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Total Folate Content and Retention in Rosehips (*Rosa* ssp.) after Drying

Lena Strålsjö,*,† Charlotte Alklint,‡ Marie E. Olsson,§ and Ingegerd Sjöholm‡

Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051,
SE-750 07 Uppsala, Sweden, Department of Food Engineering, Lund Institute of Technology,
Lund University, P.O. Box 124, SE-221 00 Lund, Sweden, and Department of Crop Science,
Swedish University of Agricultural Sciences, P.O. Box 44, SE-230 53 Alnarp, Sweden

Folate concentrations in rosehips and commercial rosehip products and factors affecting folate retention during drying were investigated. On the basis of the raw material studied during 3 years, rosehips were shown to be a rich folate source, $400-600 \mu g/100$ g based on dry matter and $160-185 \mu g/100$ g based on the fresh weight (edible part). Rosehips are not often consumed fresh; therefore, drying to produce stable semimanufactures is a crucial step. The degradation of folate was shown to be dependent on the drying time until the water activity was below 0.75. The required drying time was reduced by cutting the rosehips in slices and to some extent also by increasing the temperature. Retention of folate and ascorbic acid was affected by the same factors, and high content of ascorbic acid could provide a possible protection for folate degradation.

KEYWORDS: Folate retention; folic acid; ascorbic acid; freeze drying; air drying; temperature; sliced rosehips; water activity; water content

INTRODUCTION

Folate is a water soluble B vitamin required for all cellular replication and growth. Several health benefits of folate such as prevention of neural tube defects in babies, cardiovascular diseases caused by elevated plasma homocysteine, and certain forms of cancer have been recognized (I). A good folate status may also positively affect cognitive functions (2). The fact that the average daily intake of folate in many Western populations is often far below the recommendations emphasizes the need for a critical evaluation of all kinds of dietary folate sources. In a review by de Bree et al. (3) plant foods (vegetables, fruit, and potatoes) were stated to be the predominant contributors for the folate intake in Europe.

Rosehips (*Rosa* spp.) have traditionally been used as a vitamin supplement or for health food products in many European countries, since the fruits (hips) are a rich source of ascorbic acid. Recent studies have also shown rosehips to be rich in other antioxidants such as carotenoids and phenolics (4, 5). Rosehip extracts also possess high antioxidative capacity in all tested assays (6) as well as antimutagenic effects (7). Nevertheless, there is a lack of reliable information on folate levels in rosehips. The few data found in different European food tables show discrepancies ranging from 2 to 200 μ g folate per 100 g dried rosehips (8, 9). The folate content in various types of powdered rosehip soups, popular desserts in Sweden, is reported to be around zero (9). Factors such as heat, light, and oxidation during processing might explain the decrease in folate content in these products. Processing methods are probably important with respect to maintaining the content of this vitamin. The reported content of ascorbic acid in the Swedish food table is 2.7 mg/g (9), and in a study by Gao et al. (6), ascorbic acid levels up to 60 mg/g were observed. The content of ascorbic acid is known to stabilize various folate forms in liquid model systems (10) and is also suggested to be related to folate stability in foods (11).

This study was designed to obtain reliable data for the folate content in rosehips and to compare different factors affecting folate retention in rosehips during various procedures of drying. Traditionally, rosehips are dried whole at a temperature of 70 °C, a drying process requiring over 10 h (12, 13). Alklint (12) showed that the retention of ascorbic acid increased when cutting the rosehips into slices prior to drying to reduce the drying time. Therefore, in the present study, the effect of sample size and temperature on folate retention during air drying was investigated. Freeze drying was used as a reference method. Water activity (a_w) and moisture content were analyzed as parameters of drying efficiency. The total content of ascorbic acid was quantified by high-performance liquid chromatography (HPLC) as a possible parameter related to folate stability. Total folate concentrations were quantified using a modified commercial radioprotein binding assay (RPBA) optimized and validated for

^{*} To whom correspondence should be addressed. Tel: +46-18-671453. Fax: +46-18-672995.

[†] Department of Food Science, Swedish University of Agricultural Sciences. [‡] Lund University.

[§] Department of Crop Science, Swedish University of Agricultural Sciences.

Table 1. Content of Folate (μ g/100 g DM and FW) and Ascorbic Acid (mg/g DM) in the Edible Part of Sliced Fresh Rosehips^a

		DM ^c	folate ^d (µg/100 g)		ascorbic acid ^e (mg/g)	
sample ^b	year	(g/100 g)	FW	DM	DM	
<i>R. dumalis</i> mean <i>R. rubiginosa</i>	1999 2000 2001 1999 2000 2001	31.8 27.8 30.7 38.5 25.7 29.8	172 ± 13 167 ± 18 184 ± 15 174^{f} 164 ± 8 na 158 ± 7	$542 \pm 40 600 \pm 64 599 \pm 49 580' 427 \pm 20 na 531 \pm 24$	43 ± 1 na 58 ± 2 33 ± 1 na 41 ± 3	
mean			161 ^{<i>f</i>}	479 ^{<i>f</i>}		

^{*a*} FW, fresh weight; DM, dry matter; na, not analyzed. ^{*b*} All samples were freezedried prior to folate quantification by RPBA according to Strålsjö (*14*) and ascorbic acid quantification with minor modifications according to Wimalasiri and Wills (*16*). ^{*c*} DM in the edible part of fresh rosehips. ^{*d*} Folate values are means of triplicates ± standard deviation. ^{*e*} Total ascorbic acid values (ascorbic acid and dehydroascorbic acid) are means of duplicates ± standard deviation. ^{*f*} Mean value from the different years.

folate analysis in berries (14). This optimized RPBA is a fast and easy method suitable for analyzing total folates in foods containing mainly 5-CH₃-H₄folate (14–16). It has, with satisfying results, been compared to a robust HPLC method presented by Jastrebova et al. (17). The same HPLC method has also been used to characterized the folates in rosehips, and the main folate form was shown to be 5-CH₃-H₄folate (>95%) (14, 15).

MATERIAL AND METHODS

Samples and Study Design. Two different rosehips, *Rosa rubiginosa* and *Rosa dumalis*, were obtained from a local grower (O. Torstensson Farm, Grödby, Sweden) in the years 1999, 2000, and 2001. Fully ripe rosehips were harvested from the same row in the field, picked by hand, stored, and packed in plastic bags in the dark at 4 °C in a refrigerator until use. The fresh rosehips had a volume density of 520 g/L, a length of 19-32 mm, a diameter of 11-16 mm, and a thickness of the flesh of 2-3.5 mm.

Drying of whole or sliced roschips was performed during a period of 4-10 days after harvesting. The roschips were sliced in 2 mm thick slices in a domestic food processor (Moulinex kitchen slicer), with an edged knife. After the roschips were sliced, the kernels were easily removed before drying. Whole, respectively sliced roschips (2 mm slices), were dried in a laboratory convective dryer (*18*) at constant temperatures between 70 and 90 °C. The relative humidity was between 8 and 10% at a temperature of 85 °C, and the used air velocity was 4.5 m/s except for the drying of the whole roschips, where it was 3.0 m/s. Each drying procedure started from an initial weight of 350-370 g of fresh roschips, and the weight was continuously registered. The original dry matter (DM) of the material varied between 25 and 40 g/100 g (see DM in **Table 1**). Each drying procedure was ended when the balance in the drier showed 96 g/100 g DM. The dried samples were stored at -20 °C until folate and ascorbic acid analyses.

Reference Drying Procedure. Freeze drying (~72 h, 0.2–0.4 mmHg) was used as a reference method (Nino lab, pilot dryer, Sweden). The temperature increase was set to 1 °C/h from -20 °C to maximum +20 °C. Initially, analyses showed no variations in folate content after freeze drying of whole or sliced rosehips. Therefore, to reduce the drying time, all control samples were cut into slices prior to freeze drying.

We observed freeze drying to be essential prior to folate analyses to obtain results with satisfactory repeatability achieving coefficient of variations (CV%) below 10%. This procedure was required due to the strong waxy cuticle cover of rosehips, hard to homogenize unless the samples were completely dried. Moreover, we observed higher folate concentrations in freeze-dried samples as compared to fresh indicating the technique for folate extraction not to be efficient enough for fresh rosehips.

Drying Procedures. To study the effects of temperature on folate retention during drying, sliced rosehips of *R. dumalis* and R. *rubiginosa* were dried at four different temperatures (70, 80, 85, and 90 °C). The product temperature (sliced rosehips) during the experiments varied from approximately 56 °C at a drying temperature of 70 °C to 65 °C at a drying temperature of 90 °C. The effect of cutting rosehips into slices prior to drying was checked by drying rosehips of both cultivars, whole at 70 °C and sliced at 85 °C.

To study the kinetics of folate degradation during drying, sliced rosehip samples of *R. dumalis* were dried in a randomized order at 85 °C for 5, 10, 15, 25, 45, 70, and 100 min. The weight of the samples was continuously logged, and the DM and water activity were examined for each set of the partially dried sample. The original DM of the rosehips was 28 g/100 g. After the air drying procedure, all samples were subsequently dried according to the reference drying procedure, prior to folate analysis.

Commercial Products. Two commercial rosehip products (dessert soups), Ekströms nyponsoppa original (A) and Ekströms extra prima nypon soppa (B) were kindly provided by Procordia Foods, Eslöv, Sweden, for folate quantification. Rosehip soup A was made of dried rosehips imported from Chile and contained 9% rosehips, and rosehip soup B was made of rosehip purée from Germany and contained 15% rosehips based on fresh weight (FW). Both products, with a shelf life of 7 months, contained 200–250 kJ, 13–15% carbohydrates (sugar), and <0.5% protein/fat, and both were fortified with ascorbic acid to a content of >25 mg/100 mL. The water content was approximately 90% (9).

Chemical Analyses. Folate Analysis. Dried rosehips were ground in an ice-cold mortar into powder and stored at -20 °C no longer than a week. Dried samples (0.3 g) or rosehip soup (4 g) were extracted and enzyme-treated in triplicate using chicken pancreas conjugase as described by Strålsjö et al. (15). A modified commercial RPBA kit (SimulTRAC-SNB Radioassay kit; Vitamin B12 [57Co]/folate [125I] (ICN Pharmaceuticals Inc., Costa Mesa, CA)) with external calibration, (6S)-5-CH₃-H₄folate, was used for quantification of total folate (14). The standard (0.5-10 ng/mL, purity corrected) and samples were diluted in 0.1 M phosphate buffer, pH 6.1, containing 1% (w/v) ascorbic acid. As no disturbing effect by the rosehip matrix was shown during the RPBA quantification, no sample clean up was included. The precision of the optimized method including sample pretreatment and RPBA quantification showed CV% below 8% (interassay), and the recovery was 90-106%. Initially, samples from 1999 had been quantified using a (6R,S)-5-CH₃-H₄folate standard calibration (15). To be able to compare results from different assays after method optimization, these results were recalculated against (6S)-5-CH₃-H₄folate standard used in the optimized assay procedure (14).

Ascorbic Acid Analysis. Samples for analysis of ascorbic acid were homogenized in an ice-cold mortar in dim-green light and extracted in 2% m-phosphoric acid. The samples were centrifuged at 16 500g for 15 min at 4 °C, and the supernatant thereafter was filtered through a C18 Sep-Pak column. The first 3 mL were discarded, and an aliquot of the following 1 mL was used for HPLC quantification. The HPLC system used consisted of a Waters 600 pump, a Maraton autosampler with a rheodyne injector, and a Hewlett-Packard UV-1100 detector. The solvents for separation and wavelength used for monitoring the ascorbic acid were used with some modifications according to Wimalasiri and Wills (19). Waters Carbohydrate analysis 3.9 mm \times 300 mm column with a Waters C18 precolumn was used. The separation was performed with isocratic running 1.2 mL/min at room temperature for 5 min. The mobile phase was acetonitrile with 35% (v/v) 15 mM NH₄-PO₄, pH 4.3, adjusted with 1 M H₃PO₄. Detection was carried out at 248 nm. The peak of ascorbic acid in the samples was identified comparing the retention time with ascorbic acid standard. Integrated peaks were calculated by comparison with an external standard solution of known concentrations. The total ascorbic acid concentration was determined using a reduction procedure according to Esteve et al. (20).

DM and Water Activity. The DM was determined after drying the sample for 16 h in a vacuum oven (Forma Scientific, Germany) at

70 °C and 100 Pa of vacuum. The water activity was measured in Rotronic Hygroskop DT (Rotronic Ag, Switzerland) at 22 °C.

Calculations and Statistical Analysis. Results were presented as mean values from triplicate \pm standard deviation based on DM or FW. The retention of both nutrients was calculated from the means of triplicates or duplicates according to the following equation:

nutrient retention (%) =

$$nutrient_{drvineprocedure}/nutrient_{referencedrving} \times 100$$

Statistical analysis was performed with Tukey's pairwise comparison ($\alpha = 0.05$) using the software Minitab release 13 (Minitab Inc., State College, PA). Significant variations were considered from p < 0.05.

RESULTS AND DISCUSSION

Folate Content in Rosehips. The main purpose of this study was to investigate the level of folate in Swedish rosehips. Folate concentrations in two rosehips, R. dumalis and R. rubiginosa, yielded between 427 μ g/100 g DM and 600 μ g/100 g DM (edible part) after the reference drying procedures (Table 1). Corresponding figures based on FW were 158 μ g/100 g and 184 μ g/100 g, respectively. A high variation in water content was observed in samples from different years. Folate concentrations expressed as $\mu g/100$ g DM were significantly higher in R. dumalis than in R. rubiginosa (p < 0.05). Both sources showed a higher content in 2001 as compared to 1999, indicating seasonal variations from weather and growing conditions to affect the folate levels. Similar observations were made comparing ascorbic acid content within both sources of rosehips and samples from different years (R. dumalis, 43-58 mg/g DM, and R. rubiginosa, 33–41 mg/g DM) (Table 1).

Folate data published in national food tables today are unreliable presenting folate content in the edible part of dried rosehips between 2 and 210 μ g/100 DM (8, 9). In an earlier study by our group, we reported folate concentrations in fresh rosehips R. dumalis grown at Balsgård, Sweden, to be around 100 μ g/100 g FW (14). More profound studies regarding growing conditions and postharvest handling are required to obtain reliable data on folate content in rosehips. Procedures for sample pretreatment prior to folate quantification are also needed for further investigation. However, on the basis of results in this study, we can conclude that rosehips are a rich folate source. Obtained concentrations of ascorbic acid in our study were in the same range as earlier studies (6, 21). Gao et al. (6)reported ascorbic acid contents in R. dumalis between 20 and 40 mg/g DM and in R. rubiginosa between 10 and 25 mg/g DM.

Folate Retention in Rosehips during Drying. During air drying, folate retention was shown to be dependent on the drying time influenced by cutting of rosehips into slices and to some extent also the drying temperature (Tables 2 and 3). Even if folate degradation was shown to be affected by temperature (22), the effect of shorter drying time at a higher temperature was larger than the increased degradation because of the temperature. The required length of drying time could be reduced by 40% using a temperature of 90 °C instead of 70 °C (Table 2). A higher retention of both folate and ascorbic acid was observed after cutting the rosehips into slices and increasing the temperature (Table 3). Similar drying times for rosehips have been recommended earlier for whole berries (12, 13). Ochoa et al. (13) reported a stronger effect from temperature on length of drying time than from the two parameters velocity and relative humidity. However, they did not investigate the effect of cutting the samples into slices. In our study, the cutting of rosehips

Table 2. Content and Retention of Folate (μ g/100 g DM) and Ascorbic Acid (mg/g DM) in the Edible Part of Sliced Rosehips Air-Dried at Different Temperatures

	drying		DM ^b	folate ^c		ascorbic acid ^d	
rosehips ^a	(°C)	drying	(g/100 g)	μg/100 g DM	% ^e	mg/g DM	% ^e
R. dumalis	*	*	100	542 ± 40	100	43 ± 1	100
R. rubiginosa	70	2 h 40 min	96.4	420 ± 108	77	38 ± 2	89
	80	2 h 20 min	95.9	505 ± 87	93	38 ± 2	90
	85	1 h 55 min	97.0	515 ± 88	95	39 ± 2	90
	90	1 h 45 min	93.6	450 ± 49	83	38 ± 1	90
	*	*	100	427 ± 20	100	33 ± 1	100
	70	2 h 50 min	95.2	352 ± 27	82	32 ± 0	97
	80	2 h 20 min	95.0	374 ± 64	88	31 ± 0	92
	85	2 h 00 min	97.4	359 ± 31	84	31 ± 1	94
	90	1 h 45 min	95.4	392 ± 31	92	30 ± 1	89

^{*} For 72 h, standard freeze drying procedure, set to 100%. ^{*a*} Rosehips harvested 1999. ^{*b*} DM in edible part of dried rosehips. ^{*c*} Folate values are means of triplicates \pm standard deviations analyzed according to Strålsjö et al. (*14*). ^{*d*} Total ascorbic acid values (ascorbic acid and dehydroascorbic acid) are means of duplicates \pm standard deviations analyzed with minor modifications according to Wimalasiri and Wills (*16*). ^{*e*} Nutrient retention (%) = nutrient_{dryingprocedure}/nutrient_{referencedrying} × 100.

Table 3. Content and Retention of Folate (μ g/100 g DM) and Ascorbic Acid (mg/g DM) in Whole or Sliced Rosehips after Different Air Drying Procedures

	drying procedure	drying	folate ^{b,d,e}		ascorbic acid ^{c,d,e}	
rosehips ^a	(°C)	time	μg/100 g	%	mg/g	%
R. dumalis	*	*	$599 \pm 49^{\text{A}}$	100	$58\pm2^{\text{A}}$	100
	85, slices	1 h 40 min	$515\pm09^{\mathrm{AB}}$	86	48 ± 1^{B}	83
	70, whole	11 h	435 ± 33^{B}	72	27 ± 1 ^C	47
R. rubiginosa	*	*	531 ± 24^{A}	100	41 ± 3^{A}	100
	85, slices	1 h 40 min	416 ± 10 ^B	78	37 ± 1^{A}	90
	70, whole	11 h	391 ± 17 ^B	74	19 ± 1^{B}	46

^{*} For 72 h, standard freeze drying procedure, set to 100%. ^{*a*} Rosehips harvested 2001. ^{*b*} Folate values are means of triplicates ± standard deviations analyzed according to Strålsjö et al. (*14*). ^{*c*} Total ascorbic acid values (ascorbic acid and dehydroascorbic acid) are means of duplicates ± standard deviations analyzed with minor modifications according to Wimalasiri and Wills (*16*). ^{*d*} Different superscript letters indicate significance (*p* < 0.05). ^{*e*} Nutrient retention (%) = nutrient_{dryingprocedure}/nutrient_{referencedrying} × 100.

into slices reduced the required drying time from 11 h to 1 h and 45 min (**Table 3**).

The most effective drying procedure tested in our study regarding optimal folate retention was using sliced rosehips and high temperature. The same drying procedure was also superior for retention of ascorbic acid, which also was reported earlier when studying ascorbic acid retention during drying in whole, halved, and sliced rosehips (12). The degradation of ascorbic acid in low moisture food systems has found to be influenced by the water activity down to water activities as low as 0.1, depending on temperature (23). Therefore, we aimed to control whether the available water in the product, expressed by water activity, also affected the rate of folate degradation. Folate concentrations (μ g/100 g DM), water content (g/100 g DM, %), and water activity $((0-1) \times 100)$ were plotted as a function of time during air drying at 85 °C between 5 and 100 min (Figure 1). The results showed that folate degradation in rosehips is high when the water activity is exceeding 0.75. This was also demonstrated by depicting folate retention and water content as a function of water activity (Figure 2).

The degradation of folate was largest at the high water activities during the first 25 min (Figure 1). González-Martínez,

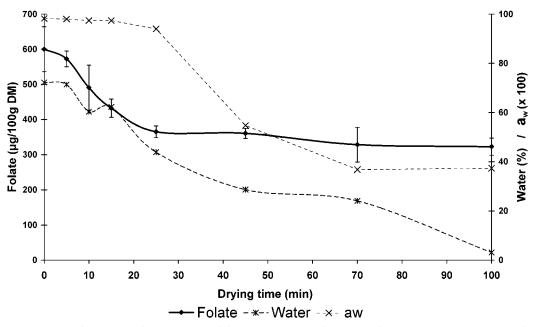
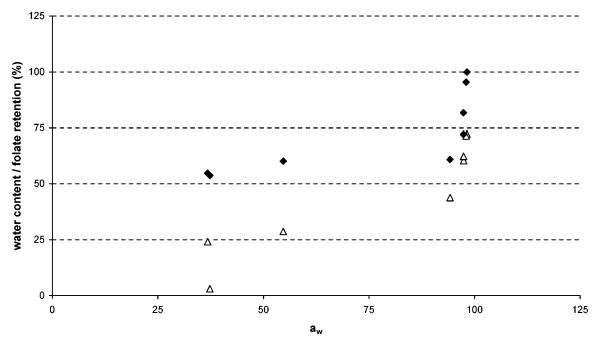


Figure 1. Folate concentrations (μ g/100 g DM), water content (%), and water activity ($a_w \times 100$, %) in sliced rosehips *R. dumalis* (year 2000) during air drying (85 °C) for 100 min.



△ water content ◆ folate retention

Figure 2. Folate retention (%, \blacklozenge) and water content (%, \bigtriangleup) as a function of water activities (a_w) × 100 (22 °C) reached after 5, 10, 15, 25, 45, 70, and 100 min of air drying (85 °C) of sliced rosehips *R. dumalis* (year 2000).

Dejmek, and Gekas (24) have shown that the cell walls in plant material are intact at temperatures below 45 °C. The time to reach a temperature above 45 °C in the flesh of the rosehips during drying in our study was approximately 3 min. During the following 7 min, the temperature increased to 65 °C and the temperature of the flesh was then kept at 65 °C until the water content was below 45%. This means that the time between 45 and 65 °C in our study will be 22 min. After 25 min, the folate retention was around 65%. Calculated according to figures presented by Barret and Lund (22) for degradation of folate in the presence of oxygen at 60 °C, this corresponds to a retention of 63%. Such good correlation is surprising since the temperature and water activity in the sample changes during drying

and the oxygen transportation in the tissue are limited. The thermal destruction of the folates in the cell structures might also be influenced by degradation of other different cell components during the drying process. Folate in plant cells has been found to be localized mainly in the mitochondria, the cytosol, and the nucleus (25).

After 25 min, as mentioned above, the folate retention was around 65% and the water content had dropped from approximately 75 to 44%, but more importantly, the water activity was quickly dropping from around 0.95 to 0.75. Thereafter, the folate retention was relatively stable, remaining at 54% after 100 min when the drying procedure was interrupted at a water activity of 0.37 and a water content of 3%.

Folate Retention in Commercial Rosehip Soups. In two commercial rosehip soups (Ekströms original and Ekströms extra prima), the folate content was $11 \pm 1 \,\mu g/100$ g FW and $16 \pm 2 \,\mu g/100$ g FW, respectively. Calculating theoretical folate retention using folate concentrations in fresh rosehips obtained in our study (~160 $\mu g/100$ g) and eq 1 (material and methods), the folate retention in both products is 76 and 66%, respectively. Expressed as folate content per serving portion (approximately 250 g), rosehip desserts provide about 10% of the recommended daily intake (25–40 μg).

On the basis of the raw material studied over 3 years, rosehips were shown to be a rich folate source. However, rosehips are not consumed fresh. Therefore, drying to produce stable semimanufactures is a crucial step, especially when considering retention of nutrients. The folate retention in rosehips during different drying procedures as checked in our study was inconsistent. Drying of rosehips cut into slices at 85 °C showed variable retention from 50 to 95% in the three different studies from 1999 to 2001. The reasons for this high variation need further investigations. One explanation might be that the DM content varied between the years and the differences might be an effect of different environmental conditions in the year 2000 with a summer with very high precipitation (26). The folate stability was shown to be dependent mainly on the drying time until the water activity was reduced below 0.75. The length of drying time was reduced by cutting rosehips into slices and to some extent also by increasing the temperature. The levels of ascorbic acid seemed to follow the same pattern as the folate levels. However, to gain reliable knowledge on folate retention in rosehips and also other berries, more systematic studies regarding factors affecting folate stability are required.

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