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Effect of Germination and Thermal Treatments on Folates in Rye

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Effects of germination conditions and thermal processes on folate contents of rye were investigated. Total folate contents were determined microbiologically with *Lactobacillus rhamnosus* (ATCC 7469) as the growth indicator organism, and individual folates were determined by high-performance liquid chromatography after affinity chromatographic purification. Germination increased the folate content by 1.7−3.8-fold, depending on germination temperature, with a maximum content of 250 μg/100 g dry matter. Hypocotylar roots with their notably high folate concentrations (600−1180 μg/100 g dry matter) contributed 30−50% of the folate contents of germinated grains. Germination altered the proportions of folates, increasing the proportion of 5-methyltetrahydrofolate and decreasing the proportion of formylated folate compounds. Thermal treatments (extrusion, autoclaving and puffing, and IR and toasting) resulted in significant folate losses. However, folate levels in grains that were germinated and then were heat processed were higher than for native (nongerminated) grains. Opportunities to optimize rye processing to enhance folate levels in rye-based foods are discussed.

KEYWORDS: Folate; rye; grain; germination; thermal treatment

INTRODUCTION

Recent studies have detailed the health benefits of whole grains and the importance for an increase in whole-grain consumption (*1*−*3*). Whole-grain foods are important sources of dietary fiber and many bioactive compounds, including folates. Adequate intake of folate is associated with prevention of megaloblastic anaemia and neural tube defects, protection against certain cancers, and decreasing the risk for cardiovascular diseases by maintaining normal plasma homocysteine concentrations. However, among many populations, the average folate intake is lower than recommended (*4*).

The folate content of rye is relatively high, approximately 65−135 μg/100 g dry matter (*5*−*7*), and the bioavailability of folates is good (*8*). Folates are located in the outer layers of the grain that are often lost in milling fractionation (*9*, *10*). Although the food industry has introduced new types of rye products, for instance, snacks and functional foods, the selection is still rather limited. Processing methods offering healthy and convenient products with good sensory properties need to be developed to increase the consumption of whole grain.

Minimal processing represents one way of improving vitamin retention in foods. By means of certain processes, such as fractionation, fermentation, and germination, it is possible to maintain or even naturally enhance the folate contents of foods or food ingredients (*10*−*12*). In addition, combination of processes remains a promising, yet rather unexplored, research area.

During germination, folate synthesis is accelerated because of increased demand for C1-units as the metabolic activities of the developing seedling increase (*13*, *14*). Germination (or malting) is mostly used in beer and whiskey production to enzymatically degrade starch to sugars that are suitable for yeast fermentation. In bakery and confectionary products, malted cereals contribute to creating a certain flavor, color, or desirable texture. A crunchy texture can also be obtained by puffing or by some other heat treatments. In any case, to avoid microbiological spoilage, germinated grains have to be thermally processed. Unfortunately, folates are very susceptible to heat, oxidative degradation, and leaching.

The aim of this study was to investigate the effect of germination at different temperatures on folate contents of rye and to study the extent of folate losses and changes in folate form distribution during thermal treatments. The contribution of folates in hypocotylar roots to the total folate content of germinated grain was also estimated.

MATERIALS AND METHODS

Germination Experiment I. Two rye cultivars, small-grained Akusti and large-grained Amilo, were germinated at 5, 10, and 25 °C in commercial malting equipment (Joe White Malting Systems, Mel-

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bourne, Australia). The grains were first steeped for 8 h followed by a 16-h air rest. After that, a second steep of 6 h was applied. Grain moisture content was approximately 42−46%. The total germination time, including steeping, was 6 days. Sampling points were 1, 2, 4, and 6 days. The samples were frozen, were subsequently freeze-dried, and were stored at −20 °C until folate analysis. Roots were not removed after germination.

Germination Experiment II. In this experiment, rye cultivar Amilo was used as the starting material. Roots were removed after germination, and from some batches roots were also analyzed for folates. Liukkonen et al. (*15*) have presented a detailed description of the experimental design. The grains were first steeped for 8 h followed by a 16-h air rest and then were steeped again for 8, 6, or 4 h depending on the germination temperature (8, 15, and 22 °C, respectively). Grain moisture content after steeping was 44−50.5%. Three variables representing typical germination conditions were temperature (8, 15, and 22 °C), total germination time (3, 5, and 7 days), and temperature of subsequent drying (50, 75, and 100 °C or freeze-drying). A central composite design with four replicates at the center point was applied in the statistical experimental design. Results were analyzed by multiple regression methods (MLR or PLS). Regression analysis was performed, and response surfaces were plotted with the Modde 4.0 (Umetrics AB, Umeå, Sweden).

Thermal Processes. Thermal processing studies were performed with both native and germinated Amilo cultivar (from germination experiment II: 5 days at 15 °C, drying at 75 °C).

Extrusion. Native and germinated rye flour were extruded with APV MPF 19/25 twin screw extruder using process parameters which produced expanded and relatively crispy extrudate without any detected toasted taste. The diameter of die was 3 mm. The temperatures of four heating/cooling barrel zones calculated from die were set at 140 °C, 140 °C, 120 °C, and 20 °C. The screw speed was 225 rpm. The retention time of rye in the extruder was about 30 s. The total load (% from maximum allowable load) during the extrusion was 60% for native and 76% for germinated rye.

Autoclaving and Puffing. Native and germinated rye grains were mixed with water (1:1, w/w) in open steel vessel and were autoclaved at 120 °C for 0.5 h to gelatinize most of the starch. The expanded grains were dried and toasted in rack oven (Sveba Dahlin 900) at 175 °C for 30 min. The final products were crispy and slightly puffed grains.

IR and Toasting. The native and germinated rye grains were moistened to a moisture content of 20−30% at 80 °C. Grains were then heat-treated under radiated heat from 375 W Osram lamps for 5 min followed by heating in rack oven at 200 °C for 10 min. The final products were toasted but not puffed grains.

Calibrants. (6S)-Tetrahydrofolate (H_4folate, sodium salt), (6S)-5-methyltetrahydrofolate (5-CH_3-H_4folate, calcium salt), and (6S)-5-formyltetrahydrofolate (5-HCO-H_4folate, sodium salt) were obtained from Eprova AG (Schaffhausen, Switzerland). 10-Formylfolic acid (10-HCO-PGA), folic acid (PGA), and pteroyl-L-glutamic acid ($PteGlu_3$) were obtained from Dr. Schirck's Laboratories (Jona, Switzerland). Calibrants were dissolved as described by van den Berg et al. (*16*), and the purities were checked using molar absorptivity coefficients at pH 7.0 (*17*). Standard stock solutions were prepared to 0.01 M acetate buffer containing 1% (w/v) sodium ascorbate (pH 4.9). Stock solutions were flushed with nitrogen and were stored at −20 °C. 10-Formyldihydrofolate (10-HCO-H_2folate) was synthesized from 5,10-methenyltetrahydrofolate hydrochloride (5,10-CH^+-H_4folate, chlorine hydrochloride, Eprova AG) as described by Pfeiffer et al. (*18*) and modified by Kariluoto et al. (*11*). Standard solution was flushed with nitrogen and was stored in 0.05 M Tris/HCl, pH 8.4 at −20 °C.

A mixture of standards for high performance liquid chromatography (HPLC) analysis was prepared by diluting standard solutions with 0.01 M acetate buffer containing 1% (w/v) sodium ascorbate (pH 4.9). The mixture was then flushed with nitrogen and was stored at −20 °C.

Sample Extraction. Extraction and trienzyme treatment have been published in detail elsewhere (*6*, *11*). Briefly, samples (0.8−2 g) were extracted in duplicate with 50 mM Ches/50 mM Hepes, containing 2% (w/v) sodium ascorbate and 10 mM 2-mercaptoethanol, pH 7.85, by boiling for 10 min. In trienzyme treatment, samples were first incubated with hog kidney conjugase and α-amylase and then with protease. Hog kidney conjugase was prepared according to Gregory et al. (*19*), and conjugase activity was tested according to Vahteristo et al. (*20*) using $PteGlu_3$ as substrate. Procedures were carried out under subdued light or samples and calibrants were covered with aluminum foil. Sample extracts were kept under nitrogen atmosphere whenever feasible.

Microbiological Assay. Samples were analyzed for total folates by a microbiological method on 96-well microtiter plates (tissue culture treated; Costar Corporation, Cambridge, MA) at pH 6.1 using glycerol-cryoprotected *Lactobacillus rhamnosus* (ATCC 7469) as the growth indicator organism. Two dilutions were made from each sample extract, and eight levels of calibrant (0−80 pg 5-HCO-H_4folate/well) were included into each plate. Plates were incubated for 18−20 h at 37 °C. The turbidity was measured with a microplate reader (iEMS Reader MF; Labsystems, Helsinki, Finland) at 595 nm (*11*). Over 10% difference between the results of two duplicates was considered unacceptable. Certified reference material CRM 121 (wholemeal flour, obtained from the Institute for Reference Materials and Measurements, Geel, Belgium) was analyzed as a quality control sample in each incubation. A control chart was constructed on the basis of 10 determinations (*11*), and results were rejected if the folate content of CRM differed more than 1.5 standard deviations from the average (51.1 ± 6.5 μg/100 g dry matter; certified value 50 ± 7 μg/100 g dry matter).

Sample Purification and HPLC. Distribution of different folates was determined from selected germination samples. Thermally processed grains were also analyzed for folate forms. Affinity chromatography with folate binding protein coupled to agarose was used as a purification method for sample extracts. Preparation and use of affinity columns have been published previously (*6*). The folate load applied into the affinity columns was not allowed to exceed one-fourth of the binding capacity to ensure quantitative binding of 5-HCO-H_4folate.

The HPLC equipment consisted of Waters 510 and 515 HPLC pumps and Waters 717 plus Autosampler (Waters, Milford, MA). Waters 2487 Dual λ Absorbance detector was set at 290 nm and Waters 470 fluorescence detector was set at 290-nm excitation and 356-nm emission wavelengths for reduced folates and at 360 nm/460 nm for 10-HCO-PGA. Waters Millennium 2020 Chromatography Manager data acquisition system was used to collect and calculate data. Quantification was based on an external standard method with calibrants purified with affinity chromatography.

Folates were separated on a ThermoQuest (Cheshire, United Kingdom) Hypersil ODS column (150 mm × 4.6 mm; 3 μm). Gradient elution was performed with acetonitrile−30 mM phosphate buffer, pH 2.2, at 0.9 mL/min flow rate (*11*). Injection volumes varied from 25 to 200 μL. Peaks were identified by retention times, and their identities were confirmed by spiking and comparing ratios of fluorescence and UV peaks.

RESULTS

Effect of Germination on Folates. Total folate content increased markedly during germination I (**Figure 1**). Germination led to 1.7−3.8-fold increase in folate content in comparison with the native grain. The influence of the germination temperature was clear: generally, the higher the germination temperature, the higher the folate content at a certain sampling point. The increase in total folate content (1.8−2.5-fold) during germination II was smaller than in the first germination experiment, probably because the roots were removed. The highest folate contents were achieved when rye grains were germinated at 14−16 °C for 7 days and were dried below 75 °C (*15*). Increasing the drying temperature gradually decreased folate contents.

The increase in folate content during germination was associated with increased amounts of H_4folate and 5-CH_3-H_4-folate (**Table 1**). Interestingly, during germination, the relative proportions of H_4folate and 5-CH_3-H_4folate remained roughly the same, 23−25% and 49−55%, respectively.

Folates in Hypocotylar Roots. The folate concentrations in roots were markedly high, 600−1180 μg/100 g dry matter

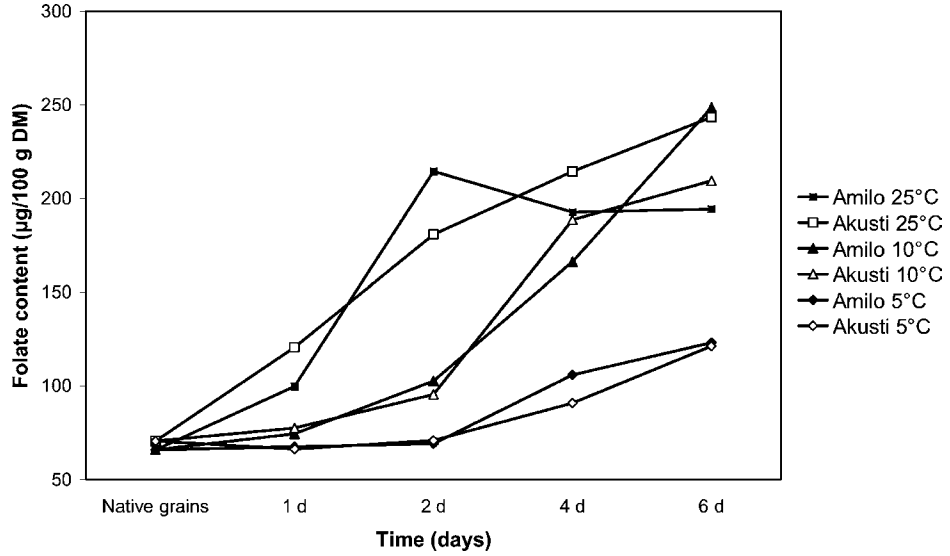

Figure 1. Effect of germination time and temperature on folate levels of two rye cultivars (Amilo and Akusti; germination experiment I).

Table 1. Folates in Rye Grains after Germination at 15 °C and Subsequent Freeze-Drying (Germination Experiment II)

	germination time		
folates (μg/100 g dry matter)[a]	3 days	5 days	7 days
H4	16.7	21.9	23.4
5-CH3-H4	39.3	49.4	46.1
10-HCO-H2	6.6	9.0	12.9
10-HCO-PGA	3.2	3.7	4.0
5-HCO-H4	2.5	2.8	4.6
PGA	2.9	3.3	3.4
sum of folates	71	90	94

[a] Mean of duplicate analysis.

(**Table 2**). The roots were only 5−10% of total germinated grain weight but contributed 30−50% of the folate content. Total folate contents of roots were 6−10-fold higher than in the respective germinated grains and 10−19-fold higher than in native (nongerminated) grains. Roots from grains germinated at 22 °C contained less folates than those germinated at 15 °C.

The main folate in roots was 5-CH_3-H_4folate, corresponding to 67−77% of the sum of the folate forms (**Table 2**). H_4folate contributed only to 4−10%, but surprisingly, the combined proportions of 5-CH_3-H_4folate and H_4folate were almost the same in both roots and in the respective grains. The difference was two percentage units at the most. In our analytical system, 10-HCO-H_4folate could not be quantitated as such. However, the amounts of 10-HCO-H_2folate and 10-HCO-PGA give an estimate of the 10-HCO-H_4folate content. The sum of these folates was 13−18%, whereas 5-HCO-H_4folate contributed 4−6%.

Folate Losses in Thermal Processes. Folate losses varied between different thermal processes (**Figure 2**), but the relative losses were similar for both germinated and native (nongerminated) grains. Folate losses were greatest in autoclaving and puffing treatment (45−54% compared to either germinated or nongerminated control). IR treatment followed by roasting resulted in losses of 35−50%, whereas in extrusion the losses were moderate, 26−28%. Although folates were very sensitive to thermal processing, the rise in folate content during germination ensured a high folate concentration in the end products.

The most distinctive differences between the folate form distributions of native and germinated grains after heat processing were the large proportion of 5-CH_3-H_4folate and the lower proportion of formylated folates in germinated, heat-processed samples (**Table 3**). The relative proportion of 5-CH_3-H_4folate in germinated and then heat-processed samples was roughly the same as in germinated control (non-heat-processed). Thermal treatments caused an increase in the proportion of PGA, whereas the amount of H_4folate decreased drastically. The sum of 10-HCO-H_2folate and 10-HCO-PGA constituted 20−22% of the sum of folate forms in germinated and heat-processed grains.

DISCUSSION

Statistical Evaluation and Analytical Findings. The fit of model in germination experiment II was evaluated by the coefficient of determination (R^2) and the predictive power of model (Q^2; should be over 0.5). The coefficient of determination was 0.87 and the predictive power was 0.73. In addition, the reproducibility of the germination process was studied by analyzing the four replicates at the center point of the statistical design (15 °C, 5 days, dried at 75 °C) both by microbiological assay and HPLC. Germination process was well reproducible: the average total folate content was 147 ± 1.0 μg/100 g (coefficient of variation (CV) percent = 0.7) and the sum of folate forms was 78 ± 3.2 μg/100 g (CV% = 4.1).

Differences between duplicate samples were generally satisfactory. The microbiological assay of a particular sample was repeated if the difference between the results of two duplicates exceeded 10%. In HPLC analyses, the difference between duplicates varied from 2 to 9%, except for autoclaved and puffed samples (17 and 18%).

In germinated samples, the sum of folate forms determined by HPLC varied from 61 to 81% of the total folate content determined by microbiological assay. Folate concentrations were often near the quantitation limits, which caused uncertainty to the results. The main problems were peak purities and identification: despite the selective purification method based on folate binding protein, some unidentified peaks existed in the chromatograms (**Figure 3**). The two most common unidentified were eluted approximately 1.5 and 3.2 min after 5-CH_3-H_4folate. A third peak was often found 0.6 min before PGA, which matches the retention time of 5,10-methenyl-H_4folate. However, there was no corresponding peak in the fluorescence chromatogram (360 nm/460 nm).

In thermally processed grains, the sum of folate forms varied from 33 to 79% of the total folate content determined by

Table 2. Folates in Rye Roots after Germination (Germination Experiment II)

	germination parameters: temperature, time, and drying				
folates (μg/100 g dry matter)[a]	15 °C, 3 days, freeze-drying	15 °C, 7 days, freeze-drying	15 °C, 5 days, 50 °C	15 °C, 5 days, 75 °C	22 °C, 5 days, 75 °C
H4	98	46	41	n.a.[b]	n.a.[b]
5-CH3-H4	631	610	729	n.a.	n.a.
10-HCO-H2	116	114	84	n.a.	n.a.
10-HCO-PGA	33	42	36	n.a.	n.a.
5-HCO-H4	56	50	36	n.a.	n.a.
PGA	10	13	23	n.a.	n.a.
sum of folates	944	876	948	n.a.	n.a.
total folates by MA	1184	1168	1064	1130	607

[a] Mean of duplicate analysis. Residual moisture not determined (residual moistures in the respective grains 5.6–7.5%). [b] n.a. = not analyzed.

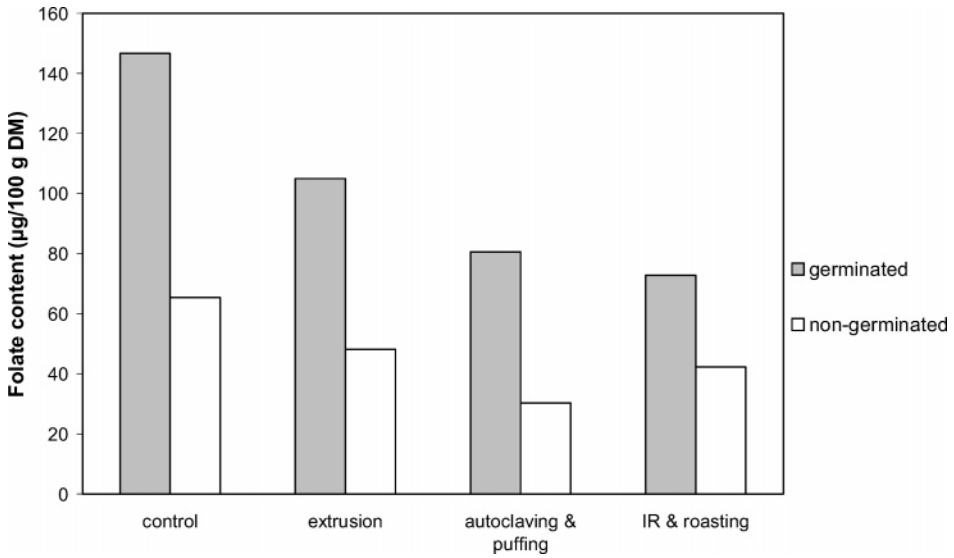

Figure 2. Effect of thermal processing on folate levels in germinated and nongerminated rye grains.

Table 3. Folates in Native Or Nongerminated and Germinated Rye Grains after Thermal Processes (Germinated 5 Days at 22 °C, Dried at 75 °C)

	extruded		autoclaved & puffed		IR & roasted	
folates (μg/100 g dry matter)[a]	native	germinated	native	germinated	native	germinated
H4	2.8	4.4		1.4	0.9	1.3
5-CH3-H4	7.6	48.5	1.8	27.5	3.0	14.2
10-HCO-H2	4.0	7.8	2.0	3.6	2.9	
10-HCO-PGA	8.5	8.4	5.0	6.5	2.4	4.9
5-HCO-H4	7.0	3.7	4.2	1.5	1.8	0.8
PGA	8.2	9.1	5.3	4.6	3.2	2.9
sum of folates	38	82	18	45	14	24
total folates by MA	48	105	30	81	42	73

[a] Mean of duplicate analysis.

microbiological method. The difference was not dependent on low folate concentrations as such. For instance, the ratio was better for nongerminated, extruded grains (79%) than for germinated, autoclaved and puffed grains (56%), although the total folate content was higher in the latter. The highest ratios were found for extruded grains where the folate losses were smallest. Kariluoto et al. (*11*) found that in sourdough fermentation and baking process the lowest ratio was seen in bread sample (44%) whereas at other stages of the baking process the ratios were over 70%. High temperatures may lead to formation of compounds, either folate or nonfolate, that cannot be quantitated by HPLC but have response by *Lactobacillus rhamnosus* in the microbiological assay.

Impurities derived from the sample matrix commonly masked 5-HCO-H_4folate peak in the fluorescence signal. The signal of this folate in the UV detector was sometimes too weak to allow reliable quantification either. Pfeiffer et al. (*18*) found that interfering peaks can be derived from reagents but also from nonfolate compounds retained in the affinity chromatography matrix, and Gujska and Kuncewicz (*7*) also faced difficulties in quantification of 5-HCO-H_4folate in cereal-grain products, especially whole-grain rye flour.

5-CH_3-H_4folate peak sometimes existed as a double peak. Spiking the purified sample extract confirmed that 5-CH_3-H_4-folate was the first peak of the doublet. The latter peak interfered with the separation and quantification of 10-HCO-H_2folate which is present in some cereal products and vegetables (*11*, *18*, *21*). Doherty and Beecher (*22*) reported a similar interfering peak in BCR 421 dried milk powder. There are at least three possible explanations. First, the peak could simply be a matrix-

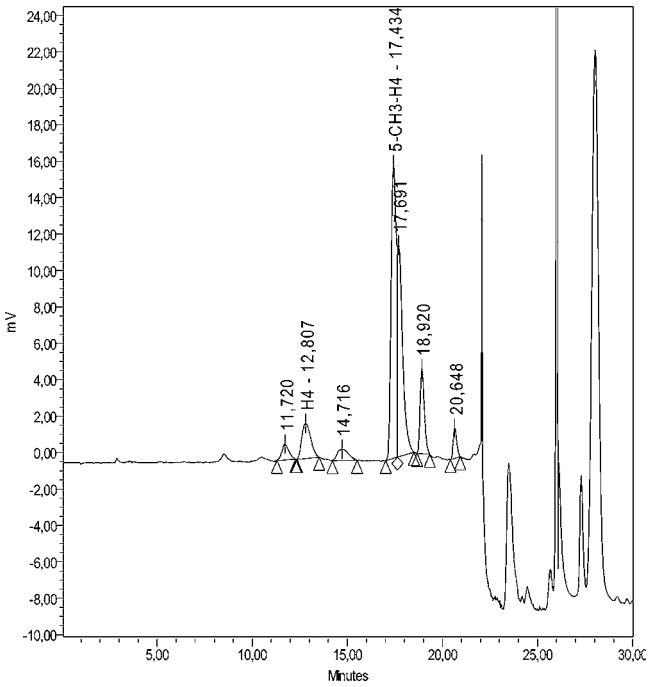

Figure 3. HPLC-chromatogram with fluorescence detection of rye germinated 5 days at 15 °C and subsequently dried to a final temperature of 75 °C (H4, tetrahydrofolate; 5-CH3-H4, 5-methyltetrahydrofolate).

derived impurity. Second, the content of highly conjugated folate derivatives is notably increased during germination (*13*, *23*), and hog kidney conjugase may not have completely deconjugated 5-CH_3-H_4folate polyglutamates. However, on the basis of the enzyme activity test result showing that 50 μL of conjugase was able to convert 20 μg of $PteGlu_3$ to folic acid, the amount of conjugase added to the samples was at least 400-fold to that theoretically needed. In addition, the incubation time for samples was several times longer than in the activity test. It is possible, though, that highly conjugated endogenous folates are not as effectively deconjugated as $PteGlu_3$. The existence of conjugase inhibitors in the samples is unlikely but cannot be fully ruled out. Third, the latter peak may also be a folate compound, for example, 5-CH_3-H_2folate. The samples were not analyzed "fresh" but were either freeze-dried or dried at elevated temperatures which could have caused destruction or interconversion of folates. Further research is thus required to clarify this possibility. Shortly, our findings once again highlight the need for careful peak identification as well as regular monitoring of analytical performance.

Effect of Germination on Folates. The influence of the germination temperature on folate contents is largely explained by the proportion of germinated grains (in other words, the rate of germination): at 5 °C, 100% of grains were germinated only after 3 days, whereas at 10 °C, 100% germination was accomplished after 2 days and at 25 °C already after 1 day. The folate content of Amilo cultivar germinated at 25 °C reached its maximum in 2 days after which the folate content slightly decreased, presumably because of folate destruction by continuous aeration (presence of oxygen).

Jägerstad et al. (*12*) have reported similar folate concentrations to ours for industrially malted rye grains (140−330 μg/100 g fresh weight) and somewhat lower concentrations for malted wheat and oat (140 and 70 μg/100 g, respectively). In barley beer malts, the folate concentrations were 200−300 μg/100 g, and the increase took place mainly during the first 2 days of germination.

Effects of germination on folate concentration of dicotyledonous seeds have also been measured. Jabrin et al. (*14*) found a 1.5-fold increase in folate content in pea seeds imbibed for 18 h, which agrees well with the 0.9−1.7-fold increase at day 1 in our experiment. This initial increase that was associated with the de novo synthesis of H_4folate appeared in cotyledons only. Root tips contained 5-fold more folates that mature roots, which indicates the high capacity of proliferating tissues to synthesize and accumulate folates (*14*).

In cereal-derived products, formylated folates tend to dominate (*6*, *7*, *18*, *21*). On the other hand, in vegetables, 5-CH_3-H_4folate is typically the main folate contributing to >90% of the folate content (*21*, *24*). In this sense, germinating grain with its relatively high 5-CH_3-H_4folate and H_4folate proportions (approximately 50% and 25%) can be regarded as an intermediate phase between grain and metabolically active, photosynthetic organism. For instance, in whole-meal rye flour, the proportion of 5-CH_3-H_4folate is only ∼20−25% and that of H_4folate ∼5% (*6*, *11*).

Folates in Hypocotylar Roots. The high folate content in roots (600−1180 μg/100 g dry matter) found in this study was similar to results from other studies with proliferating tissues. Spronk and Cossins (*13*), for instance, found folate contents of approximately 740 and 1340 μg/100 g dry matter in etiolated and green 8-day-old wheat leaves, respectively. Roots from grains germinated at 22 °C contained less folates than those germinated at 15 °C. It is likely that the folate content reached its maximum earlier at higher temperatures after which folates began to break down. However, because of experimental design, this could not be detected and confirmed. The decrease may result from folate destruction by oxidation or metabolic processes inherent to physiological development of the plant.

Folate form distribution in roots (67−77% of 5-CH_3-H_4folate, 4−10% of H_4folate, 13−18% of 10-HCO-H_2folate and 10-HCO-PGA, and 4−6% of 5-HCO-H_4folate) agreed well with results of earlier studies. Roos and Cossins (*25*) found that the main folate in 3-day-old pea cotyledons germinated in the dark was 5-CH_3-H_4folate corresponding to 76% of the total folate, whereas 10-HCO-H_4folate and 5-HCO-H_4folate contributed to 19% and 5%, respectively. Spronk and Cossins (*13*) reported slightly different patterns for radish cotyledons, pea leaves, and spinach leaves: 45−67% of 5-CH_3-H_4folate, 16−35% of 10-HCO-H_4folate, 11−17% 5-HCO-H_4folate, and 1−10% of H_4-folate. They also found that in etiolated leaves the folate contents were significantly lower and the proportion of methylated derivatives was higher than in leaves grown in the normal light−dark cycle.

In many food processes, roots loosen easily and are then removed, which means that a fraction rich in folates could easily be separated and collected. On the other hand, the bitter taste of roots may limit their use in foods. However, Yang et al. (*26*) have shown that steeping and germination conditions can be optimized to achieve a product with acceptable sensory properties and high antioxidant contents.

Folate Losses in Thermal Processes. Although folate losses in thermal processing were significant, the folate contents in germinated and subsequently heat-processed grains always exceeded the folate content of the native (nongerminated) grain. The greatest losses occurred during autoclaving and puffing where the presence of oxygen is combined with high temperatures. The temperature in extrusion was as high as 140 °C, but the processing time was very short, only 30 s, which resulted in better retention of folates than in the two other treatments. Similarly, Håkansson et al. (*27*) found folate losses of 33−39%

and 26−72% in whole-grain wheat autoclaving and popping, respectively, depending on the severeness of process conditions. Extrusion cooking of white flour resulted in 19−22% losses, little less than in our experiment.

Results suggest that high-temperature−short time or low-temperature processes retain folates better than high-temperature and long time processes such as popping and autoclaving. However, in bread baking, folate losses are still moderate, 20−35% (*9*, *11*, *28*), although the baking temperatures are typically high. This may be due to smaller surface area in baking compared with thermal processing of rye grains.

Nongerminated, heat-processed grains contained less 5-CH_3-H_4folate and 5-HCO-H_4folate but more PGA than whole-meal rye flour (*6*). The sum of 10-HCO-H_2folate and 10-HCO-PGA corresponded to 33−40%. Similar trends in folate pattern were found in rye bread baking where significant losses of H_4folate, 5-CH_3-H_4folate, and 5-HCO-H_4folate occurred whereas the proportions of 10-HCO-H_2folate, 10-HCO-PGA, and PGA increased (*11*).

Leaching can also present a significant source of water-soluble vitamin losses. In our study, leaching was probably not an important factor since the added water was removed mainly by evaporation. One possible mechanism for folate destruction at high temperature was suggested by Schneider et al. (*29*), who found that up to 50% of PGA was converted to N^2-[1-(carboxyethyl)]folic acid (CEF) at 100 °C in the presence of different sugars because of the reaction between reducing carbohydrates and exocyclic amino group of folate molecule. The reaction was more pronounced with disaccharides than with monosaccharides, which is relevant information regarding germination where β-amylase releases maltose from the non-reducing end of the starch polymer. Thus, in thermal processing of germinated rye, the conditions may favor the formation of CEF. In addition to the well-established oxidative cleavage of C9−N10 bond and photodegradation (*30*), CEF formation may also be an important way of folate degradation.

Germination was an efficient way in increasing folate content of rye and ensured high folate levels even in subsequently thermally processed grains. Germination shaped the folate distribution of rye from a pattern commonly found in cereal grains toward that usually found in vegetables. In addition to preventing or minimizing vitamin losses, it is possible to enhance folate contents by means of novel processing methods, using traditional processes in new applications, and combining processes. The potential use of roots, an easily separable fraction with notably high folate levels, as a food ingredient is worthy of research.

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