Effect of Baking Method and Fermentation on Folate Content of Rye and Wheat Breads

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ABSTRACT

The effect of baking method on folates of rye and wheat breads, as well as the effect of sourdough fermentation of rye, were examined. Sourdough fermentations were performed both with and without added yeast, and samples were taken throughout the baking process. Samples were analyzed microbiologically for their total folate content after tri-enzyme extraction. Individual folate vitamers were determined by HPLC after affinity chromatographic purification. The lowest folate contents for both rye and wheat breads were found from breads baked without added yeast. Total folate content increased considerably during sourdough fermentation due to increased amounts of 10-HCO-H_2folate, 5-CH_3-H_4folate, and 5-HCO-H_4folate. Baker's yeast contributed markedly to the final folate content of bread by synthesizing folates during fermentation. Proofing did not influence total folate content but changes in vitamer distribution were observed. Folate losses in baking were ≈25%. The variety of sourdoughs and baking processes obviously lead to great variation in folate content of rye breads. The possibilities to enhance natural folate content of rye bread by improving folate retention in technological processes and by screening and combining suitable yeasts and lactic acid bacteria should be further investigated.

Folates are derivatives of folic acid, one of the B vitamins. Folic acid fortification in several countries throughout the world, as well as accumulating scientific findings related to functionality of folates beyond vitamin activity have stressed the need for an in-depth understanding about factors affecting folate contents in food. Low folate concentrations in biological materials and occurrence of various chemical forms with different stabilities are challenges to food folate analysis.

In Finland where fortification is not common, folate intake is at a moderately sufficient level. An average of 11% of the daily folate intake derives from rye alone (Laurinen 2000). Consumption of whole grain rye has been boosted by studies that have strengthened the image of rye as a healthy dietary choice, and the potential for formulating novel rye products is growing as the effects of various processes and their variables are better understood (Liukkonen et al 2003). Rye is an important source of dietary fiber as well as many bioactive compounds such as phenolic acids, lignans, and phytosterols. Consumption of whole grain has been associated with reduced risk of coronary heart disease (Anderson et al 2000), certain cancers (Slavin 2000), and diabetes (Liu et al 2000). We have recently shown that endogenous folates from rye products and orange juice are bioavailable as well as synthetic folic acid from fortified white bread (Vahteristo et al 2002).

The majority of rye in Finnish diet is consumed as sourdough fermented bread baked from whole meal rye flour. Sourdough is classically made by mixing rye flour and water and allowing it to ferment. Bakeries typically have their own sourdoughs which are maintained by back-slopping procedure. The microorganisms (lactic acid bacteria and yeasts) originate mainly from the flour but also from the microflora associated with baker's yeast often added to the sourdough (Lönner and Ahrne 1995).

Baking technology of rye is different from that of wheat. Absence of gluten in rye is compensated by pentosans, the solubility of which is optimal at lower pH values. Acidity also reduces enzymatic activity, which improves dough processability and enables the use of rye with low falling number (Lönner and Ahrne 1995). In addition to technological interests, fermentation creates a typical flavor and increases the shelf life of bread, mainly due to lactic and acetic acid production by lactic acid bacteria (Lönner and Preve-Åkesson 1989). Nutritional value is improved by better bioaccessibility of minerals due to destruction of phytic acid (Lönner and Ahrne 1995).

Folate content of rye grain is relatively high at ≈65–70 µg/100 g (Hegedüs et al 1985; Kariluoto et al 2001) although contents as high as 92 µg/100 g (Cerna and Kas 1983) and 143 µg/100 g (Müller 1993) have also been reported. Yet information on folates in rye bread is scarce, and the effect of baking process on folates is largely unknown. From wheat baking, it is known that a major portion of folates in bread derives from yeast and that bread often contains more folate than its flour (Keagy et al 1975). Recently, this has been confirmed by Osseyi et al (2001) and Arcot et al (2002). The effect of relatively long fermentation associated with the growth of microorganisms, as well as decrease of pH is therefore of interest as regards folates in rye baking.

The objective of this study was to examine the effect of fermentation and baking in particular on endogenous folates of rye and wheat. In this study, wheat served as a general point of comparison for rye. Information about total folate content and distribution of folate vitamers was evaluated to characterize changes and identify critical steps during the whole baking process of rye and wheat breads.

MATERIALS AND METHODS

Experiment I: Rye and Wheat Breads

The objective of this experiment was to study the effect of baking method on folate content of rye and wheat breads. Breads and the flours used for baking were analyzed for total folates by microbiological assay and for folate vitamer distribution by HPLC.

Rye Breads

Three rye breads were baked using different fermentations: 1) bread leavened with yeast; 2) traditional sourdough fermentation with baker's yeast *Saccharomyces cerevisiae* and lactic acid bacteria (bread fermented with yeast and lactic acid bacteria); and 3) sourdough fermentation without added yeast (bread fermented with lactic acid bacteria). The dough formula in all three breads was similar: 792 g of whole meal rye flour (commercial flour, obtained from a Finnish milling company), 710 g of water, and 12 g of salt. The amount of baker's yeast in bakings 1 and 2 was 15 g.

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Sourdough seed used in bakings 2 and 3 was a multistrain laboratory starter from the laboratory of Cereal Technology at the Department of Food Technology, University of Helsinki. It contained a *Lactobacillus plantarum* strain and a *Candida milleri* yeast strain according to identifications performed earlier with standard procedures in that laboratory (Haapanen 1998). Sourdough seed was stored at 4°C and renewed weekly by fermentation for 16 hr at 30°C.

Bread leavened with yeast. Baker's yeast and water were mixed, after which flour and salt were added and mixed to form a sticky dough. The dough was then proofed for 30 min at 30°C (100% rh). Pieces of dough (450 g) were molded by hand, panned, and proofed for 15 min at 30°C. Breads were baked at 200°C for 60 min.

Bread fermented with yeast and lactic acid bacteria. Sourdough was prepared by mixing 36% whole meal rye flour, 59% water, and 5% sourdough seed. This mixture was left to ferment for 16 hr at 30°C. Dough was prepared by mixing 505 g of the resulting sourdough with the rest of the whole meal rye flour, yeast, water, and salt. Proofing and baking conditions were as in bread leavened with yeast.

Bread fermented with lactic acid bacteria. As in bread fermented with yeast and lactic acid bacteria, but no yeast was added.

Wheat Breads

Wheat breads were baked using three methods: 1) sponge-dough method; 2) straight-dough method; and 3) straight-dough method with baking powder. The dough formula in all three breads was similar: 900 g of all purpose wheat flour (obtained from a Finnish milling company), 520 g of water, 18 g of salt, and 9 g of margarine. In addition, 45 g of baker's yeast and 9 g of sugar were added to sponge dough and straight dough bakings. The amount of baking powder in baking 3 was 10 g.

Baking 1. Sponge-dough bread: 150 g of flour, 520 g of water, and 45 g of baker's yeast were mixed and fermented for 2 hr at 35°C, after which the rest of flour and sugar were added. Pieces of dough (400 g) were molded by hand, panned, and proofed for 30 min at 30°C (80% rh). Breads were baked at 200°C for 15 min.

Baking 2. Straight-dough bread: Baker's yeast, salt, and sugar were mixed with water. Flour and margarine were then added. Proofing and baking was performed as in baking 1.

Baking 3. Bread leavened with baking powder: Flour, baking powder, and salt were mixed, after which margarine and water were added. The dough was divided into 400-g pieces that were panned and baked at 200°C for 20 min.

Experiment II: Sourdough Fermentations

In this experiment, the effect of two different sourdough fermentations on folate content in rye baking were studied. Samples were taken at six points of the baking process: 1) flour, 2) sourdough start, 3) sourdough end, 4) dough, 5) proofed dough, and 6) bread. Samples were freeze-dried before analysis. Total folates were measured using microbiological assay. Changes in vitamer distribution during fermentation 1a were determined by HPLC. Total titratable acidity and pH were also measured.

In sourdough fermentation 1, the sourdough was prepared by mixing 3,036 g of whole meal rye flour and 5,058 g of water. Rye flours used in fermentations 1a and 1b differed. In 1a, the cultivar was small-grained Akusti; in 1b, the cultivar was large-grained Amilo. Both cultivars were grown in the same year at the same location. Falling numbers of the two flours were 110 and 200, respectively. Baker's yeast (*S. cerevisiae,* 0.4% dough weight), *L. brevis* (Chr. Hansen, Denmark; 0.04% sourdough weight), and *L. plantarum* (Chr. Hansen, Denmark; 0.04% sourdough weight) were added. Sourdough was left to ferment for 20 hr at 30°C. After fermentation, a part of the resulting sourdough (3,217 g) was mixed with 3,960 g of whole meal rye flour and 1,980 g of water. Baker's yeast (0.9% dough weight) and salt (0.6% dough weight) were added. After a floor time of 45 min at 28°C, the dough was divided into 600-g pieces that were molded by hand and panned before proofing for 75 min at 35°C (70% rh). Breads were baked at 220–240°C for 40 min.

In sourdough fermentation 2, the sourdough seed was the same as used in previously described rye breads (experiment I). Sourdough was prepared by mixing 1,500 g of whole meal rye flour, 2,476 g of water, and 200 g of sourdough seed. After fermentation (16 hr at 30°C), 4,116 g of the resulting sourdough was mixed with 1,287 g of rye flour, and 49 g of salt (0.9% dough weight). After a floor time of 60 min at 30°C (85% rh), the dough was divided into 450-g pieces that were molded by hand and proofed for 30 min at 30°C (85% rh). Breads were baked at 210°C for 60 min.

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 B. Schirck's Laboratories (Jona, Switzerland). Calibrants were dissolved as described by van den Berg et al (1994) and the purities were checked using molar absorptivity coefficients at pH 7.0 (Blakley 1969). Standard solutions were flushed with nitrogen and stored in 0.01*M* acetate buffer containing 1% (w/v) sodium ascorbate (pH 4.9) 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 (1997), except for the reaction time, which was increased from 1.5 to 2.5 hr. Purity, as calculated using molar absorptivity coefficient $\varepsilon_{234} = 3.4 \times 10^4\ M^{-1}\ cm^{-1}$ at pH 7.4 (Baggott et al 1995) was 90%. Standard solution was flushed with nitrogen and stored in 0.05*M* Tris/HCl, pH 8.4 at –20°C.

Sample Extraction

All analytical procedures were conducted under subdued light or samples and calibrants were covered with aluminum foil. Sample extracts were kept under nitrogen atmosphere whenever feasible.

Extraction and trienzyme treatment have been published in detail elsewhere (Kariluoto et al 2001). Samples (0.8–2.0 g), in duplicate, were extracted with 50 m*M* Ches, 50 m*M* Hepes, containing 2% (w/v) sodium ascorbate, and 10 m*M* 2-mercaptoethanol, pH 7.85, by boiling for 10 min. In trienzyme treatment, samples were first incubated with hog kidney conjugase and α-amylase (EC 3.2.1.1, A-6211 Sigma, St. Louis, MO) at pH 4.9, and then with protease (EC 3.4.24.31, P-5147, Sigma) at pH 7.0. Hog kidney conjugase was prepared according to Gregory et al (1984). Conjugase activity was tested according to Vahteristo et al (1996) using $PteGlu_3$ as substrate; enzyme batch was discarded if the proportion of folic acid after 40 min incubation at 37°C did not reach 90%.

Microbiological Assay

Samples were analyzed for total folates by microbiological method on 96-well microtiter plates (tissue culture treated; Costar Corp., Cambridge, MA) using glycerol-cryoprotected *L. rhamnosus* (ATCC 7469) as the growth organism. Cryoprotection was performed as described in draft of European Standard for determination of folate by microbiological assay (prEN 14131 2001) with one exception: instead of autoclaving, the culture medium was sterile filtered (acrodisc syringe filters, 0.2 µm; Pall Gelman Laboratory, Ann Arbor, MI). Assay medium (folic acid casei medium; Difco, Becton Dickinson and Co, Sparks, MD) was used as 75% strength of the recommended amount, and 0.075 g of ascorbic acid was added after heating (Molloy and Scott 1997). After adjusting to pH 6.1 with acetic acid, the medium was sterile filtered. Sterile saline (2.5 mL) was added (prEN 14131 2001) to 1 mL of cryoprotected inoculum, and 300 µL of this solution was added to 100 mL of culture medium.

Sample extracts were diluted with 0.5% (w/v) sodium ascorbate (Molloy and Scott 1997). The sodium ascorbate solution was adjusted to pH 6.1 with acetic acid. Two dilutions, typically varying from 1:400 to 1:50, were made from each sample extract, and 100 µL of each dilution was applied to four wells. Eight levels of calibrant (0–80 pg 5-HCO-H_4folate/well), four wells of each level, were included into each plate and 0.5% (w/v) sodium ascorbate (pH 6.1) was added to the wells so that the final volume in each well before adding the inoculated medium was 100 µL. Inoculated medium (200 µL) was added to each well. Plates were incubated for 18–20 hr at 37°C after which turbidity was measured with a microplate reader (iEMS Reader MF; Labsystems, Helsinki, Finland) at 595 nm. Mixing the plates before measurement was not necessary.

Sample Purification and HPLC

Affinity chromatography was used to purify and concentrate sample extracts. Preparation and use of affinity columns have been published elsewhere (Kariluoto et al 2001). Affinity columns were prepared by coupling folate binding protein (FBP) from bovine milk (Scripps Laboratories, San Diego, CA) to agarose (Affi-Gel 10; Bio-Rad Laboratories, Richmond, CA). Binding capacity of the columns was 4.6 µg of folic acid, from which no more than one fourth was used to ensure quantitative binding of 5-HCO-H_4folate.

During the experimental part of the study, the HPLC equipment was changed. At the beginning, a Varian Vista 5500 liquid chromatograph and cooled Waters 712 Satellite Wisp autosampler were used. These were replaced by Waters 510 and 515 HPLC pumps and Waters 717 plus Autosampler (Waters, Milford, MA). Detection was accomplished with Waters 2487 Dual λ absorbance detector set at 290 nm and Waters 470 fluorescence detector set at 290 nm excitation and 356 nm emission wavelengths for reduced folates and 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 using eight levels of calibrants purified with affinity chromatography.

Folate monoglutamates were separated on a Shandon (Cheshire, UK) Hypersil ODS column (150 mm × 4.6 mm; 3 µm). Gradient elution was performed with acetonitrile and 30 m*M* phosphate buffer, pH 2.2, at 0.9 mL/min flow rate. Column temperature was kept at 30°C. The gradient was started at 5% (v/v) acetonitrile that was maintained isocratically for the first 9 min and then raised to 7% within 13 min. Thereafter, the acetonitrile concentration was raised to 16% within 9 min and maintained for 2 min. Injection volume was 50–200 µL. Peaks were identified by retention times and identity was confirmed by spiking and comparing ratios of fluorescence and UV peaks.

Analytical Performance

Short-term stability (1–5 days) of folates was investigated at –20 and 4°C in affinity chromatography elution solution (0.02*M* trifluoroacetic acid and 0.01*M* dithiothreitol neutralized with 1*M* piperazine, containing sodium ascorbate and 2-mercaptoethanol as reducing agents). The concentrations of folates were 80 ng/mL for PGA and 40 ng/mL for other vitamers. In addition, stability of folates (20 ng of each vitamer/mL) was studied in extraction buffer (pH 7.85) and after heat extraction and after adjusting to pH 4.9. Both solutions were flushed with nitrogen and stored at –20°C before analysis.

Recovery tests were made for all six samples from rye fermentation 1a by spiking samples before extraction with folate standards at the same level as present in samples, ≈60–120 µg/100 g. Spiked samples were treated as normal samples and analyzed by HPLC. Recovery in microbiological assay was tested by spiking a certified reference material (CRM 121, whole meal flour) with 5-HCO-H_4folate (n = 2) and also by analyzing a known amount of 5-HCO-H_4folate as a sample (n = 2).

Certified reference material, CRM 121, was analyzed microbiologically in each set of samples. The average folate content calculated from 10 determinations was 51.1 ± 6.5 µg/100 g on dry matter basis (certified value 50 ± 7 µg/100 g on dry matter basis), and a control chart was constructed based on this information. Results were rejected if the folate content of CRM differed more than 1.5 standard deviations from the average.

Other exclusion criteria in microbiological assay were >10% difference between the results of two duplicates, minimum turbidity of 0.7 for the highest level of calibrant (80 pg of 5-HCO-H_4folate) and maximum turbidity of 0.05–0.06 for the lowest level (0 pg of 5-HCO-H_4folate). The coefficient of variation (CV %) between all four dilutions of the same sample averaged 6% as calculated from 12 randomly selected results covering the whole analysis period.

Validation data for affinity chromatography and HPLC have been reported previously (Kariluoto et al 2001). CRM 121 was also analyzed by HPLC (n = 4), and the result expressed as a sum of five vitamers was 30.7 ± 1.0 µg/100 g on dry matter basis

Statistical Analysis

Differences between folate values of breads baked using different methods (experiment I) and values of different baking steps (experiment II) were evaluated using Fisher's 95% least significant difference (LSD) procedure.

RESULTS AND DISCUSSION

Analytical Performance

Short-time stability of folates in affinity chromatography solution was excellent. No loss was observed after five days at –20°C or two days at 4°C which represent typical storage conditions (samples waiting for HPLC analysis in freezer or in autosampler). Folate stability was better after heat extraction and adjustment to pH 4.9 than in extraction buffer, pH 7.85. After extraction and adjustment to pH 4.9, no significant losses occurred during the first two weeks at –20°C. Hence, we justified storing the extracted samples at pH 4.9, –20°C, over a weekend or even longer, if necessary, before further analysis.

Recoveries for different vitamers in rye samples were mostly satisfactory, 84–104%, except for H_4folate (average for different type of samples 72 ± 5%) and for 5-HCO-H_4folate in sponge and bread samples (52–60%). Recovery for microbiologically determined 5-HCO-H_4folate was 105%, both when added to CRM 121 and when analyzed alone. Results were not corrected with recoveries.

There existed a difference between microbiological total folate content and the sum of vitamers determined by HPLC, even after molecular weight corrections. In this case, where an exact comparison between folates determined by the two methods was needed, the individual vitamers determined by HPLC were converted to 5-HCO-H_4folate, the calibrant used in microbiological assay. The sum of vitamers was ≈80–120% of the microbiologically determined total folate content for wheat breads and

TABLE I
Total Folates in Wheat and Rye Breads and Flours

Bread Type	Total Folates (µg/100 g)[a]
Wheat bread	
Flour	27
Sponge dough	50
Straight dough	45
Baking powder	17
Rye bread	
Flour	44
Yeast fermented	42
Yeast and LAB[b] fermented	42
LAB fermented	29

[a] Mean of duplicate analysis by microbiological assay, values on a dry basis.
[b] Lactic acid bacteria.

the majority of rye samples. However, for rye breads the ratio was only 60–70%, in one case even as low as 45%. Part of this discrepancy can be explained by insufficient separation of 10-HCO-H_2 and 5-HCO-H_4folate from interfering peaks derived from sample matrix. Other possibilities include the presence of unidentified folate vitamers in samples and different responses of *L. rhamnosus* to different vitamers or responses to nonfolate compounds. These results are in line with recent reports showing that folate contents in food measured by HPLC can be as much as 30% smaller than microbiological results, for reasons that remain unclear (Konings et al 2001).

Experiment I: Rye and Wheat Breads

For both rye and wheat breads the lowest folate contents were found in breads baked without added yeast (Table I). Wheat breads baked using sponge-dough or straight-dough method contained 2.5× more folates than bread leavened with baking powder. In rye breads, the difference was also clear, although not as pronounced as among wheat breads; rye bread baked using lactic acid bacteria fermentation contained 31% less folates than the two other rye breads. Total folate contents for the wheat breads baked using sponge-dough and straight-dough method were similar, 50 and 45 μg/100 g on dry matter basis, respectively. Neither did the total folate contents in rye breads baked using yeast and lactic acid bacteria fermentation and leavening with yeast differ from each other. Inclusion of yeast resulted in higher folate content of bread that would have been expected based on the folate content of flour. Hence it was presumed that folates in both rye and wheat breads derive not only from flour but also from yeast. This observation is consistent with previous results of Butterfield and Calloway (1972) and Keagy et al (1975), who estimated that as much as 53–65% of folates in wheat bread derive from yeast and that bread contains more folate than its flour.

In rye bread, 5-HCO-H_4folate was the most abundant vitamer, whereas in wheat breads 5-CH_3-H_4 and 10-HCO-H_2folate contributed most to the folate content (Table II). Low amounts of 5-CH_3-H_4folate and 5-HCO-H_4folate and the absence of H_4folate characterized both rye and wheat breads baked without added yeast (lactic acid bacteria fermented rye bread and wheat bread leavened with baking powder). The most prominent difference was seen in 5-CH_3-H_4folate content. In addition, in the rye bread fermented with yeast and lactic acid bacteria the amount of 5-CH_3-H_4folate was lower than in rye bread leavened with yeast only (1.9 and 4.4 μg/100 g, respectively). The reason for this remains unclear but it is possible that lactic acid bacteria growing in sourdough might have utilized the folate synthesized by yeast.

In rye bread chromatograms 10-HCO-H_2folate was often masked. Anyhow, it seemed to be an important vitamer in yeast and lactic acid bacteria fermented rye bread (23% of the sum of vitamers) and its proportion in wheat bread leavened with baking powder (51%) was also noteworthy.

Experiment II: Effect of Fermentation

Folates were synthesized to a great extent during sourdough fermentation when yeast was added (Table III), and the highest folate contents on dry matter basis were determined at the end of the fermentation before more flour and water were added. During sourdough fermentations 1a and 1b with added *S. cerevisiae* folate content increased by 54 and 128%, respectively. Changes in folate content during sourdough baking of rye has not been published, but data related to wheat baking are available. In wheat baking, Osseyi et al (2001) reported a folate increase of 73% when folate content of fermented sponge was compared with that of flour. However, sampling points differ from those used in this study. For fermentations 1a and 1b, comparable figures would be 80 and 161%, respectively.

Folate concentration remained unchanged during fermentation 2 (without added yeast) which, together with the results from experiment I, further suggests to the role *S. cerevisiae* as a major contributor to folates in baking. The folate content of *S. cerevisiae* is very high, >2,000 μg/100 g (Seyoum and Selhub 1998). Even assuming folate content of the two yeasts to be similar, it is possible that conditions in sourdough fermentation favored the growth of *S. cerevisiae*. It is also likely that the initial amount of *C. milleri* in sourdough was smaller than the amount of *S. cerevisiae* added to sourdough. The effect of lactic acid bacteria cannot fully be excluded either. In fermented milk products, some lactic acid bacteria strains synthesize folates that then can stimulate the growth of others. Other strains, on the contrary, utilize folates from the medium (Lin and Young 2000; Smid et al 2001).

The magnitude of folate increase during fermentations using flours derived from different cultivars (fermentations 1a and 1b) was different, 54 and 128%. It can be speculated that this could partly be explained by the development of acidity during fermentation. There was a clear difference between falling numbers (110 and 200). In sourdough baking, low values for falling number are even desirable to a certain extent because high amylolytic activity ensures a strong and rapid start for fermentation and development of a characteristic flavor (Lönner and Ahrne 1995). In fermentation 1a, pH declined from 6.3 to 3.9 and total titratable acidity increased from 3.6 to 17.1. In fermentation 1b, pH declined from 6.4 to 4.2 and total titratable acidity increased from 2.1 to 9.1. In breads, pH values were 4.9 and 5.1 for fermentations 1a and 1b, respectively. The development of acidity during fermentation 1a took place very rapidly: pH declined to <6 in ≈3 hr, whereas in fermentation 1b, the same change in pH took 12 hr. Lactic acid concentration after fermentation 1a was ≈1.1%, which was twice as high as after fermentation 1b. Lactic acid bacteria compete with yeasts for nutrients and produce organic acids, thus gradually retarding the growth of yeast (Ticha and Holas 1982). It can be hypothesized that the more acidic conditions in fermentation 1a could result in either suppressed synthesis of folates or, perhaps more likely, destruction of labile folates. 5-CH_3-H_4folate, especially, is less stable in acidic

TABLE II
Folate Vitamers in Wheat and Rye Breads

Bread Type	Folate Vitamer (μg/100 g)[a]						
	H4	5-CH3-H4	10-HCO-H2	10-HCO-PGA	5-HCO-H4	PGA	Sum of Vitamers
Wheat							
Sponge dough	0.8	13.2	11.9	4.8	9.3	0.5	41
Straight dough	1.5	14.8	10.7	4.9	9.0	0.7	42
Baking powder	· · ·	2.4	10.5	3.0	4.3	0.5	21
Rye							
Yeast fermented	0.4	4.4	msk[b]	5.3	11.7	2.1	24
Yeast and LAB[c] fermented	tr[d]	1.9	6.7	6.6	12.5	2.0	30
LAB fermented	tr	0.8	· · ·	5.0	7.9	1.8	15

[a] Mean of duplicate analysis, values on a dry basis.
[b] Masked by impurities.
[c] Lactic acid bacteria.
[d] Traces.

pH than in moderately alkaline conditions (O'Broin et al 1975). Moreover, fermentation is a biological process that cannot be fully controlled, and the experiments should have been repeated.

Increase in folate content during fermentation resulted mainly from the increased amounts of 10-HCO-H_2folate and 5-CH_3-H_4folate (Table IV). An increase, although not statistically significant, was also seen in 5-HCO-H_4folate content. Seyoum and Selhub (1998) showed that folate in baker's yeast is comprised mainly of 5-CH_3-H_4folate (77%), H_4folate and 5-HCO-H_4folate as well as 10-HCO-H_4folate polyglutamates. Interestingly, Osseyi et al (2001) reported that higher folate content in fermented sponge in comparison to wheat flour is a result of higher amounts of 5-CH_3-H_4folate and H_4folate, probably derived from yeast. In this study, the concentrations of 5-CH_3-H_4folate and H_4folate definitively increased if rye flour and initial sourdough (t = 0) are compared, but the amount of H_4folate did not increase during the actual fermentation phase, as the amount of 5-CH_3-H_4folate did.

After fermentation, the addition of other ingredients had a dilution effect on the folate content. Proofing itself did not affect the total folate content, but remarkable changes in vitamer distribution were observed. The changes in vitamer distribution might have been induced by the addition of yeast after fermentation. Most prominent change was the threefold increase in the H_4folate amount during proofing. The amount of 10-HCO-H_2folate, on the contrary, decreased considerably. Osseyi et al (2001) also reported a clear increase (28%) in H_4folate content in wheat baking from the stage of fermented sponge to proofed dough.

Data on the effect of proofing in wheat baking is available; however, the comparison of data is complicated because baking methods and sampling points vary. Arcot et al (2002) reported a 68% increase in total folate content during proofing. Keagy et al (1975) compared freshly mixed dough with proofed dough and found 73% higher folate content for bread baked using straight-dough method but only 20% increase for bread baked using sponge-dough method. In some studies, no increase has been found (Cerna and Kas 1983); Osseyi et al (2001) reported a 15% decrease in folate content from fermented sponge to proofed dough stage.

Baking losses in this study were ≈25%, although not constant. The magnitude of losses agrees well with previously reported losses of 20–34% for wheat bread (Butterfield and Calloway 1972; Keagy et al 1975; Osseyi et al 2001; Arcot et al 2002). After baking, practically no H_4folate was detected, and the losses of 5-CH_3-H_4folate (67%) and 5-HCO-H_4folate (76%) were also significant. Losses of H_4folate and 5-HCO-H_4folate in this study were somewhat higher than losses reported for wheat bread baking (61% for H_4folate and 45% for 5-HCO-H_4folate) by Osseyi et al (2001). However, in our study, the amount of 5-CH_3-H_4folate decreased, whereas in the study of Osseyi et al (2001) it increased 150%. The amounts of 10-HCO-H_2folate, PGA, and especially 10-HCO-PGA were higher in bread than in proofed dough. One of the folate vitamers in rye, 10-HCO-H_4folate, is prone to oxidation and is readily converted to 10-HCO-PGA and 10-HCO-H_2folate (Robinson 1971; Maruyama et al 1978; Pfeiffer et al 1997). 10-HCO-H_4folate could not be quantitated in our analytical system as such but as 10-HCO-PGA. Thus, the amounts of 10-HCO-PGA and 10-HCO-H_2folate reflect the amount of 10-HCO-H_4folate, oxidized either previously (in milling process, for example) or during analytical procedures.

In summary, large differences between folate contents of commercial rye breads are readily understandable bearing in mind the variety of (bakery) sourdoughs and their microflora. In addition, the microflora of a particular sourdough is prone to change in the course of time (Rosenquist and Hansen 2000). Other factors affecting folate content include differences in the amylolytic and microbial activity of rye flour, addition of baker's yeast, and differences in the baking process (fermentation time, baking temperature, etc).

CONCLUSIONS

More information and better understanding about the effect of different baking processes and fermentation on folate content was gained. Folate content increased during sourdough fermentation commonly used in rye baking and the increase was associated mainly with the growth of yeast. Yeast was able to compensate folate losses both in rye and wheat bakings not only by its high folate content itself but also by synthesizing folates. There was no difference between total folate contents of wheat breads baked using either sponge-dough or straight-dough method. Changes in folate content during rye baking were accompanied by changes in vitamer distribution. Sourdough fermentation affects not only sensory and microbiological but also nutritional quality. The results suggest that by the means of screening and selecting appropriate

TABLE III
Total Folates During Sourdough Fermentation Process of Rye

	Total Folates (µg/100 g)[a]		
Baking Step	Fermentation 1a *S. cerevisiae* Added	Fermentation 1b *S. cerevisiae* Added	Fermentation 2 Endogenous *C. milleri*
Flour	66	62	62
Sourdough, start	77	71	58
Sourdough, end	119	162	61
Dough	94	106	56
Proofed dough	93	111	54
Bread	93	79	40

[a] Mean of duplicate analysis by microbiological assay, values on a dry basis.

TABLE IV
Folate Vitamers During Sourdough Fermentation Process[a] of Rye

	Folate Vitamer (µg/100 g)[b]						
Baking Step	H4	5-CH3-H4	10-HCO-H2	10-HCO-PGA	5-HCO-H4	PGA	Sum of Vitamers
Flour	3.2	15.0	16.4	6.6	17.6	11.9	71
Sourdough, start	8.9	19.7	10.7	9.5	15.6	10.5	75
Sourdough, end	8.7	25.7	20.7	4.2	19.3	7.3	86
Dough	5.7	21.7	26.3	5.1	9.2	5.1	73
Proofed dough	17.7	25.6	12.4	2.4	9.5	2.2	70
Bread	0.7	8.4	15.8	10.1	2.2	3.3	41

[a] Fermentation 1a, *S. cerevisiae* added.
[b] Mean of duplicate analysis, values on a dry basis.

yeasts and lactic acid bacteria it could be possible to enhance natural folate content in rye bread.

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