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Influence of Processing on Total, Monoglutamate and Polyglutamate Folate Contents of Leeks, Cauliflower, and Green Beans

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Bioavailability of dietary folate might be impaired by the polyglutamate chain to which ~70% of dietary folates are bound. This chain must be removed enzymatically in the intestine before folate is absorbed as a monoglutamate. To increase formation of monoglutamate folate in vegetables, the vegetables were subjected to various processing treatments. Treatments included freezing (−18 °C, 16 h) and thawing (4 °C, 24 h) and hydrostatic high-pressure treatment (200 MPa, 5 min). Both freezing/thawing and high-pressure treatment increased the proportion of folate in the monoglutamate form in leeks, cauliflower, and green beans 2−3-fold. However, loss of total folate after these treatments was >55%. It is concluded that conversion of folate polyglutamate to the monoglutamate form in vegetables is possible by certain processing treatments. Potentially this could lead to vegetables with higher folate bioavailability. However, to prevent folate loss into processing water, processing in a closed system should be applied.

KEYWORDS: Folate; monoglutamate; polyglutamate; processing; freezing; high-pressure treatment; blanching; steaming; vegetables

INTRODUCTION

Vegetables and fruits are rich sources of dietary folate, which is the natural form of folic acid. This is a B vitamin that has proven capacity to decrease the risk of neural tube defects in newborns (*1*, *2*) and can lower plasma homocysteine concentrations in humans. High plasma homocysteine concentrations are associated with cardiovascular diseases (*3*). Currently the impact of lowering plasma homocysteine levels by folic acid on disease risk is under study (*4*). Folate intake is also studied in relation to certain forms of cancer (*5*).

Various studies have shown that the bioavailability of dietary folate is lower than that of synthetic folic acid (*6*, *7*). One of the main factors impairing the bioavailability of dietary folate may be that food folate is present mainly as polyglutamate conjugate (*8*, *9*). Before folate is absorbed from the gut, all but the proximal glutamate moiety must be removed by the enzyme glutamate carboxypeptidase II (also known as γ-glutamyl hydrolase or folate conjugase), present in the brush border of the small intestine (*10*). Synthetic folic acid, used as a supplement and in food fortification, is a monoglutamate, which may explain why it is absorbed more easily (*11*). Study results vary, however, with study designs, and some studies show hardly any difference in absorption rate of polyglutamate as compared to monoglutamate folate (*9*, *12*). Other important factors that can impair dietary folate bioavailability are the matrix of the food, the intestinal pH, the fiber content, and the presence of organic acids or folate binding protein in the food (*8*, *9*).

In vegetables, folate is present in various vitamers, including tetrahydrofolate (H_4folate) and 5-methyltetrahydrofolate (5-CH_3-H_4folate). These vitamers exist to a small extent as monoglutamates but are mainly conjugated to a chain of two to eight glutamate moieties. The γ-glutamyl hydrolase enzyme is also present in vegetables. A few decades ago it was noted that during homogenization preceding analysis of fresh vegetables, spontaneous conversion of folate polyglutamate to the monoglutamate form occurs (*13*). Although this process interfered with the determination of the natural monoglutamate folate content of vegetables, we speculated that it could be used to

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Table 1. Description of the Processing Treatments Used in the Pilot Study with Leeks

treatment		description
A	blanching	submersion of the cut and washed vegetables in boiling water (1 L of water/kg of fresh vegetable weight) for 10 min using household utensils on an electric stove
B	freezing (–80 °C), thawing	freezing at –80 °C for 16 h, followed by 24 h of thawing in the refrigerator at 4 °C and then blanching (treatment A)
C	freezing (–18 °C), thawing	freezing at –18 °C for 16 h, followed by 24 h of thawing in the refrigerator at 4 °C and then blanching (treatment A)
D	freeze-drying	freeze-drying for 120 h, followed by re-addition of the evaporated water, storage in the refrigerator at 4 °C for 6 h, and then blanching (treatment A)
E	high-pressure treatment	high-pressure treatment at 50, 100, 150, and 200 MPa for 5 min; pressure established by compression of glycol surrounding the vegetable, which was vacuum-packed in a plastic bag; treatment followed by 6 h of storage in the refrigerator at 4 °C and then blanching (treatment A)

produce vegetables rich in monoglutamate folate, thereby increasing the bioaccessibility of folate from vegetables. Bioaccessibility is defined as the proportion of an ingested nutrient, which is (in the absorbable form) presented to the intestinal brush border for absorption.

It is not clear where γ-glutamyl hydrolase is located within plant cells. Although in animals it is known to be lysosomal (*14*−*16*), in plants it is probably located mainly in the cytosole and extracellularly (*17*, *18*). We hypothesized that folate bioaccessibility from vegetables could be increased by disruption of the cell structure. Cell disruption will establish contact between polyglutamate folate and the enzyme, thereby stimulating hydrolysis of folate polyglutamate to the monoglutamate form.

Thus, the aim of this study was to process vegetables in such a way that the endogenous γ-glutamyl hydrolase activity would be stimulated, resulting in vegetables with an increased content of monoglutamate folate. In a later stage, folate bioavailability from such vegetables could then be tested in humans and be compared to that from vegetables processed in the same way (similar matrix, pH, etc.) but with a low amount of folate in the monoglutamate form.

MATERIALS AND METHODS

Materials. From a list of products that contribute >75% to the folate intake of the Dutch population (*19*), we selected several vegetables with a high folate content, a high proportion of polyglutamate folate, and different plant structures (color, type of tissue). The vegetables chosen were leeks, cauliflower, and green beans. The processing conditions used were chosen because of their ability to damage cell structure. In addition, the vegetables were blanched either before or after processing to destroy enzymatic activity, thereby preventing any further conversion of folate polyglutamate to the monoglutamate form.

Pilot Study. Leeks (20 kg) were purchased from a local supermarket, cut into 5-mm rings, washed, and subjected to various experimental treatments as described in **Table 1**. High-pressure treatment is a novel processing technique used to preserve food products in a mild way. Because this technique affects membrane porosity, it can affect the enzymatic conversion of folate polyglutamate to the monoglutamate form. High-pressure treatment at 100−200 MPa results in crystallization of phospholipids in cell membranes, resulting in permeabilization of membranes (*20*). Freezing also damages the plant cell structure by expansion of the intracellular fluid. In theory, slow freezing causes more damage to cell structure because of the larger ice crystals formed in comparison to rapid freezing at lower temperatures. Therefore, we applied freezing at both −18 and −80 °C.

All treatments were started on the same day to prevent changes in folate content caused by pretreatment storage. Storage in the refrigerator after the treatments was applied to allow enzymatic activity to take place. After blanching, draining, and airtight-packed cooling at room temperature, samples ($n = 1$) were stored in freezer bags at −80 °C until analysis.

High-pressure treatment was carried out at the industrial test plant of the Agrotechnologic Research Institute (ATO), Wageningen, The Netherlands. All other treatments were performed at the Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands.

Main Study. From the pilot study, the best treatments for conversion of folate polyglutamate to the monoglutamate form were selected. Criteria were, first, the best results in converting polyglutamate folate into monoglutamate folate and, second, the applicability of the processing method on a larger scale. These included freezing at −18 °C followed by refrigerated thawing during 24 h and hydrostatic high-pressure treatment of 200 MPa for 5 min, now followed by 24 h of refrigerated storage (pilot study, 6 h). For high-pressure treatment, the start temperature was set at 22 °C and the maximum temperature during the treatments was 30 °C. Both freezing and thawing and high-pressure treatments were either preceded or followed by blanching for enzyme inactivation. This was done to produce vegetables either high in polyglutamate folate or high in monoglutamate folate, but not different in other aspects. The separate effects of 24 h of refrigerated storage or blanching on folate vitamer content were studied, as was the effect of hydrostatic high-pressure treatment without subsequent storage. The effects of steaming the vegetables instead of blanching were also studied. All treatments applied during the main study are summarized in **Table 2**.

Freshly cut and washed leeks (rings, 5 mm), cauliflower (florets, 2−4 cm), and green beans (pieces, 5 cm) were purchased in batches of 5 kg each from a wholesale greengrocer. After thorough mixing, random samples of 200 g were taken from these vegetable batches and processed. Five of these samples were packed in freezer bags and immediately frozen at −80 °C, until analysis. Folate vitamer content after each of the treatments G−N (**Table 2**) was compared to the mean content of these five raw samples. All treatments started on the same day. The time needed for blanching cauliflower and green beans was established by placing five thermocouples to the innermost part of the vegetables. The vegetables were put in boiling water, and the temperature was measured every second using a datalogger until a temperature of >90 °C was reached. For leeks, from which the thermocouples always became detached, it was assumed that the time required for heating the vegetable internally would be shorter than for cauliflower and green beans because of the higher water content and smaller cross section. To inactivate enzymes and microorganisms effectively, blanching periods were calculated as the time required for heating the innermost part of the vegetable plus 4 min. For leeks, cauliflower, and green beans, blanching periods of 5, 8, and 6 min were used, respectively. To establish steaming periods, the same approach was used: leeks, cauliflower, and green beans were steamed for 5, 7, and 6 min, respectively. After blanching or steaming, the vegetables were drained in a colander for a few seconds. Vegetables were immediately packed in freezer bags, cooled on ice water, and stored in the freezer at −80 °C until analysis.

Analysis. Frozen samples were homogenized each with ∼1 L of liquid nitrogen in a 4 L Waring blender for 3−5 min until a homogeneous powder was developed. Samples of ∼100 g were put in duplicate 100 mL plastic pots and stored at −80 °C. Homogenization was done within one week after storage of the processed vegetables.

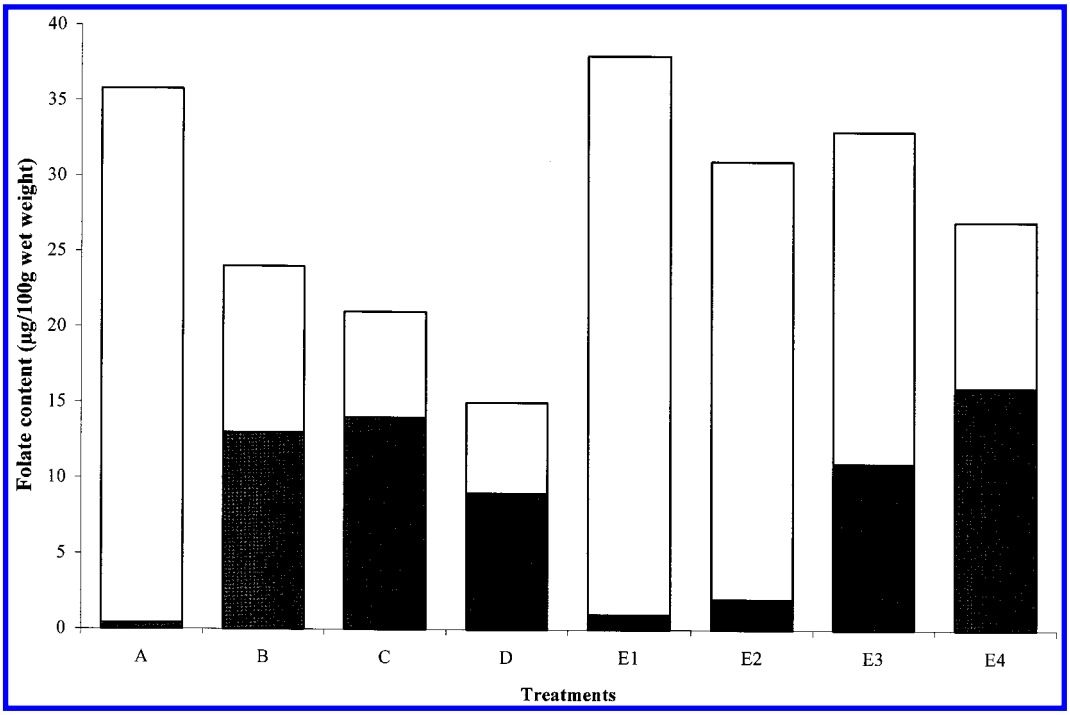

Figure 1. Folate content (total, monoglutamate, and polyglutamate) of leeks after various processing treatments used in the pilot study ($n = 1$): (shaded bars) monoglutamate folate; (white bars) polyglutamate folate; (A) blanching; (B) freezing (–80 °C), thawing; (C) freezing (–18 °C), thawing; (D) freeze-drying; (E) high-pressure treatment [(E1) 50 MPa; (E2) 100 MPa; (E3) 150 MPa; (E4) 200 MPa]. See **Table 1** for description of treatments. Polyglutamate content was calculated as the total folate content (after deconjugation) minus the monoglutamate folate content (before deconjugation).

Table 2. Description of the Processing Treatments Used in the Main Study with Leeks, Cauliflower, and Green Beans

	treatment	description
F	raw	no treatment
G	storage	storage for 24 h in a refrigerator at 4 °C
H	blanching	blanching in an industrial blanching kettle (10 L of water/200 g of fresh vegetable weight) for 5 min (leeks), 8 min (cauliflower), or 6 min (green beans)
I	steaming	steaming in a steaming sieve of 200 g of vegetable above 1 L of boiling water for 5 min (leeks), 7 min (cauliflower), or 6 min (green beans)
J	high-pressure treatment	high-pressure treatment at 200 MPa for 5 min; pressure established by compression of water surrounding the vegetables
K	freezing, thawing, blanching	freezing at –18 °C for 16 h, followed by thawing during storage (treatment G) and then blanching (treatment H)
L	high-pressure treatment, blanching	high-pressure treatment (treatment J) followed by storage (treatment G) and then blanching (treatment H)
M	blanching, freezing, thawing	blanching (treatment H) followed by freezing at –18 °C for 16 h and thawing during storage (treatment G)
N	blanching, high-pressure treatment	blanching (treatment H) followed by high-pressure treatment (treatment J) and storage (treatment G)

Samples from the pilot study were transported on dry ice to the Inspectorate of Health Protection and Veterinary Public Health, The Netherlands, for analysis. Samples from the main study were analyzed at the RIKILT, Wageningen, The Netherlands. Both institutes used the same analysis method. In short, after thawing of the vegetable, powder folates were extracted from the samples by further homogenization in a 50 mM Ches/Hepes buffer (pH 7.8), containing 2% ascorbic acid and 2 M 2-mercaptoethanol as antioxidants with an Ultra Turrax under nitrogen for 15 s. This homogenate was subjected to heat treatment (10 min under nitrogen in a boiling water bath) and homogenized again with an Ultra Turrax (under nitrogen) for 15 s. A first aliquot was analyzed without addition of any enzymes (treatment 1) to estimate the monoglutamate content of the samples. In a second aliquot, folate concentrations were quantified after the addition of rat plasma conjugase for conversion of folate polyglutamate to the monoglutamate form, as well as protease and amylase (treatment 2). In this way, the sum of monoglutamates and polyglutamates was established. The difference between the amount of folate assayed in treatments 1 and 2 were assumed to represent the folate polyglutamate content. Deconjugation was 100% complete for each sample as checked by external addition of triglutamate folic acid. After purification by affinity chromatography, folate monoglutamates were determined using an HPLC method with fluorescence and diode array detection (*21*). This procedure was used to quantify the levels of several folate forms naturally present, including tetrahydrofolate, 5-methyltetrahydrofolate, 10-formyldihydrofolate, and 5-formyltetrahydrofolate. The method showed recoveries of 81−87% with externally added 5-methyltetrahydrofolate for raw samples of leeks, cauliflower, and green beans.

RESULTS

Pilot Study. The pilot study with leeks showed that all processing treatments applied (treatments B−E) yielded higher monoglutamate folate content in leeks compared to blanching (treatment A; **Figure 1**; $n = 1$). On the basis of relative monoglutamate folate yield, theoretically expected larger ice crystals after slow freezing, and applicability of the processing method on a larger scale, we chose freezing at −18 °C (treatment C) and high-pressure treatment at 200 MPa for 5

Table 3. Folate Vitamer Content of Raw Vegetables (Micrograms per 100 g), Based on Wet and Dry Weight (Mean ± SD, $n = 5$)

		monoglutamate			polyglutamate[a]	
	total	5-CH_3-H_4folate	H_4folate	total mono as % of total folate	5-CH_3-H_4folate	H_4folate
wet weight						
leeks	49.6 ± 4.4	16.1 ± 3.3	<DL[b]	29.5 ± 8.0	27.9 ± 6.6	5.6 ± 1.3
cauliflower	53.5 ± 8.2	4.8 ± 1	<DL	8.5 ± 1.2	45.7 ± 6.7	3.0 ± 1.1
green beans	39.1 ± 5.1	12.7 ± 0.4	<DL	27.8 ± 4.7	19.2 ± 4.0	7.3 ± 1.8
dry weight						
leeks	580 ± 56	187 ± 38	<DL	32.8 ± 9.4	326 ± 78	66 ± 16
cauliflower	696 ± 111	62 ± 11	<DL	9.0 ± 1.3	595 ± 95	39 ± 14
green beans	526 ± 65	170 ± 7	<DL	32.9 ± 5.1	257 ± 52	98 ± 23

[a] Polyglutamate content was calculated as the total folate content (after deconjugation) minus the monoglutamate folate content (before deconjugation). [b] <DL = under detection limit.

Table 4. Folate Content (Total, Monoglutamate, and Polyglutamate) of Vegetables after Various Processing Treatments[a]

	total			monoglutamate		polyglutamate[c]
treatment[b]	dry matter %	μg/100 g of dry wt	% loss	μg/100 g of dry wt	% of total	μg/100 g of dry wt
leeks						
raw (F)	8.6 ± 0.1	580 ± 56	0	187 ± 38	33 ± 9	392 ± 81
storage (G)	8.6	491	15	260	53	231
blanching (H)	6.5	417	28	23	6	394
steaming (I)	8.7	431	26	49	11	382
high-pressure treatment (J)	6.5	236	81	174	74	62
freezing, thawing, blanching (K)	5.6	85	85	85	100	0
high-pressure treatment, blanching (L)	5.3	86	85	56	65	30
blanching, freezing, thawing (M)	6.5	418	28	40	10	378
blanching, high-pressure treatment (N)	5.6	359	38	31	9	328
cauliflower						
raw (F)	7.7 ± 0.3	696 ± 111	0	62 ± 11	9 ± 1	634 ± 105
storage (G)	7.7	519	25	23	4	496
blanching (H)	6.9	626	10	16	3	610
steaming (I)	7.7	640	8	10	2	630
high-pressure treatment (J)	6.8	394	43	48	12	346
freezing, thawing, blanching (K)	6.7	246	65	62	25	184
high-pressure treatment, blanching (L)	6.3	311	55	27	9	284
blanching, freezing, thawing (M)	6.7	587	16	18	3	569
blanching, high-pressure treatment (N)	6.1	576	17	23	4	553
green beans						
raw (F)	7.4 ± 0.2	526 ± 65	0	170 ± 7	33 ± 5	355 ± 71
storage (G)	7.4	560	–7	231	41	329
blanching (H)	7.9	414	21	27	7	387
steaming (I)	7.8	471	10	10	2	461
high-pressure treatment (J)	7.4	277	47	226	82	51
freezing, thawing, blanching (K)	8.2	108	79	83	77	25
high-pressure treatment, blanching (L)	7.4	102	81	73	72	29
blanching, freezing, thawing (M)	8.5	340	35	16	5	324
blanching, high-pressure treatment (N)	8.1	397	24	15	4	382

[a] Values for raw vegetables expressed as mean ± SD ($n = 5$). All other data are based on single measurements. [b] See **Table 2** for a description of treatments. [c] Polyglutamate content was calculated as the total folate content (after deconjugation) minus the monoglutamate folate content (before deconjugation).

min (treatment E4) as the best methods for our further experiments.

Main Study. In **Table 3**, the total, monoglutamate, and polyglutamate folate contents of raw leeks, cauliflower, and green beans are shown. Variations in total folate within five samples, expressed as standard deviations, were small in all vegetables (leeks, 49.6 ± 4.4 μg/100 g; cauliflower, 53.3 ± 8.2 μg/100 g; green beans, 39.1 ± 5.1 μg/100 g). In raw leeks, 29.5% of the folate was in the monoglutamate form; for raw cauliflower and green beans this was 8.5 and 27.8%, respectively. 5-Methyltetrahydrofolate was the predominant folate form present in all vegetables. Tetrahydrofolate was present only in the polyglutamate fraction of the vitamin.

The effect of the processing treatments on the total, monoglutamate, and polyglutamate folate contents of the vegetables, based on dry weight, is shown in **Table 4** and visualized in **Figure 2**.

Effects on Total Folate. Storage of the cut and washed vegetables for 24 h in the refrigerator (treatment G) resulted in folate losses of 0−25%. Blanching (treatment H) caused folate losses of 10−28%, whereas for steaming (treatment I) this ranged from 8 to 26%. Folate loss in green beans was less after steaming (10%) than after blanching (21%). High-pressure treatment (treatment J) led to greater losses of folate in all vegetables, ranging from 47 to 81%. Freezing and thawing or high-pressure treatment followed by blanching (treatments K and L, respectively) resulted in folate losses >55%, whereas processing preceded by blanching (treatments M and N, respectively) resulted in no more loss of folate than blanching alone (<35%).

Effects on Monoglutamate and Polyglutamate Folate. Refrigerated storage (treatment G) showed a moderate increase in monoglutamate folate in leeks (from 33 to 53%) and green beans (from 33 to 41%), but not in cauliflower. Compared to raw vegetables, the proportion of folate in the monoglutamate form was lower after blanching (treatment H) and steaming (treatment I): in leeks, 6 and 11%, respectively; in cauliflower, 3 and 2%, respectively; and in green beans, 7 and 2%, respectively. Neither

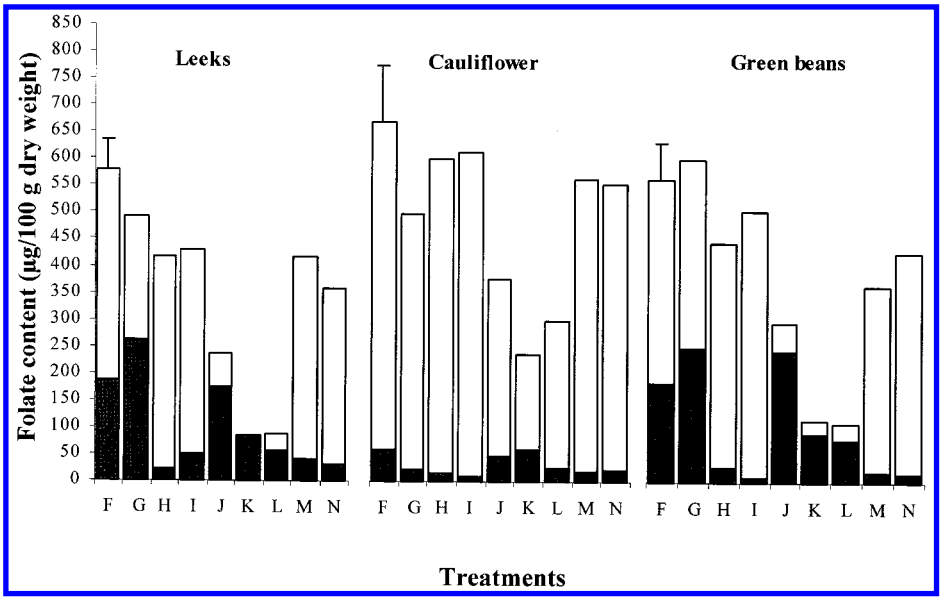

Figure 2. Folate content (total, monoglutamate, and polyglutamate) of leeks, cauliflower, and green beans before and after processing treatments used in the main study, based on dry weights: (shaded bars) monoglutamate folate; (white bars) polyglutamate folate; (F) raw; (G) storage; (H) blanching; (I) steaming; (J) high-pressure treatment; (K) freezing, thawing, blanching; (L) high-pressure treatment, blanching; (M) blanching, freezing, thawing; (N) blanching, high-pressure treatment. Values for raw vegetables are shown as mean ($n = 5$) with SD in error bars. See **Table 2** for description of treatments. Polyglutamate content was calculated as the total folate content (after deconjugation) minus the monoglutamate folate content (before deconjugation).

blanching nor steaming caused changes in the absolute amount of polyglutamate folate in the vegetables, whereas the absolute amount of monoglutamate folate decreased strongly. The proportion of folate in the monoglutamate form was increased after high-pressure treatment (treatment J) to 74% in leeks, 12% in cauliflower, and 82% in green beans. After freezing and thawing or after high-pressure treatment, both followed by blanching (treatments K and L, respectively), the proportion of folate as monoglutamate was increased: in leeks to 100 and 65%, respectively; in cauliflower to 25 and 9%, respectively; and in green beans to 77 and 72%, respectively. Blanching followed by processing (treatments M and N, respectively) resulted in a low percentage of folate in the monoglutamate form, namely, for leeks, 10 and 9%, respectively; for cauliflower, 3 and 4%, respectively; and for green beans, 5 and 4%, respectively.

DISCUSSION

In the present study we were able to stimulate the hydrolysis of folate polyglutamate to the monoglutamate form in vegetables by various processing treatments. We saw a marked increase of the proportion of monoglutamate folate relative to total folate after freezing and thawing and also after high-pressure treatment. However, both processing treatments also led to substantial loss of total folate.

The high folate losses are probably caused by leakage of folate from the vegetables during freezing and high-pressure treatments and subsequent loss into the liquid during blanching. Blanching and steaming led to major losses of monoglutamate folate, whereas the amount of polyglutamate folate in the vegetables remained practically unchanged. Freezing and thawing or high-pressure treatment enhanced loss of folate during subsequent blanching. This implies that folate in the monoglutamate form may be lost preferentially when the vegetable is in direct contact with water. Destruction of the vitamin by exposure to heat might also have led to loss of folate. However, Petersen (*22*) showed that blanching of broccoli for 5 min in a closed system results in only minor folate losses. Therefore, leakage of folate mainly in the monoglutamate form must have been the most important reason for loss of folate in our experiments. Because of the preferential leakage of monoglutamate folate, our data most probably give an underestimation of the real conversion of polyglutamate to monoglutamate by stimulated deconjugase activity due to treatments K and L.

From the pilot study with leeks, we concluded that the best way of converting folate polyglutamate to the monoglutamate form was to freeze it at −18 °C or to use high-pressure treatment. Freezing at −18 °C showed results similar to those of freezing at −80 °C. In theory, slow freezing is assumed to cause greater damage to cell structures than rapid freezing, when the small ice crystals formed impart less.

Loss of total folate in leeks was greater in the main study as compared to the pilot study. This is probably due to the difference in the proportion of blanching water used in the two studies: 1 L/kg of vegetable in the pilot study versus 50 L/kg in the main study. The latter proportion of water was chosen keeping in mind scaling up of the process at a later stage. Furthermore, in the pilot study we used a hydrostatic high-pressure apparatus with a small sample container for which glycol was used as pressure medium. For the use of this apparatus the vegetable samples were packed in vacuum plastic bags. In our main study we intended to do the same with larger amounts of vegetables in an apparatus with a larger container and with water as pressure medium. However, we did not succeed in keeping the vacuum plastic bags from leaking, and therefore we decided to carry out the experiments without prior packaging. This might also have led to greater losses of folate during high-pressure treatment in the main study compared to those in the pilot study. We considered analyzing the folate content in the processing water after high-pressure treatment and blanching. However, calculations revealed that the folate concentration would be far below the detection limit.

To our knowledge no earlier studies have reported on processing of vegetables with the aim of converting polyglutamate folate to the monoglutamate form. However, data have been published on folate retention in vegetables during storage

or processing. Storage of blanched vegetables in the frozen state decreases folate retention by 15%, whereas fresh vegetables lose ~25% of folate when stored frozen (*23*, *24*). As yet, few data are available on the impact of refrigerated storage on the folate content of vegetables. Chen et al. (*23*) report an increase of the monoglutamate folate content of 30% after refrigerated storage of whole leaf spinach for 7 days. We found increases in the same range for cut leeks and green beans after refrigerated storage for only 1 day. Recently, Konings et al. (*19*) also reported increases in the monoglutamate folate content of chopped spinach stored at room temperature. Storage for up to 60 min increased the amount of monoglutamate folate 2-fold. Chen et al. (*23*) found an increase in the monoglutamate folate content of 57% in whole leaf spinach stored at room temperature for 10 h.

The folate retention figures for steaming we found (75−90%) are in accordance with those of others (*24*). For blanching, we found higher folate retention (70−90%) than others (30−70%) (*23*, *24*). Differences in vegetables and methods make it difficult to draw conclusions. In general, the longer vegetables are exposed to heat or water, the lower the folate retention will be. Therefore, processing techniques such as processing in a closed system, microwave, and vacuum-packed high-pressure treatment, by which direct or prolonged exposure to heat or water is avoided, are preferred.

The results from this study are based on analysis of single samples, except for the raw samples in the main study. However, all samples were taken from the same vegetable batches that were carefully homogenized before sampling. The small standard deviations of the mean folate content in five raw vegetable samples show that our sampling strategy has been successful.

In conclusion, freezing as well as high-pressure treatments are promising approaches for improving the bioaccessibility of folate from vegetables. Potentially this could lead to production of vegetables with a higher folate bioavailability. However, leakage of folate into the processing water, especially of the monoglutamate form, counteracts these positive effects. To prevent loss of folate into the processing water, processing in a closed system should be applied. The effect of refrigerated storage of cut vegetables on the conversion of polyglutamate folate to the monoglutamate form deserves further research.

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