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Effects of Processing and of Storage on the Stability of Pantothenic Acid in Sea Buckthorn Products (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) Assessed by Stable Isotope Dilution Assay

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A stable isotope dilution assay for quantification of pantothenic acid in sea buckthorn berries, juice, and concentrate using a four-fold labeled isotopologue of vitamin B_5 as the internal standard was adopted using reversed phase liquid chromatography−mass spectrometry with electrospray ionization. Because of a rapid sample clean up procedure without the necessity of external calibration, this methodology permits the accurate analysis of a high number of samples within a short time. Sea buckthorn juice was stored at 25 and 40 °C for up to 7 days to determine the effects of storage temperature on the stability of pantothenic acid. Analysis of kinetic data suggested that the degradation follows a first-order model. The results of the experiments showed that storage of sea buckthorn juice for 7 days at ambient temperature (25 °C) already resulted in a significant degradation of pantothenic acid of about 18%. The processing effects of juice production and subsequent concentration revealed a decrease of about 6−7% in the juice and of 23% in the juice concentrate.

KEYWORDS: Pantothenic acid; isotope dilution assay; SIDA; HPLC-ESI-MS-MS; *Hippophaë rhamnoides*; sea buckthorn products; process stability; storage stability

INTRODUCTION

The berries of *Hippophaë rhamnoides* (Elaeagnaceae) are a rich source for vitamins such as vitamin C, tocopherols, vitamins B_1, B_2, and B_6, nicotinamide, biotin, folic acid, and pantothenic acid (*1*). (*R*)-Pantothenic acid, also known as vitamin B_5, is essential for humans and animals for growth, maintenance of physiological functions, and reproduction. The vitamin was first investigated as a growth factor for yeast (*2*) and later as a so-called antidermatitis factor (*3*). Pantothenic acid is a biosynthetic precursor of coenzyme A (*4*) and also occurs in phosphopantetheine of the acyl carrier protein (*5*). Hence, coenzymes containing pantothenic acid are involved in several vitally important biological functions affecting the metabolism of carbohydrates, lipids, and amino acids (*6*).

The determination of pantothenic acid in foods has commonly been performed by microbiological assays (*7*). This method has been accepted by the Association of Official Analytical Chemists as the official method for the determination of pantothenic acid in vitamin preparations and in milk-based infant formula (*8*, *9*). Other methodologies for measuring pantothenic acid include the radiometric microbiological assay (RMA) (*10*), radioimmunoassay (RIA) (*11*), enzyme-linked immunosorbent assay (ELISA) (*12*, *13*), optical biosensor inhibition immunoassay (*14*), capillary electrophoresis (CE) (*15*, *16*), and capillary isotachophoresis (*17*). The applications of biospecific methods have several drawbacks in being time-consuming (2−3 days) and in the need for radioisotopes (RIA and RMA). In the case of ELISA assays, antisera are not commercially available. Moreover, analytical interferences caused by cross-reactions between antibodies and components of the complex food matrices may invalidate the results. The determination of pantothenic acid by CE or high-performance liquid chromatography (HPLC) (*18*) using UV detection is hampered by low UV absorption of the analyte above 230 nm. Some studies applied sensitive gas chromatography−mass spectrometry for analysis of pantothenic acid (*19*−*23*) including a silylation step due to the low volatility of free pantothenic acid. Further methods were developed by means of liquid chromatography coupled to fluorimetric postcolumn derivatization (*24*).

A versatile and accurate methodology for determination in plant and animal tissue materials is the HPLC−electrospray

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ionization−mass spectrometry (ESI-MS) technique using a stable isotope dilution assay (SIDA) (*25*, *26*). The SIDA procedure employing a four-fold labeled isotopologue of pantothenic acid (stable isotopes, ^{13}C and ^{15}N) as an internal standard ensures that interferences of food matrices have no influence on results of quantification (*25*, *26*).

So far, evidence of dietary deficiency has not been clinically found because pantothenic acid is widely distributed in foods of the human diet. However, the consumption habits of modern societies with an increased proportion of highly processed food products might result in decreased uptake levels of pantothenic acid in the daily diet. Moreover, for subpopulations with increased needs, such as adolescents, intake is known to be frequently below the estimated safe level stated by the Food and Nutrition Board (*27*, *28*). The adequate intake of pantothenic acid is set at 2−4 mg/day for children (*27*). In the past, the studies have concentrated on processing effects and the storage stability of legumes, meat, and milk powders (*29*−*37*). In further studies, Burger et al. (*38*) and Schroeder et al. (*39*) analyzed pantothenic acid contents in various fruits and also fruit juices after deep temperature storage. Likewise, a loss of pantothenic acid was followed in processed and preserved fruit products by Walsh et al. (*40*). Until now, sea buckthorn berries and related products such as juice and juice concentrate were not investigated regarding pantothenic acid content, its stability, and processing effects on the vitamin content. One of the characteristics of sea buckthorn berries is a low pH value of approximately 2.8 in comparison to other berry fruits. The physiological activity of pantothenic acid is lost by acidic hydrolysis, resulting in two principal cleavage products, namely, the γ-lactone of pantoic acid and β-alanine (*41*). Therefore, a fast degradation of pantothenic acid in sea buckthorn juice matrices was expected due to unfavorable processing conditions such as low pH and the thermal impact of heat pasteurization or the final concentration process of the juice.

The primary objective of the present study was to survey the effects of processing on pantothenic acid stability in sea buckthorn juice and concentrate. A second aim was to assess the influence of nonsterile consumer storage conditions and also accelerated aging conditions in reducing the content of pantothenic acid in sea buckthorn juice. In addition, quantification was investigated by comparison of the validated SIDA with an external standard method (ESTD).

MATERIALS AND METHODS

Chemicals. The hemicalcium salt of (*R*)-pantothenic acid was purchased from Sigma Aldrich (Steinheim, Germany), and sodium acetate was from Merck (Darmstadt, Germany). Calcium [^{15}N,$^{13}C_3$]-(*R*)-pantothenate (**Figure 1**) was synthesized as reported by Rychlik (*21*). For HPLC-ESI-MS analysis, HPLC grade acetonitrile (Merck) and Nanopure (Barnstead, United States) water were used.

Food Samples. Berries of two growing areas of *H. rhamnoides* were collected in south Germany (area 1) and in Romania (area 2) from commercial plantings in September 2005. The samples were stored at −20 °C prior to analysis.

Processing of Sea Buckthorn Juice. The frozen berries were preheated to 8−12 °C before mashing. The mash was subjected to a treatment with pectolytic enzymes 1−2 h at 52 °C and separated into juice and pomace by a decanter machine. The turbid juice product, highly concentrated in pulp and oil, was clarified by a plate separator. Before aseptic filling, the juice was treated in a high-temperature short-time (HTST: 90 °C, 45 s) process and rechilled immediately.

Processing of Sea Buckthorn Juice Concentrate. For the production of sea buckthorn concentrate, the juice was clarified with bentonite (8−12 h, 10−12 °C). After filtration with diatomaceous earth under vacuum, the clear juice was concentrated by thermovacuum evaporation

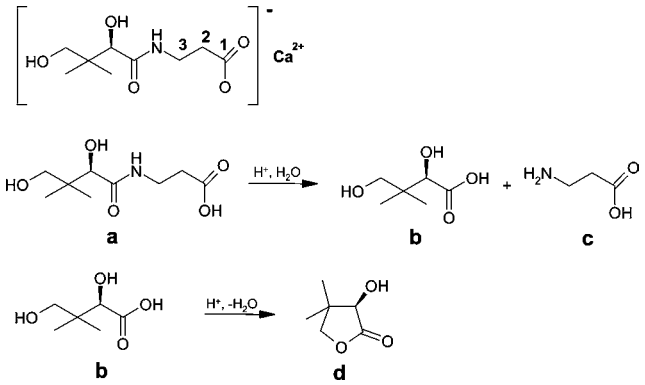

Figure 1. Structure of calcium [^{15}N,$^{13}C_3$]-(*R*)-pantothenate; 1–3, ^{13}C-labeled molecule positions and acidic hydrolysis of pantothenic acid and lactonization of pantoic acid: (**a**) pantothenic acid, (**b**) pantoic acid, (**c**) β-alanine, and (**d**) γ-lactone of pantoic acid.

(five stage evaporator, 80−85 °C). The °Brix value was 65 for clear juice concentrates. Before aseptic filling, the juice concentrate was treated in a HTST process and rechilled immediately. Solely, the concentrate of area 2 was available from the producer for analysis.

Storage Experiments. Sea buckthorn juice was weighed (20 ± 0.02 g) into Falcon tubes (50 mL) and capped with screw tops. The tubes were placed separately in two climatic test chambers (HC 4055, HC 7057) (Heraeus/ Vötsch, Balingen, Germany). Pantothenic acid degradation of sea buckthorn juice (area 1) was studied at 25 and 40 °C for 7 days. Pantothenic acid was analyzed after 72 h and then in continuation after 48 h intervals. For sampling at different time sets, new tubes were always used for investigation. To determine reference values for sea buckthorn juice samples without thermal impact, the pantothenic acid content in frozen juice (−20 °C) was analyzed on the first and on the seventh days of the study, respectively.

Sample Preparation for the Determination of Free Pantothenic Acid in Sea Buckthorn Berries, Juice, and Concentrate Using SIDA or ESTD. After removal of damaged *Hippophaë* fruits, the healthy and still frozen berries were homogenized with a T25 basic ultra turrax blender at 8000 R/min (IKA, Staufen, Germany) in sample assays of 20 g. For the ESTD measurements, samples of homogenized sea buckthorn berries, juice, and concentrate (1 g) were extracted with an aqueous solution of sodium acetate (10 mL, 0.1 mol/L, pH 8.3). For the SIDA analysis, samples of homogenized sea buckthorn berries, juice, and concentrate (1 g) were spiked with a standard solution of [^{15}N,$^{13}C_3$]-(*R*)-pantothenic acid and extracted as mentioned before. The turbid extracts were filtered through 615 1/4 paper (Macherey-Nagel, Düren, Germany) and after passage through a PTFE 0.45 μm syringe filter (Macherey-Nagel) subjected directly to HPLC-ESI-MS-MS.

Pantothenic Acid Determination by HPLC-ESI-MS-MS. For HPLC-ESI-MS-MS analysis, a G1312A binary gradient pump, a G1329A automatic liquid sampler with a cooling system (10 °C), and a G1316A column thermostat (20 °C), series 1100 from Agilent (Waldbronn, Germany), were coupled to the Agilent Ion-Trap XCT Plus LC-ESI-MS-MS system. All HPLC-ESI-MS-MS experiments were performed in positive ionization mode within a mass range between *m*/*z* 50 and 400. The drying gas was nitrogen (flow, 9.0 L/min; 350 °C), and the nebulizer pressure was set to 40 psi. For ionization, the parameters (positive mode) were applied as follows: capillary voltage, +3500 V; current end plate, 22.500 nA; and HV end plate offset, −500 V. Smart parameter settings were as follows: target mass, *m*/*z* 250; compound stability, 100%. Trap parameters were set as follows: trap drive, 31.9; scan delay, 0 μs; accumulation time, 300 ms; scan begin, *m*/*z* 50; scan end, *m*/*z* 400; averages, two spectra; ICC target, 1000000 charge control on.

HPLC-ESI-MS-MS was performed on a 150 mm × 2.1 mm i.d., 3.5 μm, Zorbax 300 Extend C_{18} column (Agilent), and the flow rate was 0.2 mL/min, using a 0.1% formic acid in water (solvent A)−acetonitrile (solvent B) gradient. Initial gradient conditions were 97% A and 3% B, held over for 10 min, followed by a linear gradient within

5 min to 100% B. Thereafter, the eluent was brought back to initial conditions within 5 min and held for 5 min for column equilibration.

Determination of Response Factors for HPLC-ESI-MS and Calculation of Pantothenic Acid Sample Amounts by SIDA and ESTD. Single ion monitoring in the mass traces *m*/*z* 220 and *m*/*z* 224 was used for monitoring both pantothenic acid isotopologues. Solutions of calcium (*R*)-pantothenate and calcium [^{15}N,$^{13}C_3$]-(*R*)-pantothenate were mixed in four mass ratios ranging from 0.4 to 2.5 to give a total volume of 10 mL and a total content of 7 μg. The response factors R_f were calculated on the basis of following equation:

$$R_f = \frac{A_{^{13}C,^{15}N-PA} \cdot m_{PA}}{A_{PA} \cdot m_{^{13}C,^{15}N-PA}}$$

The parameter A_{PA} represents the area of unlabeled pantothenic acid in the mass trace *m*/*z* 220, and by analogy, $A^{13}C,^{15}N-PA$ is the area of labeled pantothenic acid in the mass trace *m*/*z* 224. The amount of labeled pantothenic acid is abbreviated as $m^{13}C,^{15}N-PA$, and m_{PA} is the amount of unlabeled pantothenic acid.

In the case of the SIDA method, calculations of pantothenic acid amounts (*C*) in sea buckthorn juice were carried out by using the following equation:

$$C = \frac{A_{PA-sample} \cdot m_{^{13}C,^{15}N-PA} \cdot R_f}{A_{^{13}C,^{15}N-PA}}$$

The factor $A_{PA-sample}$ stands for the measured area of the sample in mass trace *m*/*z* 220, $A^{13}C,^{15}N-PA$ is the area of labeled pantothenic acid in mass trace *m*/*z* 224, respectively, and $m^{13}C,^{15}N-PA$ is the amount of the labeled pantothenic acid added during extraction. Applying the external standard calibration, the calculation of pantothenic acid amounts in sea buckthorn berries, juice, and concentrate (*C*) required the following equation:

$$C = \frac{A_{PA-sample} \cdot m_{PA}}{A_{PA}}$$

The parameter A_{PA} is the measured area of unlabeled pantothenic acid derived from mass trace *m*/*z* 220. The value for m_{PA} is denoted by the amount of unlabeled pantothenic acid and $A_{PA-sample}$ is the measured area of the sample pantothenic acid in mass trace *m*/*z* 220.

Validation of SIDA and ESTD Calibration Methods. *Linearity, Precision, Recovery, and Limits of Detection (LOD) and Quantification (LOQ) Values.* To assess the validity of ESTD quantification, amounts of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 μg of pantothenic acid were added to sea buckthorn juice (1 g) and analyzed as described before. A calibration function was based on the relationship between peak area and concentration. This relationship was approximated by linear least-square regression, and the correlation coefficients were used as the measurement of linearity. All calibration points were prepared in duplicate and were investigated by duplicate injections of each concentration.

The precision of the methods was assessed by repeatability studies. For this purpose, the standard substances were added to the matrices at amounts of 5.00 (unlabeled) and 4.54 μg/g (labeled). Each sample was prepared and analyzed six times ($n = 6$) within 1 day. The precision was expressed as the relative standard deviation (RSD, %).

Sea buckthorn juice (1 g) with a known pantothenic acid concentration was chosen as the sample for the recovery experiment. A 5.00 μg (unlabeled) amount and 4.54 μg (labeled) of pantothenic acid were added and analyzed six times as mentioned before. The recoveries in percentage (% *R*) were calculated using the following equation:

$$\% R = \left[\frac{(m_F - m_U)}{m_A}\right]100$$

where m_F represents the concentration of pantothenic acid measured in the fortified sample, m_U is the concentration of pantothenic acid in the unfortified sample, and m_A is the amount of pantothenic acid added in the fortified sample.

LOD and LOQ values were calculated according to DIN 32645 (*42*) directly by using the calibration line, which was obtained from analyzing six concentrations. Standard solutions of labeled and unlabeled pantothenic acid ranging from 0.4 to 2.4 μg were added to sea buckthorn juice (1 g) and analyzed as described before. The linearity of the calibration lines was investigated by duplicate sample preparation and duplicate injections of each concentration.

Calculations for the Stability Tests of Pantothenic Acid. Reaction rate constants were determined using the equation for first-order reactions:

$$k = \frac{1}{t} \ln \frac{C_0}{C_t}$$

where the *k* value is the reaction rate constant, *t* stands for the duration of the storage interval, C_0 is the initial concentration of pantothenic acid, and C_t is the concentration after the storage time *t* at a given temperature.

Half-life periods for pantothenic acid at 25 and 40 °C were calculated by the following equation:

$$t_{1/2} = \frac{\ln 2}{k}$$

Activation energies were calculated using the Arrhenius equation (*43*):

$$\ln \frac{k_2}{k_1} = \frac{E_a}{R}\left(\frac{T_2 - T_1}{T_2 \cdot T_1}\right)$$

where the *k* values are the reaction rate constants, *T* is the absolute temperature in Kelvin, *R* is the gas constant (8.314 J/mol K), and E_a is the activation energy. Ln *k* was plotted vs the reciprocal of the absolute temperature, and E_a was calculated from the slope of the line.

RESULTS AND DISCUSSION

Selection of the Extraction Method. In a first series of experiments, extraction with different concentrations of sodium acetate buffer (0.02, 0.1, and 1.0 mol/L) was evaluated for the analysis of sea buckthorn juice samples. The buffer with the lowest capacity resulted in a final pH value in the sample of 3.6 and gave insufficient peak shapes in liquid chromatography. Moreover, the highest ion concentration of sodium acetate buffer caused nonreproducible chromatography (final pH 5.3). Best results were obtained by a dilution ratio of 1 g of sample with 10 mL of sodium acetate solution (0.1 mol/L) resulting in a pH value of 4.7. This method extraction requires only simple sample preparation without further clean up for measuring pantothenic acid in sea buckthorn berries, juice, and concentrate and permits the analysis of a high number of samples within a short time. Thus, this sample preparation procedure is excellently suited for the routine measurement of pantothenic acid.

Comparison of Validation Data of SIDA and ESTD. *Linearity, Precision, Recovery, Response Factor (R_f), and LOD and LOQ Values.* In this study, two quantification methods were developed to analyze pantothenic acid in sea buckthorn berries and products (juice and concentrate) from two different areas. Validation parameters of both methods were compared and are summarized in **Table 1**. The correlation coefficients were 0.9886 (ESTD), 0.9957 (ESTD), and 0.9952 (SIDA) for linearity (**Table 1**). The results of both methods show that there is an excellent correlation between the peak area and the concentrations.

The precisions of measuring samples prepared by additions of the unlabeled and the labeled standards expressed as RSD (%) were found to be 4.80 and 4.77%, respectively, suggesting good precision. The obtained recovery values for the unlabeled and the labeled samples were 99.97 and 101.36%, respectively, showing the accuracy of both methods.

Table 1. Validation Parameters Determined for Pantothenic Acid by SIDA and External Standard (ESTD) Methods for Sea Buckthorn Juice[a]

validation performance criterion	SIDA	ESTD
linearity: correlation coefficients, R^2	0.9952	0.9886[b] 0.9957[c]
linearity range (mg/kg)	0.4–2.4[c]	1.0–6.0[b] 0.4–2.4[c]
precision expressed as relative standard deviation (%) ($n = 6$)	4.77	4.80
recovery rate R (%) ($n = 6$)	101.36	99.97
addition level for recovery and precision studies (mg/kg)	4.54	5.00
LOD (mg/kg)	0.240	0.225
LOQ (mg/kg)	0.794	0.750

[a] n, repetitions of analyses. [b] Linearity range for quantification. [c] Linearity range for calculation of LOD and LOQ.

Table 2. Deviation (%) of the Pantothenic Acid Concentration Calculated by Means of SIDA and External Standard (ESTD) Calibration

food samples	SIDA (mg/kg)	ESTD (mg/kg)	deviation (%)
Hippophaë berries area 1	5.46	5.31	2.75
Hippophaë berries area 2	5.09	4.95	2.75
Hippophaë juice area 1	5.13	4.99	2.73
Hippophaë juice area 2	4.74	4.62	2.53
Hippophaë juice concentrate area 2 (1:6 diluted)	3.92	3.79	3.32

The response factor (R_f) gave the value for $R_f = 0.72$ in the single ion monitoring mode (*m*/*z* 220 and *m*/*z* 224). A comparison of LOD and of LOQ for SIDA and ESTD, presented in **Table 1**, showed similar results. Likewise, the developed SIDA and an ESTD revealed similarly excellent performance data regarding linearity, precision, recovery, and LOD and LOQ values (**Table 1**).

The deviation of the pantothenic acid concentration calculated by means of SIDA and ESTD of less than 3.32% (**Table 2**) was lower than the measured precisions of both methods (SIDA, 4.77%; ESTD, 4.80%) and also proved their equivalence. The SIDA is working without external calibration standards for quantification. For routine and high-throughput analysis, efficiency is much higher than with ESTD. Conceivable influences of potential artifact formation during sample storage are eliminated by applying SIDA. Therefore, time-consuming addition experiments can be omitted.

The literature review reveals only one reference on pantothenic acid in sea buckthorn berries containing 1.5 mg/kg (*1*), which is decisively lower than our data of approximately 5 mg/kg. Although there is no information available, no matter which method was applied, it can be assumed that the cited content was determined by a microbiological assay. The discrepancy of data remains open as a recent method comparison between the microbiological assay and the SIDA revealed only slight differences between the results of both assays (*44*). However, SIDA can be considered the superior alternative, as the identity of pantothenic acid is inherently controlled by mass spectrometry and liquid chromatography−mass spectrometry is less tedious than the microbiological assay.

Storage Stability in Food Systems. The aim of our current study was to evaluate the stability of pantothenic acid in sea buckthorn juice (area 1) under the influence of freezing (−20 °C) and two nonsterile consumer storage conditions (25 and 40 °C for 7 days). The following results of quantification were obtained solely by SIDA.

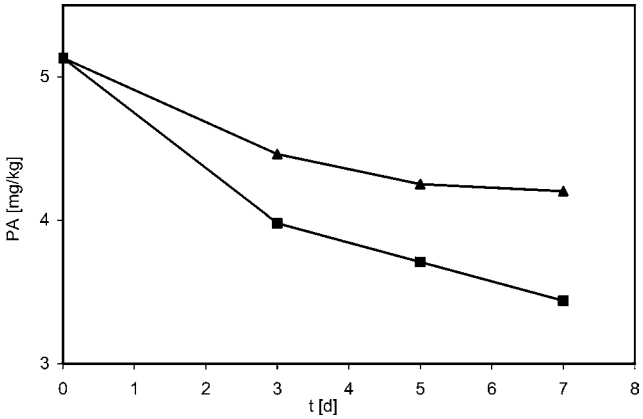

Figure 2. Effect of storage temperature on pantothenic acid degradation in sea buckthorn juice (area 1). Juice storage at 25 (▲) and 40 °C (■) for 7 days.

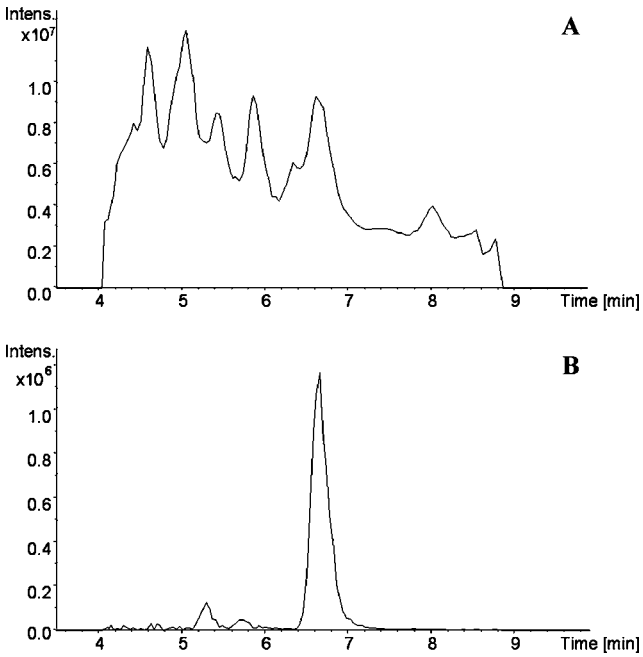

Figure 3. (**A**) HPLC-ESI-MS base peak chromatogram (positive mode) (scan region, *m*/*z* 50–400 amu) of *Hippophaë* juice (area 1) and (**B**) single ion monitoring chromatogram of pantothenic acid at *m*/*z* 220.

To study the effect of freezing (−20 °C), the pantothenic acid content in frozen sea buckthorn juice was measured on the first and on the seventh days of storage, respectively, for the determination of reference values for sea buckthorn juice samples without thermal impact. This kind of storage resulted in no alternation of the pantothenic acid concentration of the juice and confirmed the results of prior studies, which negated a pantothenic acid loss for meat (*32*), spinach (*35*), and fruits (*39*) during freezing.

Figure 2 shows the effect of storage of sea buckthorn juice at 25 and at 40 °C over 7 days. During storage at 25 °C, the pantothenic acid concentration decreased by about 18% after 7 days. In accelerated storage experiments, the stability of pantothenic acid was studied at 40 °C. After storage for 7 days, the amount decreased from 5.13 to 3.44 mg/kg, which is equivalent to a loss of 32.9% and indicated that pantothenic acid is unstable at elevated temperatures. When the logarithm

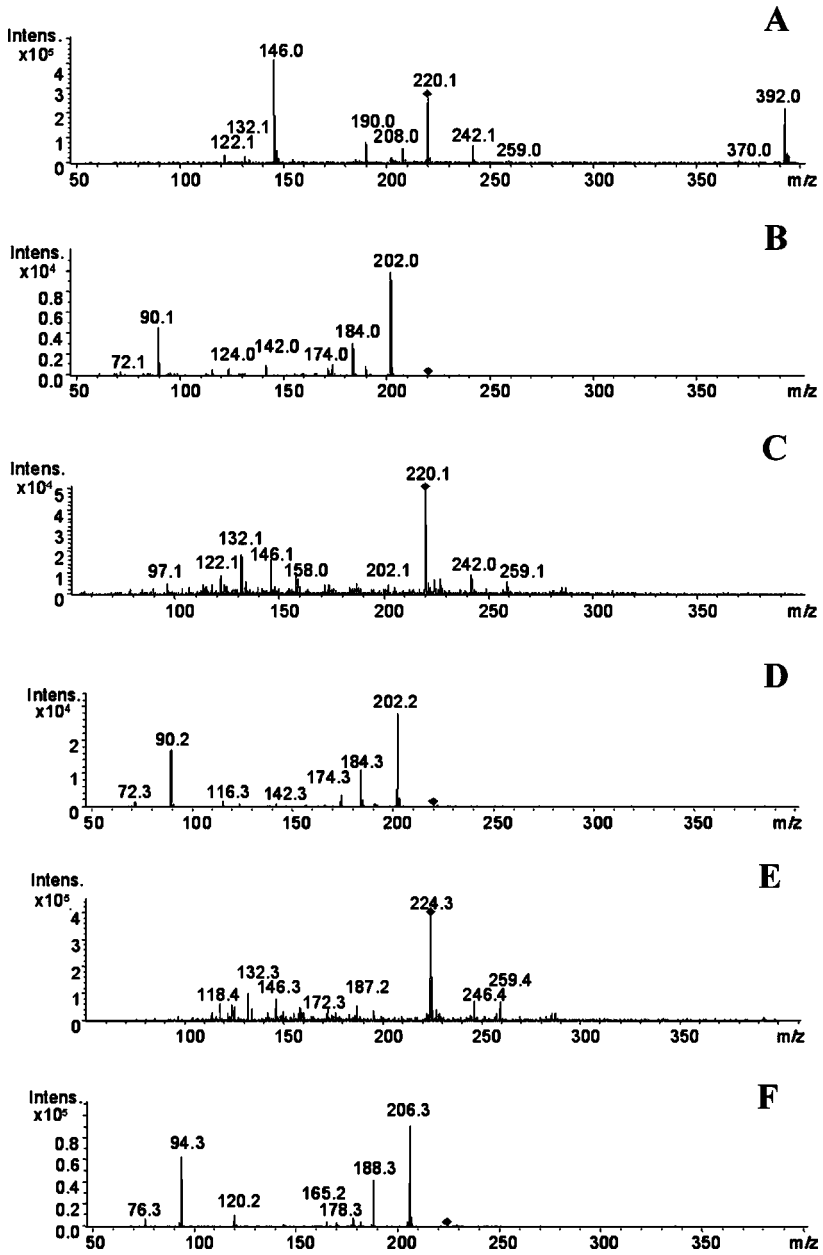

Figure 4. (**A**) Full scan mass spectra of pantothenic acid in sea buckthorn juice (area 1), (**B**) MS-MS spectrum (*m*/*z* 220) of pantothenic acid in sea buckthorn juice, (**C**) full scan mass spectra of pantothenic acid standard solution (unlabeled), (**D**) MS-MS spectrum of pantothenic acid standard solution (unlabeled) with the precursor ion at *m*/*z* 220, (**E**) full scan mass spectra of pantothenic acid standard solution (labeled), and (**F**) MS-MS spectrum of [^{15}N,$^{13}C_3$]pantothenic acid standard solution with the precursor ion at *m*/*z* 224.

of the concentration of pantothenic acid was plotted vs the time of heating at a constant temperature, linear graphs were obtained at each of the studied temperatures, which is characteristic for first-order reactions. Therefore, values for the reaction rate constant *k* were calculated using the standard first-order equation. The average values for *k* at 25 and 40 °C are 0.0373 and 0.0688, respectively. The half-life period of 18.58 days at 25 °C and 10.07 days at 40 °C proved the instability of pantothenic acid under these conditions. This is well in line with the observation of Frost et al. (*45*) and Hamm et al. (*46*), who also found that pantothenic acid destruction in buffered (pH 3.7−4.0, 5, and 6), nonbuffered (pH 3.7−4.0) model systems and in food systems (pH 5.4 and 7.0) at various temperatures (10−100 and 118−143 °C) followed first-order kinetics. Frost and McIntire (*45*) reported an E_a of 19 kcal/mol for pantothenic acid at pH 3.7−4.0 for unbuffered and buffered aqueous systems. Hamm et al. (*46*) demonstrated that a typical Arrhenius plot was obtained from the thermal inactivation data and calculated activation energies (E_a) ranging from 20 to 27 kcal/mol for model systems (pH 4.0−6.0) and from 20 to 38 kcal/mol for food systems at pH 5.4 and 7.0 (meat puree and pea puree). In our study, the calculation of the activation energy values was based on two different temperatures. The determined value for E_a of 7.4 kcal/mol is remarkably lower than the E_a values for pantothenic acid hydrolysis reported in the studies mentioned before (*45*, *46*). This finding was obviously due to the homogeneous distribution of pantothenic acid in a liquid matrix and a pH value of 2.8 for sea buckthorn juice as compared to other matrices. Prior model experiments have shown that solutions of pantothenic acid are most stable between pH 5.5 and pH 7.0 (*47*). Below and above these pH values, it

is thermolabile and shows acidic hydrolysis to β-alanine and the γ-lactone of pantoic acid (*47*−*52*) (**Figure 1**).

Comparing the results of our study with literature data, pantothenic acid loss in sea buckthorn juice at elevated temperatures, a lower pH value and homogeneous distribution appeared higher than in other food systems, such as composite meals or whole milk powder. In 5 years of storage at room temperature (22 ± 2 °C), the pantothenic acid concentration decreased by approximately 50% in canned Dutch Army meals (consisting of vegetables, meat, and pulses) (*31*). Moreover, Ford et al. (*34*) reported that the content of pantothenic acid of whole milk powder declined progressively, by approximately 18% over eight weeks at a storage temperature of 60 °C. Our results proved that a storage of sea buckthorn juice for 7 days at ambient temperature (25 °C) resulted already in a significant degradation of pantothenic acid.

Processing Effects. The pantothenic acid content of berries and juice in area 2 was 6.8 and 7.6% lower than in area 1 (**Table 2**), respectively. After preheating and mashing, a decanter machine was used for the gentle separation into juice and pomace without application of high pressure. In contrast to this, commercial sea buckthorn juice production includes a HTST process (90 °C, 45 s) before aseptic filling. As the measured pH of the juice was as low as 2.8 and due to the heating process, a significant thermal degradation of pantothenic acid in sea buckthorn juice had to be expected. Interestingly, HTST treatment only slightly decreased the concentration of both area juices and resulted in a loss of 6.0% in juice of area 1 and of 6.9% in juice of area 2. This effect could be attributed to the concentration process of water soluble components during juice production, thus compensating in part the degradation of pantothenic acid. During thermovacuum evaporation (five stage evaporator, 80−85 °C) of the juice (area 2) to obtain concentrate, the pH of the latter was reduced to 2.6, which went along with a loss of 23.0%. However, the production process of sea buckthorn juice resulted in a lower degradation than expected when considering the previously described lability toward extreme pH values and thermal impact.

Prior investigations of pantothenic acid in food were focused on the processing effects of animal products, legumes, grains, and cereals (*29*, *30*, *33*, *35*−*37*, *39*). The effects of juice and concentrate production on the pantothenic acid content were not investigated until the present. Generally, a similar or higher loss as compared to sea buckthorn juice was found during processing of other foods. For example, the average retention in the roasted beef loin was about 89%, with an average recovery in the drip of 19% (*30*). In another study, spinach retained 21% pantothenic acid after water blanching as compared with 67% retention after steam blanching. Similar trends were noted for pantothenic acid loss during blanching of broccoli (*35*). Hoppner and Lampi (*36*) investigated the effect of cooking times in conjunction with presoaking procedures in 25 dried legumes and reported a retention of pantothenic acid ranging between 14 and 78%. The average loss from canning fruit juices was as high as 50.5% (*39*).

Mass Spectrometry of Pantothenic Acid in HPLC-ESI-MS-MS. In the present study, mass spectrometric quantification of pantothenic acid was performed by ESI in positive ionization mode resulting in an abundant ion signal for $[M + H]^+$ adducts. Direct injection of sea buckthorn berry, juice, and concentrate extracts to the HPLC-ESI-MS-MS system and rapid elution parameters enabled detection in a complex matrix at a retention time of 6.7 min (**Figure 3**). In the full scan spectrum (*m*/*z* 50−400 amu), pantothenic acid showed an intense base peak for the protonated molecule at *m*/*z* 220 $[M + H]^+$. Quasimolecular ion adducts of alkali metals were also observed under the applied ESI-MS conditions leading to ion signals for $[M + Na]^+$ at *m*/*z* 242 and with lower ion intensity for $[M + K]^+$ at *m*/*z* 259 (**Figure 4A**). ESI-MS-MS fragmentation of *m*/*z* 220 yielded abundant fragment ions at *m*/*z* 202 $[M - 18]^+$ and at *m*/*z* 184 $[M - 36]^+$ by consecutive losses of two water molecules. The likewise intense MS-MS fragment ion signal at *m*/*z* 90 $[M - 130]^+$ is related to the formation of β-alanine (**Figure 4B**). In comparison to the full scan spectrum (**Figure 4C**) and the MS-MS fragmentation (**Figure 4D**) of standard pantothenic acid, the predominant ions at *m*/*z* 146 and *m*/*z* 392 at the retention time of pantothenic acid were assigned to unidentified components of the juice matrix. The highly sensitive ESI ionization in positive mode resulted in excellent ion yields for quantification due to the nitrogen content in the molecule. Moreover, the characteristic MS-MS fragmentation pattern enabled the identification and selective quantification in a complex food matrix such as sea buckthorn juice by means of an external calibration curve (ESTD). By using $[^{15}N,^{13}C_3]$-(*R*)-pantothenic acid as the internal standard, which is detectable by its base signal at *m*/*z* 224 (**Figure 4E**), pantothenic acid can be quantified in sea buckthorn berries, juice, and concentrate even more selectively. The fragmentation path is corroborated by the cleavage of four-fold labeled pantothenic acid to give the ion signal at *m*/*z* 94 corresponding to the $[^{15}N,^{13}C_3]$-β-alanine moiety (**Figure 4F**).

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