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Folate Contents of Legumes Determined by Optimized Enzyme Treatment and Stable Isotope Dilution Assays

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ABSTRACT

Stable isotope dilution assays (SIDAs) were adopted to quantify folates in legumes. Deconjugation of polyglutamic to monoglutamic vitamers was achieved by a combined treatment with rat plasma and chicken pancreas conjugase. Additionally, conjugase inhibitors were effectively removed by strong anion exchange prior to deconjugation.

Determination of various dried legume seeds by the optimized SIDA technique revealed total folate contents between 10 µg/100g (green peas/ *Pisum sativum*) and 318 µg/100g (soy bean/ *Glycine max*). Total folate concentrations of canned legumes were analyzed in a concentration range between 4 µg/100g (green beans, *Phaseolus vulgaris*) and up to 69 µg/100g (blackeyed peas, cow peas /*Vigna unguiculata*). Deep frozen products of green peas and green beans were found to contain relatively high folate concentrations up to 146 µg/100g (green peas). A comparison to literature data, analyzed by means of microbiological assays, indicated significant lower SIDA values for some dried legume seeds.

The folate pattern measured by SIDA revealed 5-methyltetrahydrofolate as the predominant vitamer in frozen peas, dried lentils, dried blackeyed peas and mung beans, whereas in fresh beans and soy beans tetrahydrofolate was most abundant and in peanuts 5-formyltetrahydrofolate was found to be the most important folate vitamer.

Key words: conjugase; folates, LC-MS/MS; legumes; stable isotope dilution assay

INTRODUCTION

In developing countries as well as in many developed regions the intake of substances belonging to the folate group is generally known to be inadequate (Konings et al., 2001). Particularly since the last decade this observation has evoked growing public concern, as folates are supposed to prevent neural tube defects (Czeizal and Dudas, 1992), alzheimer's disease (Snowdon et al., 2000) and cardio vascular disease (Robinson, 2000). Furthermore, the role of folate deficiency in inducing single strand breaks of DNA (Ames, 2001) and in favoring the activation of proto-oncogenes (Rizwana and Hahn, 1999) appear to be the cause for the meanwhile evident correlation between a low dietary intake of folates and the risk of cancer (Caudill, 2003).

In the industrialized countries the following approaches have been suggested to settle this problem and still are discussed controversely: (1) mandatory fortification, (2) recommendations to use folic acid supplements or to consume fortified foods and (3) dietary advise to increase the intake by foods rich in folates. Whereas in the USA and in further countries mainly on the American continent folate fortification is mandatory for cereal products and resulted in a significant increase of the mean folate intake (Quinlivan and Gregory, 2003), in European countries concerns over fortification with folic acid center around masking of vitamin B_{12} deficiency. Therefore, European governments prefer to recommend the intake either of supplements or of fortified foods or of foods rich in folates. When surveying the existing data on foods (Scherz and Senser, 2000), legumes are reported to contain high folate concentrations and, therefore, offer the perspective to decisevely increase folate intake. However, folate data of foods are currently called in question due to different restrictions of the existing analytical methods, which raised doubts about accuracy of the figures.

In this respect, the majority of folate data are based on a microbiological assay (MA), which is not able to distinguish the single vitamers. Therefore, errors due to different responses of the microorganisms to the vitamers may invalidate the results. Since the last decade, HPLC coupled either to fluorescence (LC-FD) (Müller, 1993) or electrochemical detection (Bagley and Selhub, 2000) or to mass spectrometry (LC-MS) have been developed to differentiate between folate vitamers. In particular, stable isotope dilution assays (SIDAs) using LC-MS detection are considered the most accurate methods as the use of isotopically labeled analogues as the internal standards enables to correct for losses of the vitamers during sample clean-up. In first applications of SIDA, a few foods were analyzed for their folate content (Freisleben et al, 2003a; Rychlik, 2004) and some data have been confirmed, whereas there are still some discrepancies for cabbage vegetables. Furthermore, method comparisons partly revealed the superiority of SIDA and partly showed significant differences to the microbiological method (Puwastien et al., 2005). Among all possible errors in folate analysis, one problem on principle is the occurrence of polyglutamic forms, which often are cleaved to monoglutamates to obtain correct data on total folates.

Bearing these problems in mind, the goals of the present study were first, to optimize folate deconjugation for analysis of legumes, second to obtain a broader survey of accurate folate data in commonly consumed legumes and, third, to evaluate the perspective of legume consumption to increase folate intake in western countries as well as in developing regions of the world.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained commercially from the sources given in parentheses: acetonitrile, formic acid, hydrochloric acid, $KHCO_3$, $KHPO_4$, 2-mercapto ethanol, methanol, Na HCO_3, NaH_2PO_4, sodium acetate, Na_2SO_4, (Merck, Darmstadt, Germany), α-amylase Type II-A from Bacillus spp., CHES, HEPES, protease Type XIV from Streptomyces, sodium ascorbate (Sigma, Deisenhofen, Germany) pteroyl triglutamate (Schircks, Switzerland).

[$^2H_4$]-5-Methyltetrahydrofolic acid, [$^2H_4$]-5-formyltetrahydrofolic acid, [$^2H_4$]-10-formylfolic acid, [$^2H_4$]-tetrahydrofolic acid and [$^2H_4$]-folic acid were synthesized as reported recently (Freisleben et al., 2002).

Extraction buffer consisted of aqueous HEPES (50 mmol/L) and aqueous CHES (50 mmol/L) at pH 7.85 and contained sodium ascorbate (2 %) and 2-mercapto ethanol (20 mmol/L).

Conditioning buffer for SAX cartridges was prepared by mixing aqueous solutions of NaH_2PO_4 (0.01 mol/l, 62 ml), of $KHPO_4$ (0.01 mol/L, 28 mL), and 2-mercapto ethanol (0.2 mL), adjusting the mixture to pH 7.5 and finally making it up to 100 mL with water.

Chicken pancreas solution was prepared by dissolving 5 mg chicken pancreas (DIFCO, USA) in phosphate buffer (30 mL, 0.1 mol/L, pH 7.0) containing 1 % ascorbic acid.

Samples and extraction

Different varieties of fresh green beans (*Phaseolus vulgaris*), and fresh green peas (*Pisum sativum*), produced in Germany, were obtained in the summer 2005 at markets in the city of Munich according to the seasonal availability. Dried products, namely green

peas (*Pisum sativum*); chick peas (*Cicer arietinum*); green and red lentils (*Lens culinaris*); white beans, black beans and kidney beans (*Phaseolus vulgaris*), soy beans (*Glycine max*); mung beans (*Vigna radiata*); blackeyed peas (*Vigna unguiculata*); jack beans (*Canavalia ensiformis*); peanuts (*Arachis hypogaea*), were bought from German suppliers and are usually imported from different countries. Deep frozen and canned green peas and beans had been produced in Germany. Further investigated canned products (white beans, black beans, chick peas, kidney beans and blackeyed peas), available at the German market, had been imported. All samples were bought in quantities of 1 kilogram. Canned vegetables had been drained until no brine dripped from them any more. Dried legume seeds were washed and picked over for removal of debris. The whole edible amount of samples was thoroughly homogenized by freezing and grinding in liquid nitrogen prior to analysis. Representative aliquots of the ground material (0.5-1g) were overlaid with 10 mL of extraction buffer containing [$^2H_4$]-5-methyltetrahydrofolic acid, [$^2H_4$]-5-formyltetrahydrofolic acid, [$^2H_4$]-10-formylfolic acid, [$^2H_4$]-tetrahydrofolic acid and [$^2H_4$]-folic acid (50-500 ng each).

Deconjugation and subsequent clean-up by strong anion exchange (D-SAX)

Sample suspensions were incubated with amylase (0.03 g) for 2 h at 37 °C and with bacterial protease (0.1 g) for 6 h at 37 °C. After enzyme digestion, the samples were heated at 100 °C for 10 min, cooled on ice and spiked with rat serum (100 μL) and chicken pancreas solution (2 mL). The deconjugation was performed at 37 °C overnight. For testing conjugase activity, pteroyl triglutamate (1 μg) was added to food extracts prior deconjugation.

At the end of the conjugase treatment, the extracts were passed through a syringe filter (0.4 μm, Millipore, Bedford, MA, USA). Subsequently, the extracts were subjected to clean-up by solid phase extraction according to Freisleben et al. (2003b), using

Bakerbond SAX cartridges (quaternary amine, 500 mg, No. 7091-3, Baker, Gross-Gerau, Germany). The cartridges were successively activated with 2 volumes of hexane, methanol and water, and then conditioned with 7 to 8 volumes of conditioning buffer. After applying the sample extracts, the columns were washed with 6 volumes of conditioning buffer, and the folates were eluted with 2 mL of aqueous sodium chloride (5 %, containing 1 % sodium ascorbate and 0.1 mol/L sodium acetate). 100 µL 2-mercapto ethanol was added to each eluate and the purified extracts were stored under argon at –30°C until analysis.

The purified extracts were then subjected to LC-MS/MS. Each food sample was analyzed in triplicate by separate extractions.

Deconjugation after strong anion exchange (SAX-D)

Enzyme treatments with amylase and protease were performed as described before. Subsequently, the samples were heated to 100 °C for 10 min, then adjusted to pH 7.0 and purified by SAX as described before. After clean-up, the eluate was adjusted to pH 7.0 by addition of aqueous sodium hydroxide (0.1 mol/L) and deconjugation was performed as detailed before.

LC-MS/MS

The samples (10 µL) were chromatographed on a Nucleosil 100-5 C-18 reversed phase column (250 x 3 mm; 5 µm, Macherey & Nagel, Düren, Germany) that was connected to a photo diode array detector and a TSQ quantum triple quadrupole mass spectrometer (Finnigan MAT, Bremen, Germany).

The mobile phase consisted of variable mixtures of aqueous formic acid (0.1 %; eluent A) and acetonitrile (eluent B) at a flow of 0.4 mL / min. Gradient elution started at 9 % B held for 4 min, followed by raising the concentration of B linearly to 25 % within 10 min and to

100 % within further 6 min. Subsequently, the mobile phase was held at 100 % B for 4 min before equilibrating the column for 5 min at the initial mixture.

During the first 4.5 min of the gradient program, the column effluent was diverted to waste. The spectrometer was operated in the positive electrospray mode using selected-reaction monitoring (SRM), the conditions of which are detailed in table 1. Spray voltage was set to 3200 V, sheath gas pressure was 60 mTorr and auxiliary gas pressure 20 mTorr. Capillary temperature was 350 °C and capillary offset 35 V. Source CID (collision induced dissociation) was used with the collision energy set at 10 V.

RESULTS AND DISCUSSION

The enormous variety of naturally occurring vitamers along with their occurrence in the µg/kg range and their instability render folates highly challenging analytes. First, their occurrence in trace amounts can be met with modern LC instrumentation coupled to sensitive mass spectrometry or fluorescence detection and, second, their lability is best considered by using stable isotopically labelled analogues of the vitamers as the internal standards to correct for losses. However, the variability still is not resolved due to the lack of respective internal standards for all vitamers. Therefore, most methods use enzymatic deconjugation to transfer polyglutamates to their monoglutamic forms, thus reducing complexity of analysis. Several types of conjugases are in current use with rat plasma or chicken pancreas being the most common ones. However, a further obstacle for folate analysis is the occurrence of conjugase inhibitors, which, depending on the respective food, may decrease efficacy of monoglutamate formation.

Optimization of SIDA methodology by adaption to legume plant matrix

Although our previous study of spinach and broccoli revealed optimal deconjugation

activity of rat plasma (Freisleben et al., 2003a), a new batch of rat plasma showed only 42 % activity in the present study (table 2). Even a higher addition of rat plasma did not yield the desired activity, which forced us to test further conjugases. Besides rat plasma, chicken pancreas and hog kidney are reported to contain conjugases. As chicken pancreas is commercially available, we added solutions thereof to pteroyltriglutamate and rat serum and achieved nearly total deconjugation (table 2). As chicken pancreas is known to degrade polyglutamates only to diglutamates (Keagy, 1985), the additional hydrolytic activity of rat serum conjugase is necessary to degrade the diglutamates to monoglutamates. Furthermore, it has to be concluded from the poor conjugase activity of the used rat plasma that each batch has to be tested separately.

In a further series of experiments, the new enzymatic mixture was tested for its activity in extracts of lentils. Interestingly, the new mixture revealed only an activity of 45 % (table 3), which pointed to the presence of conjugase inhibitors in this legumes. As higher doses of enzymes did not increase significantly the yield of monoglutamates, we probed alternative routes to remove the inhibitors from the extracts. A very effective way to purify folates is strong anion exchange (SAX) chromatography, which usually is performed after deconjugation to obtain LC-mass chromatograms free from interferences. Therefore, to separate folates from conjugase inhibitors in the extracts, we performed SAX prior to deconjugation and tested this procedure, henceforth referred to as SAX-D, for its yield of pteroylmonoglutamate from added $PteGlu_3$. As the elution buffer of SAX cartridges had a pH of 5.0 , which was not the optimum for the conjugases used, we subsequently adjusted the purified extracts to pH 7.0 and, thus, obtained an activity of about 100 % (table 3). From these results it was evident that both monoglutamic and polyglutamic forms were bound to the SAX material, whereas inhibitors were removed during the washing step. The efficiency of the new sample clean-up was confirmed for freezed beans and peas, the

extracts of which were deconjugated to a much higher extent after SAX-D (table 4). The mass chromatograms of LC-tandem MS were almost devoid of interferences and confirmed the applicability of the new procedure as shown in figure 1 for an extract of white beans.

The nature of conjugase inhibitors is still under discussion. According to studies on lima beans, Wei and Gregory (1998) proposed organic acids such as citric acid to act as inhibitors. To test this hypothesis, we analyzed the content of citric acid in a white bean extract before and after SAX treatment, which revealed amounts of 9.9 g/kg and 8.3 g/ kg citric acid, respectively. As SAX effectively removed conjugase inhibitors, this treatment should also reduce citric acid in the eluate, if it was an active compound. However, the decrease was only equivalent to 16 % in the citric acid content . Therefore, conjugase inhibition cannot mainly be attributed to citric acid. On the contrary, other bean compounds must be responsible.

Quantitative results

By applying the optimized SIDA methodology to a number of dry legume seeds, fresh pulses and different processed legume products, total folate contents summarized in Table 5 and 6, respectively, were found and compared to literature data determined by LC-FD or MA. Raw and processed products were sampled from local markets and are likely to be representative of products consumed in Bavaria.

In general, total folate concentrations of dried legume seeds analyzed by SIDA were found within a concentration range of 10 up to over 300 µg/100g. Highest value was determined for soy beans containing 318 µg/100g, followed by mung beans and chick peas with nearly 280 µg/100g and red lentils and kidney beans with approximately 180 µg folate /100g. In the concentration range of 100-150 µg/100g total folate values were analyzed for white and black beans, blackeyed peas as well as green lentils, 50-100

µg/100g folate were found for jack beans and peanuts, respectively. Lowest total folate content of 10-20 µg/100g was found for green peas. In case of fresh legumes, when green pods and seeds of beans and peas were analyzed completely, folate contents ranged between 40 and 90 µg/100g and showed a naturally high variation of the fresh samples, due to their varying dry matter fraction and climate as well as genetical influences. Canned legume samples revealed significant lower folate concentrations in comparison to the respective raw material. Depending on the thermal influence during processing and the significantly higher water content of the products, values between 4 (green beans) up to 70 µg/100g (blackeyed peas) were found. No significant folate losses were observed during cooking of fresh green beans (37 µg/100g; cf. values Table 6 and 7), only the vitamer pattern was found to be altered after cooking, which will be discussed later. Folate contents obtained for fresh green beans, dried seeds and the respective canned products in this study are not comparable, because of the naturally occuring variations of the raw material. Remarkably high folate concentrations were found for deep frozen fresh green peas and beans containing approximately 70-150 µg total folate /100g.

Comparing our results with literature data in tables 6 and 7, the majority of folate data are basing on microbiological assays, whereas data obtained by liquid chromatographical methodologies, e.g. LC-FD are still scarce. Particularly the SIDA data of the dried legume seeds show significant differences in comparison to the values determined by MA. Highest MA data (USDA 2005, Augustin and Klein 1989) were by a factor of 2 or 3 higher (chick peas, green lentils, white and black beans, kidney beans, mung beans), in case of blackeyed peas by a factor of 4 higher and in case of green peas 14-27 times higher as compared to the amounts analyzed by SIDA. However, as the MA data were rather scattered, in particular the lower figures are often in good accordance with our results. Similarly, the MA data of soy beans and red lentils are well in line with SIDA values (USDA 2005; Augustin and Klein, 1989; Yon and Hyun, 2003). The high scattering in MA

data may be attributed to natural variabiliy of the raw materials, but also to the different microorganisms used for analysis. Folate concentrations of dried legume seeds and fresh green peas and green beans determined by LC-FD (Müller 1993) were found at the upper level of the concentration range from SIDA data, with the exception of dried green peas, the content of which measured by LC-FD was significantly higher.

Literature data of canned legume products (USDA, 2005; Konings et al. 2001; Leichter 1980) are well in line with SIDA results. Concerning the data found for deep frozen products, the different processing steps used during production have to be considered. Literature data (USDA 2005, Konings et al. 2001) stem from deep frozen products, which were additionally cooked; this could explain the significant lower values in comparison to SIDA results. In case of deep frozen peas, only the seeds were investigated, whereas for the fresh product pods and seeds were used for sample preparation. Interestingly, folate concentration in the frozen seeds was significantly higher than analyzed in the entirety of pods and seeds, bearing in mind, that the frozen sample had not been prepared from the investigated raw material. To clear these findings, further studies are necessary.

Summarizing the comparison of SIDA values obtained in this study with literature data, it could be assumed, that particularly the complex food matrix of dried legume seeds leads to inaccuracies of MA and LC-FD analyses.

Besides total folate content, the distribution of single vitamers is worth being considered. Interestingly, the folate pattern in legumes proved to be rather differentiated. Whereas in frozen peas, dried lentils, dried blackeyed beans and mung beans, 5-methyltetrahydrofolate was the most abundant vitamer, in fresh beans and soy beans THF was predominant and in peanuts the vitamer 5-formyltetrahydrofolate (fig. 2).

The folate distribution is strongly dependent on the physiological state of the plants and its balance in the plants' compartiments (Cossins, 2000). Tetrahydrofolate is the first intermediate in folate biosynthesis and a central point in folate metabolism. During germination and growth, 5-methyltetrahydrofolate has been observed to be most abundant (Roos et al., 1968) as it is necessary for methionine biosynthesis (Dodd and Cossins, 1970) and methylation via S-adenosylmethionine (Dodd and Cossins, 1969). Formylated folates are mainly involved in purine biosynthesis (Rowe, 1969) and, to the actual knowledge, their prevalence cannot be assigned to a special period during growth. In particular, their prevalence in roasted peanuts and dried blackeyed peas cannot be explained.

When comparing these results with folate pattern from previous studies, decisive discrepancies can be observed: in the reports of Vahteristo et al. (1997), Melse-Boonstra et al. (2002), Konings et al. (2001) and Müller (1993), the folate vitamers in green peas, green beans, different beans as well as beans and green peas were quantified, respectively. All reports stated 5-methyltetrahydrofolate to be the most abundant vitamer, which was in agreement with our results for freezed peas or mungo beans. However, some of these studies did not report the occurrence of tetrahydrofolate (Konings et al., 2001) or 5-formyltetrahydrofolate (Vahteristo e al., 1997; Meelse-Boonstra et al., 2002). These discrepancies might be due to (1) the lability of the former or (2) the occurrence of folate interconversions during extractions of the latter authors of (3) the high detection limit of the latter in HPLC coupled to fluorescence detection. Therefore, a general dominance of 5-methyltetrahydrofolate forms appear to be not valid for all legumes.

Regarding the effect of cooking on the folate distribution in beans, we observed a decrease in tetrahydrofolate, which was paralleled by an increase in 5-methyltetrahydrofolate and 5-formyltetrahydrofolate. Taking into consideration the lability

of tetrahydrofolate against oxidation, one would expect PteGlu to increase, which was not observed. Therefore, enzymatic and non-enzymatic interconversions of these folates during cooking have to be concluded.

CONCLUSIONS

The folate contents analyzed by SIDA showed for some legumes significant differences to the literature data obtained by MA. Whether the discrepancies are due to natural variability of the foods or are due to systematic errors of the methods – this question remains open and will have to be settled in future studies. Although several method comparisons already have been reported and witnessed in part good accordance, and in part disagreement of MA and LC values, some systematic investigations have to be performed including site-by-site comparisons of identical extracts in a similar way as reported recently for the analysis of pantothenic acid (Rychlik and Roth-Maier, 2005).

Although our data may not be fully representative due to the restricted number of samples, folate content of legumes appear to be somewhat lower than the existing literature data. Therefore, the mean daily folate intake from legumes might have been overestimated in the past. However, the average daily consumption of legumes in western indutrialized countries is as low as about one or two grams. Therefore, differences in folate contents in legumes will only account for differences of less then 5 µg/day. In contrast to this, legumes play an important dietary role for populations of developing countries. For example, in Western Africa a mean daily intake of 500 g peanuts is estimated, thus delivering 400 µg of dietary folate daily. However, as the intake data in developing countries are quite unreliable, distinct conclusions are difficult to draw.

Nevertheless, our data confirm legumes to be an important source for dietary folate. Therefore, the consumption of these foods should be propagated in the western countries and could contribute to a better supply with this important group of vitamins.

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Table 1: SRM transitions of folate vitamers

Verbindung	Precursor ion [m/z]	Collison energy [V]	Product ion [m/z]
PteGlu	442	19.0	295
[$^2H_4$]-PteGlu	446	19.0	299
H_4folate	446	19.0	299
[$^2H_4$]-H_4folate	450	19.0	303
5-CH_3-H_4folate	460	21.0	313
[$^2H_4$]-5-CH_3-H_4folate	464	21.0	317
10-CHO-PteGlu	470	19.0	295
[$^2H_4$]-10-CHO-PteGlu	474	19.0	299
5-CHO-H_4folate	474	25.0	327
[$^2H_4$]-5-CHO-H_4folate	478	25.0	331

Table 2 : Rate of conversion of pteroyltriglutamate (1 µg) to folic acid using different enzymes; incubation at 37 °C overnight

Source of conjugase	Conjugase activity (%)
Rat serum [a]	42
Rat serum[a] + chicken pancreas [b]	96

[a] 100 µL

[b] 2 mL of 5 mg chicken pancreas in 30 mL phosphate buffer, pH7

Table 3: Rate of conversion of pteroyltriglutamate to folic acid in lens extract using different enzymes; incubation at 37 °C overnight

Source of conjugase	Conjugase activity (%)
Rat serum [a]	7
Rat serum[a] + chicken pancreas [b]	45
Rat serum[a] + chicken pancreas[b] after SAX treatment and controlling the pH	102

[a] 100 µL

[b] 2 mL of 5 mg chicken pancreas in 30 mL phosphate buffer, pH7

Table 4: Comparison of different enzyme treatments in various legumes

	Source of conjugase			
	Rat serum		Rat serum + chicken pancreas after SAX treatment and controlling the pH	
Legume	Total folate µg/100g	Conjugase activity	Total folate µg/100g	Conjugase activity
Red lentils	70	8 %	178	102 %
Peas, frozen	50	4 %	146	74 %
Beans, frozen	13	7 %	98	92 %

Table 5: Comparison of total folate data of dried legume seeds determined by stable isotope dilution assays (SIDA) to literature data based on liquid chromatography-fluorescence detection (LC-FD) or microbiological assays (MA) [µg/ 100 g fresh weight]

Legumes: seeds, dried		LC-FD (lit. data)	MA (lit. data)	SIDA (own results)
Botanic name	Common name			Means of different samples (± SD for n=3)
Pisum sativum	Peas, green	57[a)]	274*[b)]; 290*[c)] 36[d)]; 22*[h)]	10 (± 3), 20 (± 4)
"	Peas, green, fresh**	159[a)]	65***[b)]	87 (± 10)
Cicer arietinum	Chick peas		557[b)]; 480[c)]; 281[h)]	275 (± 50)
Lens culinaris	Lentils, green	103[a)]	479[b)]; 430[c)]; 172[d)];133[h)]	110 (± 8), 154 (± 20)
"	Lentils, red		204[b)]; 68[h)]	178 (± 13)****
Phaseolus vulgaris	Beans, green, fresh**	70[a)]	37[b)]; 39[f)]	37 (± 5), 52 (± 7), 78 (± 6)
"	Beans, white	128[a)]	388[b)]; 360[c)]	106(± 10), 164 (± 10)
"	Kidney beans		394[b)]; 371[c)]; 238[e)]; 140[h)]	182 (± 21)
"	Beans, black		444[b)]; 447[c)]	143 (± 12), 106 (± 9)

Glycine max	Soy beans	375[b)]; 250[c)]; 318[e)]; 189[h)]	318 (± 28)
Vigna radiata	Mung beans	625[b)]; 490[c)]; 237[e)]; 438[h)]	277 (± 33)
Vigna unguiculata	Blackeyed peas	633[b)]; 540[c)]; 399[h)]	149 (± 33), 152(± 41)
Canavalia ensiformis	Jack beans		94 (± 11)
Arachis hypogaea	Peanut	145[#], 240[## b)]; 169[c)]; 136[e)]; 17[g)]	94 (± 7)[#], 61 (± 8)[#]

*split peas; **pods and seeds; *** seeds, green; ****peeled; # dry-roasted; ## raw

Table 6: Comparison of total folate data of processed legume seeds determined by stable isotope dilution assays (SIDA) to literature data based on liquid chromatography-fluorescence detection (LC-FD) or microbiological assays (MA) [µg/ 100 g fresh weight]

Legumes: seeds, processed	LC-FD (lit. data)[g]	MA (lit. data)[b]	SIDA (own results) Means of different samples (± SD for n=3)
Peas, green, canned		29, 44[i)]	13, 46 (± 3)
Beans, green, canned	17	18, 32[i)]	9 (± 2), 4 (± 1)
Beans, white, canned		65	29 (± 5)
Kidney beans, canned	16	36	17, 59 (± 12)
Chick peas, canned		67	63 (± 6)
Beans, black, canned		-	32 (± 6), 35 (± 6)
Blackeyed peas, canned		51	61 (± 38), 69 (± 27)
Peas, green, deep frozen		59[#]	146 (± 20), 128 (± 9)
Beans, green, deep frozen***	41[#]	23[#]	98 (± 14), 68(± 9)
Beans, green, cooked and drained*	36, 22	33	37 (± 5)**

* Pods and seeds fresh, cooked; ** cooking time: 15 min (37 µg/100g folate analyzed for the raw material cf. table 5); *** pods and seeds; # deep frozen and cooked, drained

References:

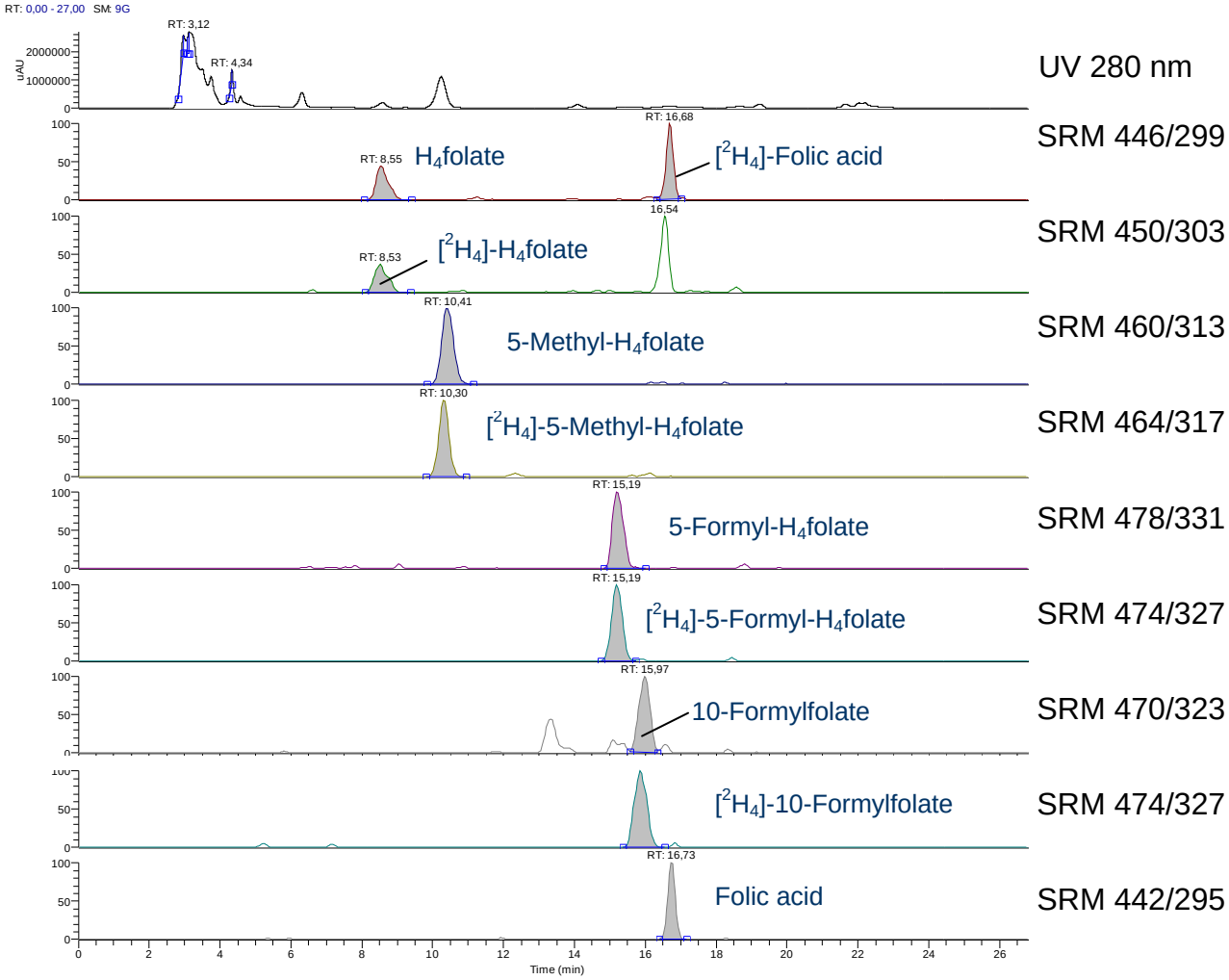
a) Müller (1993a,b)
b) USDA (2005)

c) Augustin and Klein (1989)
d) Han and Tyler (2003)
e) Yon and Hyun (2003)
f) Melse-Boonstra et al. (2002)
g) Konings et al. (2001)
h) Hoppner and Lampi (1993)
i) Leichter (1980)

LEGEND TO THE FIGURES

Figure 1. LC-MS/MS chromatogram of dried white beans. Upper trace: UV-signal; selected reaction monitoring (SRM) traces: *m/z* precursor ion/*m/z* product ion

Figure 2. Folate pattern of different legumes;H4folate, tetrahydrofolate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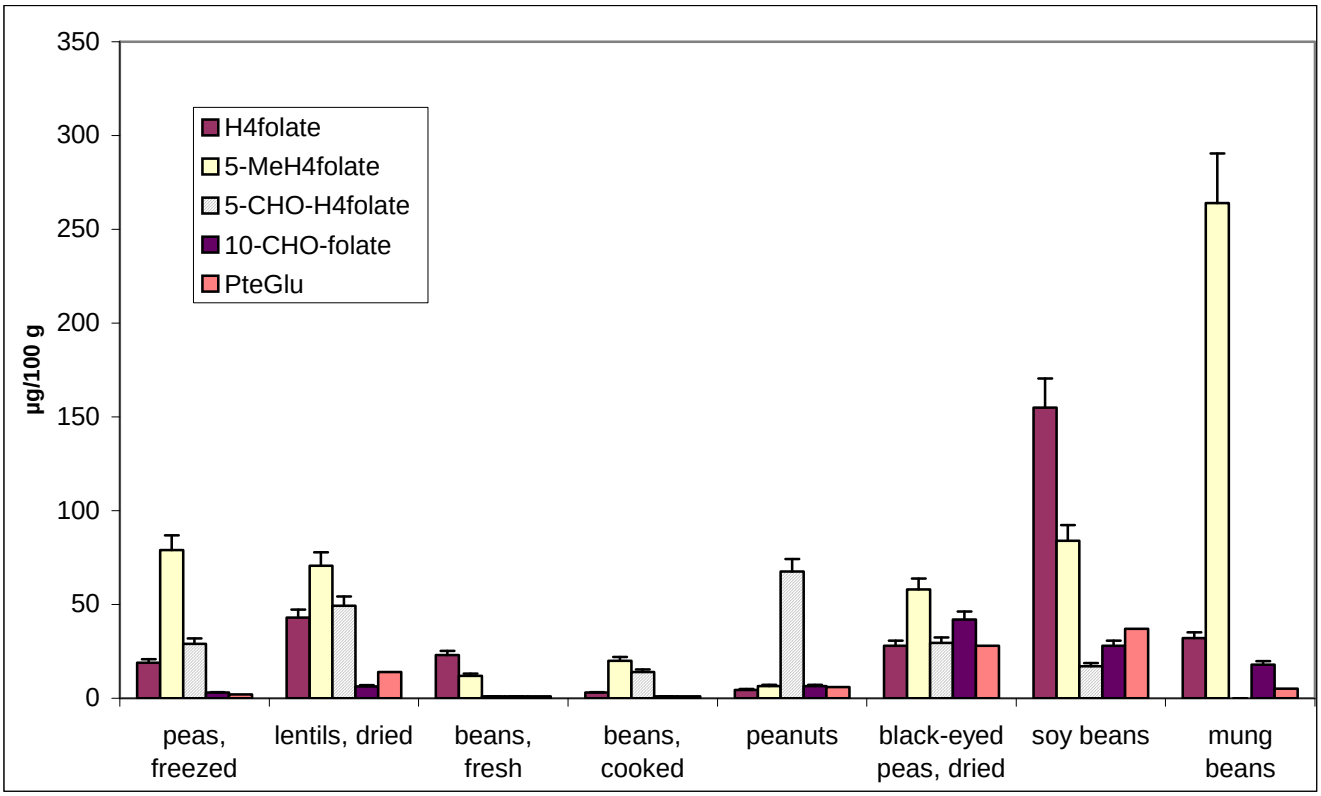
RT: 3,12
RT: 4,34
uAU
2000000
1000000
0
UV 280 nm
RT: 16,68
RT: 8,55
H4folate
[2H4]-Folic acid
SRM 446/299
16,54
RT: 8,53
[2H4]-H4folate
SRM 450/303
RT: 10,41
5-Methyl-H4folate
SRM 460/313
RT: 10,30
[2H4]-5-Methyl-H4folate
SRM 464/317
RT: 15,19
5-Formyl-H4folate
SRM 478/331
RT: 15,19
[2H4]-5-Formyl-H4folate
SRM 474/327
RT: 15,97
10-Formylfolate
SRM 470/323
[2H4]-10-Formylfolate
SRM 474/327
RT: 16,73
Folic acid
SRM 442/295
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Time (min)

Figure 1


350
300
250
200
150
100
50
0
µg/100 g
H4folate
5-MeH4folate
5-CHO-H4folate
10-CHO-folate
PteGlu
peas, freezed
lentils, dried
beans, fresh
beans, cooked
peanuts
black-eyed peas, dried
soy beans
mung beans