/kg) (Voi *et al.,* 1995). Moreover, Sochor *et al.* (2011) reported threonine as the major amino acid in apricots, which was different from aspartic acid and asparagine found in apricots as the major amino acid. The variation among the amino acid profiles of apricots may only be a consequence of cultivar differences (Voi *et al.*, 1995).

Total amino acid contents of apricot samples ranged from 3,964 to 6,872 mg/kg dw (Figure 1B). Similar total amino acid contents (2,915-8,173 mg/kg dw) were reported by Voi *et al.* (1995) in apricot purees produced from 11 apricot varieties. The lowest total amino acid content was determined in the sample containing no SO_2, while the highest amino acid content was determined in the sample containing 3,241 mg SO_2/kg. As the SO_2 concentration increased, total amino acid contents also increased (r=0.712). A reason for the increases in total amino acid contents may be the hydrolysis effect of SO_2 on the proteins in apricots, which contained proteins between 2.84 and 4.29% (Hacıseferoğulları *et al.*, 2007). Similarly, sodium metabisulphite (660 mg/ml) also resulted in the hydrolysis

Table 1. Some properties of apricots before and after storage.

Properties	Time (days)	Temperature (°C)	SO_2 concentration (mg/kg)[1]				
			0	451	832	2,112	3,241
SO_2 concentration (mg/kg)	379	30	–	41±3.395^{c}	71±12.028^{b}	133±0.0451^{d}	209±3.259^{a}
		20	–	68±6.404^{d}	179±4.257^{c}	996±19.975^{b}	1,716±0.097^{a}
		4	–	283±12.010^{d}	663±22.281^{c}	1,846±32.007^{b}	2,876±41.408^{a}
Titratable acidity (%)	0	–	0.96±0.008^{e}	1.74±0.009^{d}	1.81±0.005^{c}	2.50±0.005^{b}	3.03±0.006^{a}
	379	30	1.40±0.006^{e}	2.42±0.000^{d}	2.48±0.007^{c}	2.88±0.006^{b}	3.14±0.009^{a}
		20	1.18±0.009^{e}	1.98±0.005^{d}	1.99±0.005^{c}	2.58±0.009^{b}	3.06±0.006^{a}
		4	0.95±0.009^{e}	1.69±0.009^{d}	1.82±0.009^{c}	2.43±0.009^{b}	2.97±0.009^{a}
pH	0	–	5.37±0.007^{a}	4.59±0.000^{b}	4.51±0.000^{c}	4.09±0.007^{d}	3.95±0.007^{e}
	379	30	4.91±0.007^{a}	4.21±0.000^{b}	4.15±0.007^{c}	3.89±0.007^{d}	3.79±0.007^{e}
		20	5.18±0.007^{a}	4.50±0.007^{b}	4.44±0.007^{c}	4.04±0.007^{d}	3.87±0.007^{e}
		4	5.31±0.007^{a}	4.60±0.007^{b}	4.50±0.007^{c}	4.08±0.007^{d}	3.91±0.007^{e}
Moisture (%)	0	–	20.84±0.029^{c}	21.07±0.202^{c}	26.04±0.242^{a}	21.88±0.488^{b}	20.53±0.159^{c}
	379	30	17.69±0.016^{d}	18.52±0.124^{c}	21.81±0.029^{a}	19.01±0.395^{b}	17.72±0.083^{d}
		20	20.60±0.043^{c}	17.53±0.074^{c}	23.40±0.006^{a}	17.55±0.059^{b}	17.98±0.004^{c}
		4	22.05±0.039^{c}	22.07±0.070^{b}	24.47±0.029^{a}	19.81±0.096^{e}	20.75±0.004^{d}
Water activity	0	–	0.650±0.001^{b}	0.640±0.003^{d}	0.705±0.001^{a}	0.646±0.001^{c}	0.625±0.001^{e}
	379	30	0.609±0.001^{d}	0.627±0.001^{b}	0.693±0.001^{a}	0.624±0.001^{c}	0.602±0.001^{e}
		20	0.645±0.001^{b}	0.630±0.001^{d}	0.696±0.001^{a}	0.637±0.001^{c}	0.620±0.001^{e}
		4	0.663±0.001^{b}	0.650±0.001^{d}	0.710±0.001^{a}	0.656±0.001^{c}	0.638±0.001^{e}

[1] $^{a-e}$: the letters show statistical differences between the values in the same row.

of protein in tomato leaves, causing the decrease in protein content by 42.85% (Singh *et al.*, 1990).

Changes in aspartic acid contents during storage

Similar to the effect of SO_2 concentration on total amino acid contents of the apricot samples, aspartic acid contents of the samples also generally increased (r=0.712) as the SO_2 concentration increased. Aspartic acid content in the sample containing 3,241 mg SO_2/kg was 49% higher than that in the sample containing no SO_2 (Figure 1B). Similarly, increase (28%) in aspartic acid content of pine seedling was also observed after SO_2 treatment (1.34 mg/m^3 for 96 h) (Malhatro and Sarkar, 1979). Addition to the hydrolysis effect of SO_2 on proteins, three more different reasons for the increase in aspartic acid content may be considered, depending on SO_2 concentration.

First reason could be the activity of aspartate transaminase (also known as aspartic acid aminotransferase) which catalyses the interconversion of oxaloacetate and glutamate to aspartate and α-ketoglutarate. In fact, Pashkoulov (1993) investigated the presence of aspartate transaminase in 14 different apricot cultivars and determined its activity in all apricot cultivars. As the pH was near to optimum pH (5.5) for the enzyme, the activity of aspartate transaminase also increased (Karni-Katsadimas *et al.*, 1969). pH lower and higher than 5.5 led to the reduction in the activity of this enzyme (Karni-Katsadimas *et al.*, 1969). Thus, as the SO_2 concentration increased, activity of this enzyme decreased. Among the samples, this enzyme could have showed the highest activity in the sample (pH 5.37) containing no SO_2. However, aspartic acid contents of the samples containing SO_2 were higher than the sample containing no SO2. The results indicated that the main reason for the increases in aspartic acid contents of the samples could not be the activity of aspartate transaminase.

Second reason could be attributed to the effect of SO_2 concentration on the ethylene production in apricot samples. As known, apricots are climacteric fruits and produce high amount of ethylene. Increase in ethylene concentration caused the reduction in aspartic acid content in cocklebur seed (Clijster *et al.*, 2012). In fact, aspartic acid content of the cocklebur seed was 18% higher than that of the cocklebur seed treated with ethylene (10 cm^3/m^3, Clijster *et al.*, 2012). No study was found in the literature investigating the effect of SO_2 on ethylene production in apricots. Moreover, there is inconsistent data in the literature about the effect of SO_2 concentration on ethylene release from different plants. For example, SO_2 fumigation reduced the ethylene release from sunflower

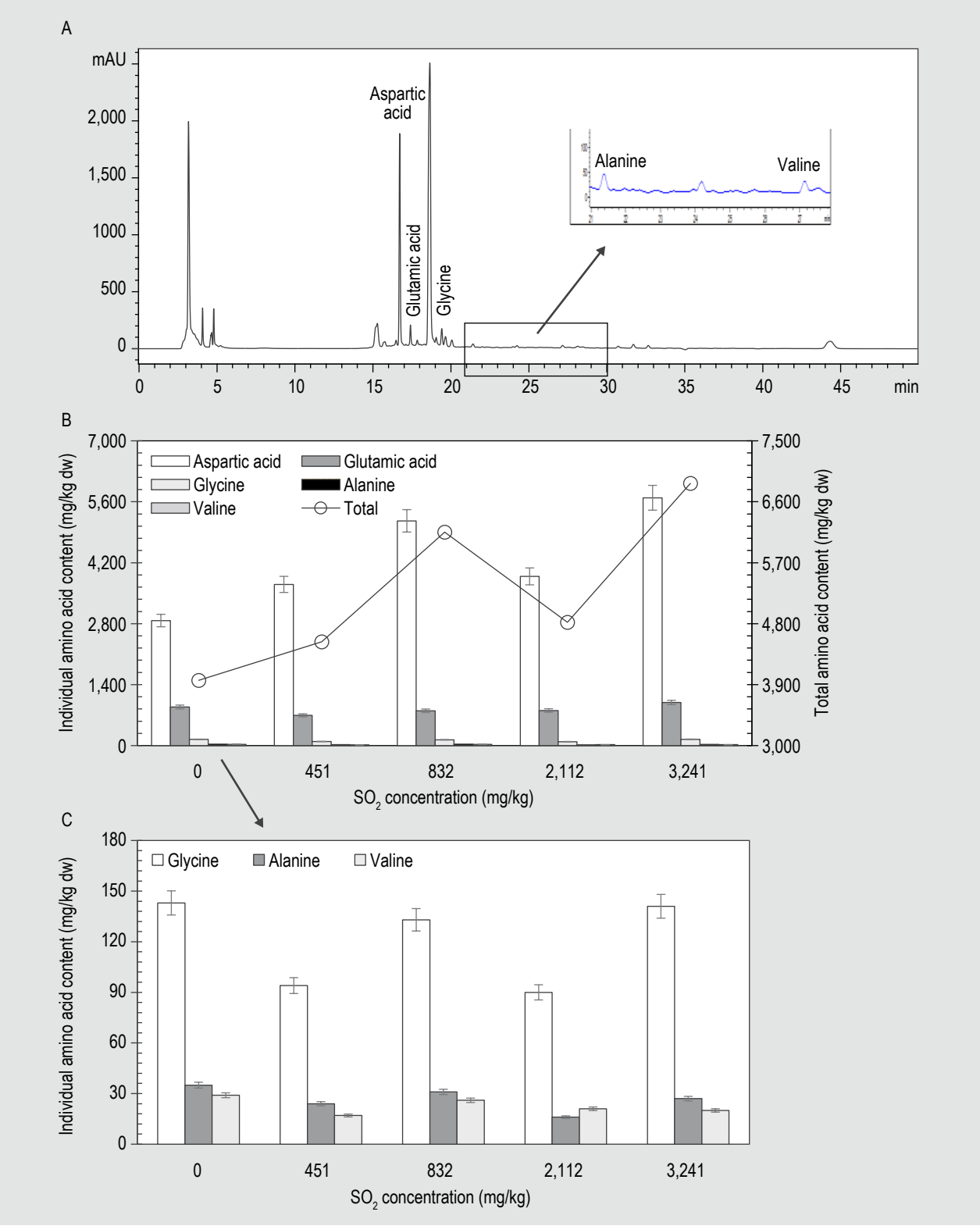

Figure 1. HPLC chromatogram of the amino acids in the sample containing 3,241 mg SO_2/kg (A) and the contents of individual amino acids (B, C) in samples before storage

(Zwoch *et al.,* 1990). In contrast, SO_2 fumigation increased the ethylene release from alfalfa plants (Peiser and Yang, 1979). Therefore, the effect of SO_2 concentration on ethylene production in sulphured dried apricots should be investigated. If the reduction in ethylene release was observed in apricots after SO_2 treatment as in sunflowers,

the reason for this could be attributed to the reduction in ethylene release.

Third reason might be the inhibiting effect of SO_2 on browning reactions in apricots since aspartic acid participates to browning reactions as a substrate (Hwang *et al.,* 2011). As the SO_2 concentration in sulphured dried apricots increases, browning reactions decelerate (Türkyılmaz *et al.*, 2013). Thus, the higher aspartic acid content of dried apricots samples with high SO_2 content would be attributed to the high SO_2 content of these samples. However, aspartic acid content of the dried apricots containing 2,112 mg SO_2/kg was lower than that of the dried apricots containing 832 mg SO_2/kg (Figure 2A). This may be due to the highest formation rate for browning which was determined in dried apricots containing 2,112 mg SO_2/kg among the dried apricots containing 0-3,241 mg SO_2/kg (Hamzaoğlu *et al.*, 2018). The effects of various compounds including amino acids on the browning in dried apricots will be discussed in our next manuscript.

Similar to SO_2 concentration, storage temperature had also significant effect on the aspartic acid content of the samples (P<0.05, Figure 2A). During storage at 20 and 30 °C, aspartic acid content decreased gradually. Similar results were reported by Babsky *et al.* (1986). The reduction in the aspartic acid contents of the dried apricot samples was fitted to a first-order kinetic model (figure not shown). The calculated k values are presented in Table 2. As the SO_2 concentration in samples increased, the k values for aspartic acid reduction at 30 °C generally increased. For example, while the k value at 30 °C for the sample containing 451 mg SO_2/kg was -1.38×10^{-3} 1/day, that in the sample containing 3,241 mg SO_2/kg was -3.68×10^{-3} 1/day. Moreover, the k value at 20 °C of the sample containing 2,112 mg SO_2/kg was 2.9 times higher than the k value at 30 °C of the same sample. As known, increases in SO_2 concentration and reduction in storage temperature lead to lower brown colour in dried apricots (Türkyılmaz *et al.,* 2013). Thus, the reduction effects of increased SO_2 concentrations and decreased storage temperature on aspartic acid contents could have not been resulted from only the participation of aspartic acid in browning reactions as a substrate. However, since aspartate transaminase shows the highest activity at 30 °C (Birolo *et al.,* 2000; Uiroga *et al.*, 1991), the reduction in aspartic acid contents of the samples stored at 30 °C might be lower than those stored at 20 °C.

Changes in glutamic acid contents during storage

SO_2 concentration had significant effect on the glutamic acid contents of dried apricot samples (P<0.05). The glutamic acid content of the sample containing no SO_2 was 886 mg/kg (Figure 1B). The content was higher (9-22%) for the samples containing SO_2, except for the sample containing 3,241 mg SO_2/kg (989 mg/kg) (Figure 1B). Similarly, the glutamic acid content of pine seedling was also higher (15%) than the sample treated with SO_2 (1.34 mg/m^3 for 96 h)

Table 2. Kinetic parameters for the changes in amino acids of samples during storage.

Temperature (°C)	Kinetic parameters	SO_2 concentration (mg/kg)				
		0	451	832	2,112	3,241
Reduction in aspartic acid content						
30	$-k \times 10^3$ (1/day)	2.30	1.38	1.84	0.65	3.68
	$t_{1/2}$ (days)	301	502	376	1,064	188
20	$-k \times 10^3$ (1/day)	2.53	2.30	1.15	2.53	1.61
	$t_{1/2}$ (days)	274	301	602	274	430
20-30	Q_{10}	0.91	0.60	1.60	0.26	2.29
Reduction in glutamic acid content						
30	$-k \times 10^3$ (1/day)	9.21	1.15	2.53	4.61	2.30
	$t_{1/2}$ (days)	752	602	274	150	301
20	$-k \times 10^3$ (1/day)	2.30	2.30	2.76	3.91	3.91
	$t_{1/2}$ (days)	301	301	251	177	177
20-30	Q_{10}	4.00	0.50	0.92	1.18	0.59
Reduction in glycine content						
30	$-k \times 10^4$ (1/day)	3.45	2.30	2.53	5.76	4.14
	$t_{1/2}$ (days)	201	301	274	120	167
20	$-k \times 10^3$ (1/day)	1.61	1.61	1.61	–	2.53
	$t_{1/2}$ (days)	430	430	430	–	274
20-30	Q_{10}	2.14	1.43	1.57	–	1.64

[1] k = reaction rate constants; $t_{1/2}$ = half-life periods; Q_{10} = temperature quotient.

(Malhatro and Sarkar, 1979). The differences among the glutamic acid contents of dried apricot samples depending on SO_2 concentrations may be due to the activities of the enzymes such as glutamate synthase and aspartate transaminase which control the glutamic acid contents of dried apricots. Although the optimum pHs of these enzymes were 7.8 and 5.5, respectively, they were active (up to 58%) at the pHs (3.95-5.37) of dried apricots (Avila *et al.*, 1987; Michuda and Martinez-Carrion, 1970). In addition to these enzymes, glutamate dehydrogenase, glutamic oxaloacetic transaminase and glutamic pyruvate transaminase have also significant effects on glutamic acid content. However, these enzymes could not be active at the pHs of dried apricots because they were inactive at the pH below 5.5 as reported by Michuda and Martinez-Carrion (1970).

The pH (5.37) of the sample containing no SO_2 was more appropriate for the activity of glutamate synthase (which catalyses conversion of α-ketoglutarate and ammonia to glutamate) than those (3.95-4.59) of the sulphured samples. At pH 5.5, the activity of glutamate synthase was 58% of its original activity (Avila *et al.,* 1987). The activity of the enzyme decreased at the pH lower than 5.5 (Avila *et al.,* 1987). Due to higher activity of glutamate synthase, glutamic acid content of the sample containing no SO_2 might be higher than those of the sulphured samples. Moreover, while the activity of aspartate transaminase at pH 5.0 was 30%, the activity at pH 4.5 was only 20% (Michuda and Martinez-Carrion, 1970). As stated before, the reduction in pH may cause the reduction in the activity of aspartate transaminase as reported by Michuda and Martinez-Carrion (1970). In other words, as the SO_2 concentration of dried apricot samples increased, the activity of aspartate transaminase may have decreased. As a result, since the conversion of glutamate to aspartate decreased in the samples with higher SO_2 concentration, glutamic acid contents of the samples containing SO_2 increased (Figure 1B).

Storage temperature also affected the glutamic acid contents of the samples during storage ($P<0.05$). Our results agree with the results of the study conducted by Babsky *et al.* (1986). Similar to aspartic acid contents, the glutamic acid content of dried apricot samples was also fitted to the first-order kinetic model (figure not shown). As the SO_2 concentration increased, the rate of reduction (Table 2) in glutamic acid at 20 °C generally increased (r=0.946). Moreover, higher *k* values were found in the sulphured dried apricots stored at 20 °C than those stored at 30 °C. For example, the *k* value at 30 °C for the sample containing 451 mg SO_2/kg was -1.15×10^{-3} 1/day, while the *k* value at 20 °C was -2.30×10^{-3} 1/day (Table 2). Although higher browning in the samples stored at 30 °C was reported by Türkyılmaz *et al.* (2013), the lower *k* values at 30 °C for the reduction in glutamic acid contents showed that these reductions for the samples stored at 20 and 30 °C could not have resulted from solely browning reactions. If that would be the case, the *k* values for the reduction in glutamic acid contents at 30 °C should have been higher than those at 20 °C. Thus, other factor/factors affected this reduction during storage. One of the factors may be the activity of glutamate synthase which showed optimum activity at 35 °C (Márquez *et al.*, 1987). Since the activity at 30 °C of the enzyme was higher than that at 20 °C, the *k* values for this reduction at 30 °C might be lower than those at 20 °C (Table 2).

Changes in contents of glycine, alanine and valine during storage

Compared with the aspartic acid (2,872-5,692 mg/kg dw) and glutamic acid (695-989 mg/kg dw) contents, the contents of glycine (90-144 mg/kg dw), alanine (16-35 mg/kg dw) and valine (17-29 mg/kg dw) in the dried apricot samples were very low (Figure 1C). The highest contents of glycine (144 mg/kg dw), valine (29 mg/kg dw) and alanine (35 mg/kg dw) were found in the samples containing no SO_2 (Figure 1C). SO_2 caused the reduction in the glycine (6-37%), valine (10-41%) and alanine (11-54%) contents. Similar to our results, glycine content of spruce seedling also decreased after SO_2 treatment (Jager and Grill, 1975). This may be due to the effect of SO_2 on the activities of glycine synthase (optimum pH was 6, Hasse *et al.*, 2007) and alanine synthase (optimum pH was 5.7-7.0, Waldmann *et al.*, 2005). As stated before, the increase in SO_2 concentration led to the reduction in the pHs of dried apricot samples (Türkyılmaz *et al.*, 2013). As the pHs of the dried apricot samples were near to the optimum pHs for these enzymes, the formations of glycine and alanine would have accelerated. Therefore, the sample containing no SO_2 which had the highest pH (5.37) among the samples might have had the highest glycine (142 mg/kg) and alanine (35 mg/kg) contents.

Similar to the effect of SO_2, storage temperature also caused the reduction in the glycine (8-48%), alanine (37-79%) and valine (10-71%) contents. As the storage temperature increased, the reductions in the contents of amino acids also increased (Figure 2C-E). For example, the reduction at 20 °C for the sample containing no SO_2 was 8%, while the reduction at 30 °C for the same sample was as high as 36%. Similar results were also reported in peach juice concentrate (Buedo *et al.*, 2000). The reductions in the contents of these amino acids during storage may be attributed to Maillard browning reaction because amino acids are the substrates for this reaction.

4. Conclusions

To date, although studies showed that bisulphite ions cause the transamination depending on their concentrations, no study investigated the effect of SO_2 on the amino acid contents of dried apricots during storage. In the present

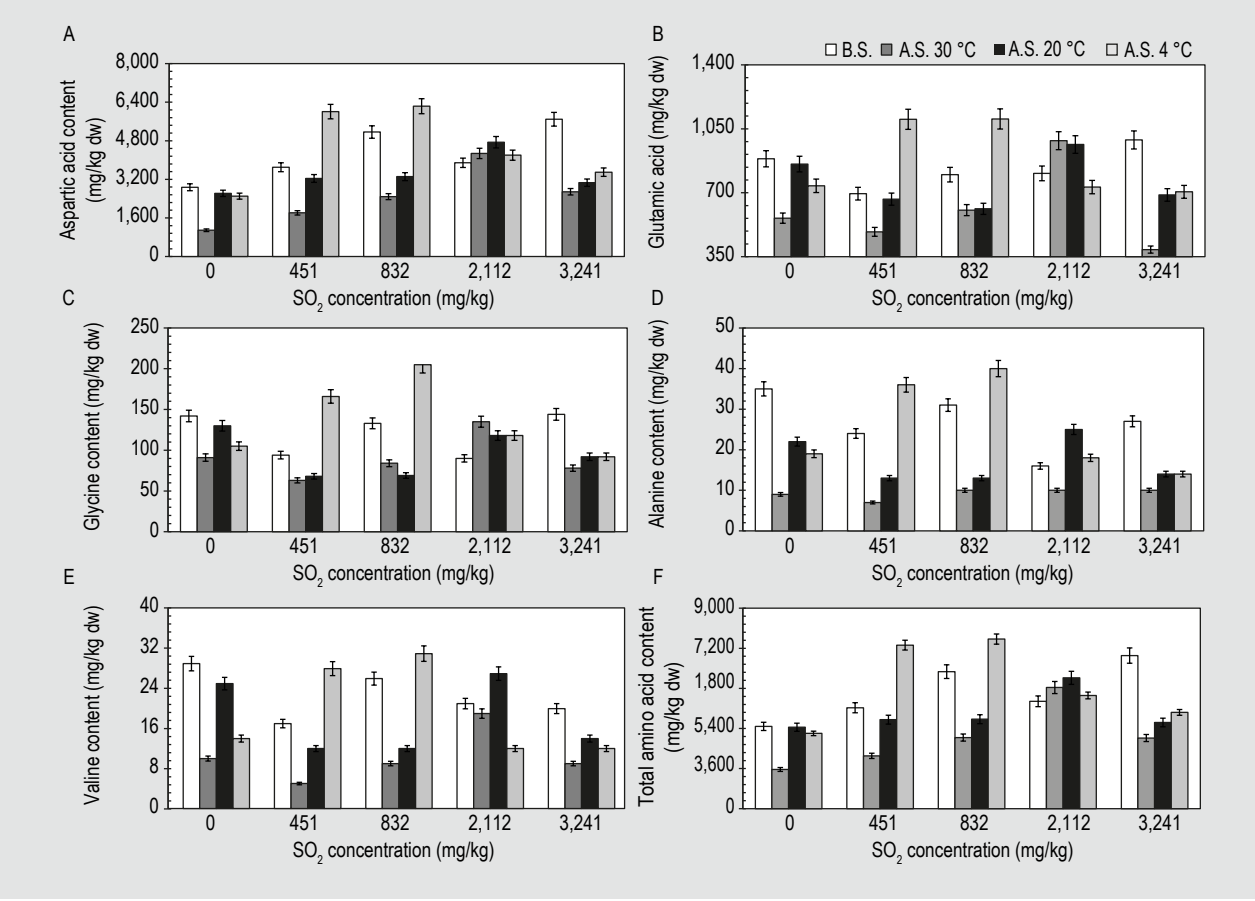

Figure 2. Changes in the contents of aspartic acid (A), glutamic acid (B), glycine (C), alanine (D), valine (E) and total amino acids (F) during storage. B.S.: at the beginning of storage, A.S.: at the end of 379 days of storage at a given temperature.

study, the changes in amino acids which are the main substrates of Maillard reaction were investigated for the first time. Aspartic acid, glutamic acid, alanine, valine and glycine were determined in all dried apricot samples. The results showed that SO_2 led to slow down the synthesis of glutamic acid, glycine, alanine and valine before storage. As known, the contribution degree of non-polar amino acids such as alanine, valine and glycine to browning was higher than the contributions that of polar amino acids such as aspartic acid and glutamic acid. Among polar amino acids, the contribution degree of glutamic acid to browning was higher than that of aspartic acid. Thus, the prevention effect of SO_2 on Maillard reaction might result from the reductions in the synthesis of glycine, alanine and valine as well as glutamic acid. However, as the SO_2 concentration increased, aspartic acid contents of the samples also increased (r=0.712). The increase may be due to protein hydrolysis despite the reduction in the synthesis of aspartic acid depending on SO_2. All amino acids determined in dried apricots decreased during storage at 20 and 30 °C. Reduction rates of aspartic acid and glutamic acid at 30 °C during storage were generally lower than those at 20 °C. The results also confirmed that the main reason for the reductions in aspartic acid and glutamic acid could not be due to Maillard reaction. Consequently, the food additive to be used to prevent browning in dried apricots in place of SO_2 should be capable of slowing down the synthesis of glycine, alanine and valine.

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