

Folate Content in Strawberries (*Fragaria* × *ananassa*): Effects of Cultivar, Ripeness, Year of Harvest, Storage, and Commercial Processing

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Folate concentrations in strawberries and folate retention during storage and commercial processing of strawberries were investigated. No previous study has focused on the effects of cultivar, ripeness, and year of harvest of strawberries with respect to the folate content. This study showed the folate concentration in strawberries to significantly depend on all of these different factors. Total folate was quantified using a modified and validated radioprotein-binding assay with external calibration (5-CH₃-H₄folate). Folate content in 13 different strawberry cultivars varied from 335 μ g/100 g of dry matter (DM) for cv. Senga Sengana to 644 μ g/100 g of DM for cv. Elsanta. Swedish harvests from 1999 and 2001 yielded higher folate concentrations than did the harvest from 2000, and the grade of ripeness affected the folate content in strawberries. This study indicated high folate retention in intact berries during storage until 3 or 9 days at 4 °C (71–99%) and also in most tested commercial products (79–103%). On the basis of these data fresh strawberries as well as processed strawberry products are recommended to be good folate sources. For instance, 250 g (fresh weight) of strawberries (~125 μ g of folate) supplies ~50% of the recommended daily folate intake in various European countries (200–300 μ g/day) or 30% of the U.S. recommendation (400 μ g/day).

KEYWORDS: Folate; folic acid; strawberries; *Fragaria* × *ananassa*; cultivar; ripeness; food processing; folate retention; radioprotein-binding assay (RPBA)

INTRODUCTION

Folates are a group of essential dietary compounds referring to all derivatives of tetrahydrofolic acid, a water-soluble B vitamin (**Figure 1**). This vitamin is necessary for cell replication and has an important role in the prevention of neural tube defects (I, 2) and possibly coronary heart diseases (3-5). There are also indications for positive effects of a good folate status for cognitive functions (6) and on prevention of certain forms of cancer (7). In a review by de Bree et al. (8) it was stated that the predominant contributors to folate intake in Europe are plant foods, for example, vegetables, fruits, and potatoes. Even in countries from northern Europe where plant food consumption is lower than in a Mediterranean diet, plant foods are estimated to contribute 45% of the total folate intake of adults (9-12).

The sensitivity to oxidative degradation enhanced by oxygen, light, and heat, resulting in splitting of the molecule into biologically inactive forms, makes folates vulnerable to losses during food handling. The very limited information available

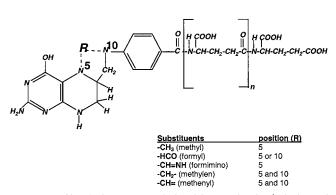


Figure 1. Chemical structure of the folate molecule (polyglutamyl tetrahydrofolate).

in this area was comprehensively reviewed by Hawkes et al. (13) and carefully supplemented with a more recently published paper by Scott et al. (14). In these reviews it is suggested that large processing losses in vegetables and legumes occur simply by leaching of folates into surrounding water used for washing, blanching, canning, or cooking and not through oxidation. Fruits and berries often are consumed fresh or only slightly processed, so losses due to leaching or any adverse effects of heat and

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oxidation are a minor risk. Furthermore, in fruit and berry products such as jam, marmalade, desserts, and canned products mainly the whole product is consumed, including also the accompanying medium. Good folate sources are strawberries and tropical and citrus fruits, which are all reported to contain between 50 and 100 μ g of folate/100 g of fruit (15).

Berries have recently been revealed to be a good source of various nutrients, focusing especially on compounds with antioxidant capacity such as ascorbic acid, carotenoids, tocopherols, flavonoids, and other phenolics. The influence of cultivar, growing conditions/area, ripening, and postharvest handling on the levels of various nutrients is of increasing interest, and several studies on especially antioxidants have been published (16-20). Strawberries are of particular interest due to their high content of antioxidants and as an important berry both for fresh consumption and for the food industry. Strawberries are cultivated in all arable regions of the globe from the Arctic to the tropics, and the quantities grown have risen steadily, doubling in the past 20 years to a world production of >2.5 million metric tons in 1997 (21). Today, there are few studies regarding the folate content of strawberries (22-24) and only one discussing folate stability (25). No studies have been found regarding variations of folate content in fresh strawberries or folate retention after processing. There is a lack of information on folate levels in all kinds of food plants during development and ripening and very few published studies on the effects of harvest and postharvest handling.

Therefore, this study was designed to quantify folate concentrations in fresh strawberries and to investigate folate retention after storage and different types of commercial processing. Variations in folate content have been studied at different grades of ripeness, in different cultivars, and between different years of harvest. Folate retention during storage of fresh berries and in commercial strawberry products such as jam, stewed desserts, and syrup has been controlled. A modified commercial radioprotein-binding assay (RPBA) optimized and validated for berries and milk has been used for total folate quantification (26). It is a fast and easy method suitable for analyzing total folates in strawberries because strawberries mainly contain 5-CH₃-H₄folate (23, 26).

MATERIALS AND METHODS

Sampling and Study Design. All Swedish strawberry cultivars, that is, Honeoye, Senga Sengana, BFr 77 111 Balsgård, Eros, Polka, Bounty, Kent, Elvira, Emily, Dania, and Elsanta, were collected from Grödby, Bromölla, except cv. Zefyr, which was collected from Fredrikslund, Uppsala. Strawberry samples of each cultivar (3000–4000 g) were harvested at the end of June at the beginning of the Swedish strawberry season in 1999, 2000, and 2001. After harvest, all samples were immediately brought to the laboratory, the calyces removed, and the strawberries frozen on trays until the central temperature was -20 °C. Thereafter, the berries were stored subsampled (200 g) under vacuum in plastic bags at -20 °C until analysis. Frozen strawberries of the cv. Senga Sengana imported from Poland were also included in this study. All strawberries and products for this study were kindly provided by Procordia Foods, Eslöv, Sweden, and transported frozen to Uppsala.

The cv. Honeoye was used for studying the effect of ripening on the folate content. Berries were harvested at three different ripening stages: unripe (white/pink), ripe (red), and fully ripe (dark red). All samples were picked on one occasion from the same row in the field and thereafter treated as described above. The study was repeated over three years.

To check folate retention during storage of fresh berries, cvs. Honeoye and Zefyr were used. Freshly harvested strawberries were stored unwrapped and subsampled in 1 L cardboard boxes at room temperature (daylight) or 4 $^{\circ}$ C (darkness) for between 0 and 9 days.

Thereafter, the strawberries were frozen on trays at -20 °C and subsampled into 500 g in plastic bags at -20 °C until analysis.

Folate losses due to processing were checked by analyzing different commercial strawberry products such as jam, stewed sauce desserts, and syrup obtained from Procordia Foods (**Table 4**). For each product the folate content in corresponding raw material (frozen strawberries) was also analyzed. Retention of folate in the products was presented as percent folate remaining in the product after processing and calculated according to the equation

folate retention (%) =

 $folate_{product}/folate_{raw material} \times conversion factor$

"Conversion factor" is that of strawberry content in the product.

To be able to compare samples on a dry matter (DM) basis, the moisture content of the strawberries was checked in duplicate according to AOAC method 920.151 (27). Strawberry samples (500 g) were homogenized, and samples (20 g) were weighed out and dried in a vacuum oven at 70 °C under a pressure of ≤ 100 mmHg until the weight difference was ± 2 mg.

Folate Standards and Chemicals. The standard substance (6*R*,*S*)-5-methyltetrahydrofolic acid (5-CH₃-H₄folate, calcium salt) was obtained from Dr. Schircks Laboratories (Jona, Switzerland), and purity was controlled as described by van den Berg et al. (28) using molar extinction coefficients at pH 7 reported by Eitenmiller (29). Standard solutions (200 μ g/mL) for calibration purposes were stored at -80 °C in phosphate buffer (pH 6.1) containing 1% l-(+)-ascorbic acid (AA) and 0.1% 2-mercaptoethanol (MCE). AA, dipotassium hydrogen phosphate, MCE, potassium hydrogen phosphate, and potassium hydroxide were purchased from E. Merck (Darmstadt, Germany). Lyophilized chicken pancreas (folate conjugase, CP) was purchased from Difco (Detroit, MI). All chemicals were of analytical purity, and the water used was of Milli-Q grade.

Analysis. Extraction and enzyme treatments using CP were performed as described by Strålsjö et al. (26). Homogenized strawberry samples (2 g) were extracted in triplicates for 12 min in a boiling water bath, in freshly prepared phosphate buffer (pH 6.1) containing 1% AA and 0.1% MCE. Samples were thereafter incubated with 2 mL of CP suspension (5 mg/mL) at pH 6.1 in a shaking water bath at 37 °C for 3 h in the dark. Placing samples in a boiling water bath for 5 min inactivated the enzyme. After centrifugation, supernatants were made to volume (50 mL) and stored at -20 °C until quantification. When folate concentrations in the samples were calculated, corrections were made for endogenous folates in the CP suspension. A modified commercial RPBA kit [SimulTRAC-SNB radioassay kit; vitamin B12 [57Co]/folate [125I] (ICN Pharmaceuticals Inc., Costa Mesa, CA)] with external calibration (5-CH₃-H₄folate) was used for quantification of total folate (26). The standards (0.5-10 ng/mL) and samples were diluted in freshly prepared phosphate buffer (pH 6.1) containing 1% AA. The precision of the method including sample pretreatment and RPBA quantification showed coefficients of variation below 8% (interassay) and addition of 5-CH₃-H₄folate standard prior to extraction resulted in recoveries of 90-106%.

Statistical Analysis. The results were presented as mean values from triplicates \pm standard deviations (SD) based on both dry (DM) and fresh weight (FW). Statistical analyses were 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

Effects of Cultivar and Year of Harvest. The total folate contents in 13 different strawberry cultivars harvested in years 1999, 2000, and 2001 are presented in **Table 1**. The folate content varied from 335 to $644 \ \mu g/100$ g of DM and from 30 to $69 \ \mu g/100$ g of FW. The water content varied from 87 to 93%. All cultivars except Zefyr and Senga Sengana (Poland) were grown at the same farm under the same conditions in southern Sweden. The results in **Table 1** were placed in

 Table 1. Total Folate Content in 13 Different Strawberry Cultivars

 Harvested in 1999, 2000, and 2001^a

	harvest	DM ^c	folate content (µg/100 g	
cultivar ^b	year	(g/100 g)	DM	FW
Elsanta	1999	10.7	644 ± 56	69 ± 6
S. Sengana (Pol)	1999	8.5	638 ± 28	54 ± 2
Honeoye	2001	9.8	631 ± 24	62 ± 2
Honeoye	1999	7.1	591 ± 40	42 ± 3
BFr 77111	1999	7.7	533 ± 42	41 ± 3
Honeoye	2000	10.0	528 ± 17	53 ± 2
Bounty	2000	9.1	525 ± 12	48 ± 1
Emily	2000	9.7	521 ± 6	51 ± 1
Eros	2000	9.2	501 ± 12	46 ± 1
Zefyr	2000	10.3	497 ± 13	51 ± 4
BFr 77111	2000	9.6	481 ± 11	46 ± 1
S. Sengana (Swe)	1999	7.7	479 ± 36	37 ± 3
Dania	2000	10.7	457 ± 16	49 ± 2
Elsanta	2000	11.7	454 ± 31	53 ± 4
S. Sengana (Swe)	2000	7.0	426 ± 8	30 ± 1
Elvira	2000	11.4	424 ± 10	48 ± 1
Kent	2000	10.6	409 ± 16	43 ± 2
Polka	2000	11.8	353 ± 19	42 ± 2
S. Sengana (Pol)	2000	10.7	335 ± 24	36 ± 3
$\text{mean} \pm \text{SD}$		9.6	$\textbf{496} \pm \textbf{89}$	$\textbf{47} \pm \textbf{9}$

^{*a*} All folate results are means of triplicates ± standard deviation. ^{*b*} All cultivars were grown in Grödby, Sweden, except for Zefyr (grown in Uppsala, Sweden) and Senga Sengana (Pol) (imported from Poland). ^{*c*} Dry matter determination in duplicates according to AOAC method 920.151 (*27*).

descending folate content (micrograms per 100 g of DM) to demonstrate the influence of harvest year on the vitamin composition of the fruit. The harvests from 1999 and 2001 yielded higher folate concentrations than the harvest from 2000. The mean folate concentration in all strawberry cultivars in this study was 496 \pm 89 μ g/100 g of DM.

Results from our study indicate that both year of harvest and cultivar are significant factors affecting the folate content of strawberries (Table 2). The folate concentrations in four strawberry cultivars harvested from the same farm with the same growing conditions were compared for years 1999 and 2000. For the cv. Honeoye the harvest from 2001 was also included. A different pattern of significance appeared when moisture content was taken into consideration. The cv. Elsanta yielded the highest folate content both in 1999 (69 \pm 6 μ g/100 g of FW) and in 2000 (53 \pm 4 μ g/100 g of FW) but when calculated on a DM basis only in 1999 (644 \pm 56 μ g/100 g). In contrast, cv. Honeoye showed the highest folate content (528 \pm 17 μ g/ 100 g) in 2000. When the folate content was expressed as micrograms per 100 g of DM, year of harvest was significant for cv. Senga Sengana imported from Poland and for cv. Elsanta. Calculated on FW, significant variations were found between the different years of harvest for all cultivars, except BFr 77111.

Effects of Ripeness and Year of Harvest. The folate content of strawberries at different grades of ripeness was studied in cv. Honeoye (**Table 3**). Strawberries were collected as unripe, perfectly ripe, and overripe fruit over three different years of harvest: 1999, 2000, and 2001. Strawberries harvested in 1999 showed significantly higher (p < 0.05) folate concentrations in unripe fruit (579 ± 22 µg/100 g of DM), compared to ripe (501 ± 22 µg/100 g of DM) and fully ripe fruits (529 ± 39 µg/100 g of DM). In contrast, the harvests from 2000 and 2001 showed the opposite order, resulting in the highest folate contents in fully ripe strawberries. For the harvest in 2001 no significant variation was found between the different grade of ripeness, either on a FW or on a DM basis. The differences in folate content between the different years could not be explained by differences in water content (88–92%).

Effects of Storage and Processing. Cv. Zefyr was used to study retention of folates in strawberries used for consumption as fresh without processing (Figure 2). The folate content was expressed as micrograms per 100 g of DM to account for evaporation effects, which could occur during storage. The retention of folate in cv. Zefyr stored in the dark at 4 °C was 84% after 3 days and still \sim 70% after as long as 9 days of storage. After 9 days, the study was finished, as the berries were considered not fit to be eaten. On the other hand, storage of cv. Zefyr in daylight at room temperature (20-25 °C), mimicking the procedure of commercial retailing of strawberries in Sweden in June and July, showed appreciable folate losses already after 1 day (folate retention = 73%). After 3 days, the folate retention was as low as 62%. To study folate retention in strawberries for industrial processing of various strawberry products the cv. Honeoye was used. No significant loss of folate could be observed, and after 3 days of storage at 4 °C in the dark folate retention was still 99% (Figure 2). These results were confirmed when the study was repeated in 2000, showing that a folate retention of 98% occurred after 3 days of storage.

Folate content in five different common Swedish strawberry products and corresponding raw material was also studied (**Table 4**). The retention of folate was high in all tested jam and stewed dessert products independent of the strawberry content in the product (79-103%). However, in syrup made from a concentrated strawberry juice no folates were detected, not even in the concentrated raw juice.

DISCUSSION

Raw Material. This study has shown that the folate concentration of strawberries is dependent on the effects of cultivar, ripeness, and year of harvest (**Tables 1–3**). The results were not surprising because similar studies of other nutrients such as ascorbic acid, anthocyanins, and phenolic compounds have also shown significant variations regarding all of these factors (*17, 20*). According to Hägg et al. (*20*), ascorbic acid content in strawberries is also highly affected by climate conditions and growing area. We have also found that the differences in folate content could not be explained by differences in water content but that different patterns of significance appeared when the data were compared on a DM basis or as FW, respectively. This important aspect is often overlooked and makes it necessary to be very careful when results from similar studies are compared.

The effect of ripeness on the folate content in strawberries is shown in Table 3 and was not that easy to evaluate. After the first year of the study, the data suggest that the higher folate content in unripe berries was caused by the fact that final ripening results in increased cell size, due to growing energy stores of carbohydrates. As a consequence, the number of cells remains constant during the ripening process and, therefore, the folate content decreased (30). After evaluation of results from the second and third years, this hypothesis cannot be supported. We did find some significant variation of the folate content between the different ripeness stages, but without any logical pattern (Table 3). Probably this folate variation is due to other external factors, for instance, weather conditions during different years of harvest. In the study by Wang et al. (17) it was shown that different ripeness states of the same strawberry cultivar result in significant variations of the content of nutrients such as anthocyanins and phenolic compounds and that different nutrients had different optimal states in relationship to ripeness

Table 2. Folate Content in Various Strawberry Cultivars Harvested in 1999, 2000, and 2001

		folate content ^{$a-c$} (μ g/100 g)						
		DM			FW			
cultivar	1999	2000	2001	1999	2000	2001		
Honeoye	591 ± 40 B1	528 ± 17 A1	631 ± 24 1	42 ± 3 C1	53 ± 2 A2	62 ± 2 2		
S. Sengana (Swe)	479 ± 36 C1	426 ± 8 B1		$37 \pm 3 C1$	30 ± 1 C2			
S. Sengana (Pol)	638 ± 28 A1	$335 \pm 24 \text{ C2}$		$54 \pm 4 B1$	36 ± 3 C2			
BFr 77111	$533 \pm 42 \text{ BC1}$	481 ± 11 AB1		41 ± 3 C1	46 ± 1 B1			
Elsanta	$644 \pm 56 \text{ A1}$	454 ± 31 B2		69 ± 6 A1	$53 \pm 4 A2$			

^{*a*} All results are means of triplicates \pm standard deviation. ^{*b*} Different letters between the rows in each column mean statistically significant differences between the cultivars (*p* < 0.05). ^{*c*} Different numbers between the columns in each row mean statistically significant differences between the year of harvest (*p* < 0.05).

	Table 3. Folate Content in Strawberr	v Cv. Honeoye at Differe	nt Grades of Ripeness	Harvested 1999	, 2000, and 2001
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		DM			FW	
ripeness	1999	2000	2001	1999	2000	2001
unripe (orange)	579 ± 22 A1	497 ± 4 A1	639 ± 28 A2	52 ± 2 A1	$51 \pm 1 \text{ A2}$	61 ± 3 A1
ripe (perfectly red)	501 ± 22 B1	$528 \pm 17 \text{ A2}$	$631 \pm 24 \text{ A3}$	47 ± 2 B1	53 ± 2 B1	$62 \pm 2 \text{ A2}$
fully ripe (dark red)	$529\pm39~\text{C1}$	$566 \pm 9 B2$	678 ± 66 A2	$41 \pm 3 \text{ AB1}$	64 ± 1 C1	69 ± 7 A2

^a All results are means of triplicates \pm standard deviation. ^b Different letters between the rows in each column mean statistically significant differences between the different grades of ripeness (p < 0.05). ^c Different numbers between the columns in each row mean significant variation between the year of harvest (p < 0.05).

berry Products
Ν

	product ^a			folate ^b		
strawberry product (brand name)	strawberry content (%)	calories (kcal/100 g)	sugar (%)	raw material (μg/100 g)	product (µg/100 g)	retention ^c (%)
strawberry jam 1 (Önos extra prima jordqubbssylt)	52 ^{<i>d</i>}	180	46	32 ± 1	15 ± 2	91
strawberry jam 2 (BOB jordgubbssylt)	35 ^d	170	41	31 ± 1	9±1	84
stewed strawberry sauce 1 (Ekströms extra fina jordgubbskräm)	20 ^{<i>d</i>}	90	23	36 ± 3	6 ± 1	79
stewed strawberry sauce 2 (Ekströms jordgubbskräm)	15 ^e	100	21	43 ± 1	7±1	103
strawberry syrup (Önos extra prima jordgubbssaft)	36 ^f (juice)	200	46	2 ± 1	nd	

^{*a*} All products contain <0.5 g/100 g of fat and protein. ^{*b*} Folate results are means of triplicates ± standard deviation. ^{*c*} Folate retention is calculated according to the equation folate retention (%) = folate_{product}/folate_{raw material} × conversion factor (strawberry content in product). ^{*d*} Frozen strawberry of the cv. Senga Sengana. ^{*e*} Frozen strawberries of the cv. Camorosa. ^{*f*} Processed strawberry juice of the cv. Honeoye.

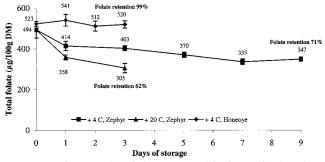


Figure 2. Folate retention during storage of fresh strawberries of cv. Zefyr at 4 and 20 °C and of cv. Honeoye at 4 °C. Folate concentrations (μ g/100 g of DM) are means of triplicates ± standard deviation (error bars)

in the berry. Unfortunately, that study gave results from only one year; it would be interesting to know if the same results would appear for subsequent years. This aspect is being studied in a work in progress on antioxidants and total antioxidative capacity of strawberry samples similar to those used in our work (Olsson et al., manuscript in preparation). Folate content in 13 different strawberry cultivars varied from 335 to 644 μ g/100 g of DM and from 36 to 69 μ g/100 g of FW. Previous published studies report folate contents (FW) from 36 μ g/100 g (23) to ~65 μ g/100 g (22, 24), and four European food data tables present a range from 20 to 99 μ g/100 g (31-34). In our study we analyzed samples harvested from the field and considered differences among cultivars and growing conditions; in the other studies no certain information about the berries is available (the samples were probably bought in a supermarket and analyzed as pooled samples). Furthermore, the present study has shown that it is difficult to compare the nutrient content in strawberries from various studies on a FW basis without considering the variation in water content. However, folate concentrations obtained by our study are similar to previously published data (22, 24, 31-34).

Strawberries cv. Senga Sengana, imported frozen from Poland, have commonly been used by the Swedish strawberry processing industry, and it was therefore interesting to include this cultivar in this study. As seen in **Table 2**, the folate content and also the water content in this cultivar fluctuated significantly between the different years of the study, much more than for the Swedish cultivars grown at the same farm and under controlled conditions throughout this study. For example, the same cv. Senga Sengana did not show significant variations of folate content during the different years (**Table 2**). Strawberries for industrial processing are purchased as frozen from strawberry farms all over Poland. The imported strawberries from different years in the present study probably derived from different farms. In the future, it would be interesting to compare the folate content in the same strawberry cultivar grown at different farms under controlled conditions. Another important factor not controlled in this study was the climate conditions. All of these aspects need to be considered whenever the content of nutrients in various plants or plant products is discussed.

Postharvest Handling. Results indicate a relatively high stability of folate in intact berries during storage at 4 °C and, as expected, the folate retention was lower when strawberries were stored at room temperature (Figure 2). Strawberries are also a rich source of ascorbic acid (35), an important antioxidant that can stabilize folates. Kalt et al. (18) reported in their study that ascorbic acid losses in different berries including strawberries were minimal after 8 days of storage at different temperatures compared to spinach, which showed losses up to 90% within 3 days after harvest (36). However, another study showed ascorbic acid retention to be moderate (25-90%) during storage of fresh strawberries, depending on the type of packaging and storage temperature (19). Nunes et al. (19) also pointed out that apparent increase in nutrient content presented on a FW basis during storage was due to water loss rather than to actual increase of the vitamin. The high to moderate stability of ascorbic acid in berries and berry products is probably due to the high content of organic acids (35) and protective effects of phenolic antioxidants (37). Probably, this antioxidative milieu in the cells also functions as a good protection for folate. Hägg et al. (20) found the retention of ascorbic acid in strawberries to be moderate (64%) after as long as 2 years of frozen storage. A similar study was performed by Vahteristo et al. (25) studying the folate content after frozen storage of strawberries at -20°C. This treatment did not affect the folate content, but unfortunately the study was terminated after only six months.

In the present study the folate retention in common Swedish processed strawberry products was studied (Table 4). The folate retention in tested products was very high overall. Strawberry syrup, which contains no folate, was one exception, but this syrup product contains only a small proportion of strawberry juice (9%) and has a very high sugar content and is therefore not a product consumed for its nutritional value. Interestingly, almost no losses of folate seemed to occur when strawberries were cooked to jam or stewed to strawberry desserts from frozen berries (Table 4). This finding is contradictory to the data reported in four European food tables, which indicate that only 3-30% of folate was retained in various strawberry products such as jam and stewed desserts (31-34). Viberg (39) estimated evaporation of water during the cooking process of strawberry jam to be \sim 5%, which has not been considered in the calculations of our study. However, this could hardly explain the much higher folate retention found in the present study, but as stated earlier in this paper, data in food data tables are generated from pooled samples and do not consider the variation in folate content between sources of raw material and various processing techniques used. There is a need for much more research in this area, but on the basis of the present study we can nevertheless recommend processed strawberry products as well as fresh and frozen strawberries to be rich sources of folate. This is a promising finding because folate relative to the

nutritional needs of humans is frequently among the most limiting of all vitamins (40). For instance, 250 g of strawberries (~125 μ g of folate) supplies ~50% of the recommended daily folate intake in different European countries (200–300 μ g/day) or 30% of the U.S. recommendation of 400 μ g/day.

ABBREVIATIONS USED

AA, ascorbic acid; 5-CH₃-H₄folate, 5-methyltetrahydrofolic acid; CP, chicken pancreas; DM, dry matter; FW, fresh weight; MCE, 2-mercaptoethanol; RPBA, radioprotein-binding assay.

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