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Effects of yeasts and bacteria on the levels of folates in rye sourdoughs

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Abstract

Fermentation of rye dough is often accompanied with an increase in folate content. In this study, three sourdough yeasts, *Candida milleri* CBS 8195, *Saccharomyces cerevisiae* TS 146, and *Torulaspora delbrueckii* TS 207; a control, baker's yeast *S. cerevisiae* ALKO 743; and four *Lactobacillus* spp., *L. acidophilus* TSB 262, *L. brevis* TSB 307, *L. plantarum* TSB 304, and *L. sanfranciscensis* TSB 299 originally isolated from rye sourdough were examined for their abilities to produce or consume folates. The microorganisms were grown in yeast extract–peptone–D-glucose medium as well as in small-scale fermentations that modelled the sourdough fermentation step used in rye baking. Total folate contents were determined using *Lactobacillus rhamnosus* (ATCC 7469) as the growth indicator organism. The microorganisms studied did not excrete folates into the media in significant amounts. Yeasts increased the folate contents of sterilised rye flour–water mixtures from 6.5 μg/100 g to between 15 and 23 μg/100 g after 19-h fermentation, whereas lactic acid bacteria decreased it to between 2.9 and 4.2 μg/100 g. Strains of *Lactobacillus bulgaricus*, *L. casei*, *L. curvatus*, *L. fermentum*, *L. helveticus*, *Pediococcus* spp., and *Streptococcus thermophilus* that were also tested gave folate contents after fermentation that varied between 2 and 10.4 μg/100 g. Although the four *Lactobacillus* spp. from sourdough consumed folates their effect on folate contents in co-cultivations was minimal. It was concluded that the increase of folate content during fermentation was mainly due to folate synthesis by yeasts. Fermentation of non-sterilised flour–water mixtures as such resulted in three-fold increases in the folate contents. Two folate producing bacteria were isolated from the non-sterilised flour and identified as *Enterobacter cowanii* and *Pantoea agglomerans*.

Keywords: Folate; Rye; Fermentation; Yeast; Lactic acid bacteria

1. Introduction

Folate is a generic term for the various forms of folic acid, one of the B vitamins. Folates are cofactors in many enzymic reactions, including the biosynthesis of nucleotides and amino acids. Folate deficiency in humans may lead to megaloblastic anaemia and neural tube defects (NTD). The daily recommended intakes of folate are between 170 and 300 μg (de Bree et al., 1997), and for pregnant women or women of childbearing age a daily intake of 400 μg is recommended (Wald and Sneddon, 1991). Several recent studies have examined the association between suboptimal folate intake and elevated plasma total homocysteine concentration, which is a risk determinant for cardiovascular diseases that can be lowered by administration of folates (Boushey et al., 1996; Ward et al., 1997; Brouwer et al., 1999).

Cereals – especially whole grain products – are major contributors to folate in the diet. In Finland, where folic acid fortification is not generally practised, cereals contribute 43% and 36% of the folate intake of men and women, respectively (The National Findiet, 2002 Study).

The consumption of rye per capita in Finland is approximately 14.8 kg/year (Balance Sheet for Food Commodities, 2002 and 2003 preliminary), which makes rye the most important single source of folate in the Finnish diet (Laurinen, 2000). The consumption of rye is common, not only in Finland and other Nordic countries but also in many parts of Central Europe. Vahteristo et al. (2002) concluded that the consump-

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tion of rye products and orange juice, even in moderate amounts, is an efficient way to improve the folate status of healthy adults. In their study the bioavailability of endogenous folates was similar to that of synthetic folic acid in fortified white bread.

Processing of rye provides interesting possibilities for natural folate enhancement (Liukkonen et al., 2003). Although the food industry has introduced new types of rye products, e.g. snacks, most of the rye in the Finnish diet is still consumed as traditionally fermented rye bread made from wholemeal flour. Sourdough is made traditionally by mixing rye flour with water and a starter from a previous sourdough and allowing the mixture to ferment. The final pH of a mature sourdough varies from 3.5 to 3.8 (Hansen, 2004). Bakeries often use their own starter cultures/sourdoughs containing bacteria and yeasts, and maintain them by back-slopping. The characteristics of sourdoughs and breads, such as the appearance, flavour, concentrations of organic acids and ethanol, acidity, etc., are dependent on the starter culture (Lönner and Preve-Åkesson, 1989).

Folate content can increase during sourdough fermentation. The increase is due mainly to the growth of yeasts which synthesize folates (Osseyi et al., 2001; Kariluoto et al., 2004). The increase may compensate for folate losses occurring during baking (Kariluoto et al., 2004). Lactic acid bacteria may also affect folate content. Studies with fermented milk products have shown that some lactic acid bacteria are able to synthesize folates whereas others may deplete them (Rao et al., 1984; Lin and Young, 2000; Crittenden et al., 2002). Lactic acid bacteria also compete for nutrients and produce organic acids that can inhibit the growth of folate synthesizing sourdough yeasts, as well as other microorganisms.

The variety of starter cultures could lead to great variation in folate content of sourdoughs but their effects have been scantly investigated. Organisms from the natural microflora of rye flour could also affect the folate level. The objective of this study was to investigate the ability of typical sourdough yeasts and lactic acid bacteria to produce or consume folates during sourdough fermentation.

2. Materials and methods

2.1. Microbial strains

The yeast strains used were a commercial baker's yeast, *Saccharomyces cerevisiae* ALKO 743, and three yeasts originally isolated from rye sourdough; *Candida milleri* CBS 8195, *Saccharomyces cerevisiae* TS 146, and *Torulaspora delbrueckii* TS 207. The four lactic acid bacteria mostly used were *Lactobacillus acidophilus* TSB 262, *Lactobacillus brevis* TSB 307, *Lactobacillus plantarum* TSB 304, and *Lactobacillus sanfranciscensis* TSB 299. In some experiments *Lactobacillus casei* TSB 1, *Lactobacillus curvatus* TSB 45, *Lactobacillus fermentum* TSB 104, *Lactobacillus helveticus* TSB 325, *Pediococcus* sp. TSB 49, and *Pediococcus* sp. TSB 260, all originally from rye sourdough, were used. Positive controls, *Lactobacillus bulgaricus* ABM 5096 and *Streptococcus thermophilus* ABM 5097, were isolated from a commercial yoghurt (Valio Ltd., Helsinki, Finland).

2.2. Intra/extracellular folate

The capability of monoculture sourdough microbes to excrete folate into a medium containing 1% yeast extract, 2% peptone and 2% D-glucose (YPD medium) was tested. The cells were grown in 50 ml of YPD on a rotary shaker, at 180 rpm and 28 °C for 19 h. The cell fractions were then collected by centrifugation for 10 min at 5000 rpm using a CF-510-A centrifuge (Labsystems, Helsinki, Finland). In addition, yeasts were grown anaerobically in tubes flushed with nitrogen. *L. bulgaricus* ABM 5096 and *S. thermophilus* ABM 5097 were grown statically as pure cultures in 50 ml of Elliker medium (Elliker et al., 1956) at 37 °C for 19 h in an anaerobic jar and the cells were collected as before. Folate contents were determined separately for the cell biomasses and the spent media.

2.3. Effect of microorganisms on folate contents of rye flour

Each of the four yeast strains and the four principal bacterial strains were tested by growing them separately and in different pairwise combinations in both sterile and non-sterile rye flour–water mixtures in 50 ml plastic tubes. The rye flour used in the experiments was a commercial wholemeal rye flour obtained from Helsinki Mills Ltd., Järvenpää, Finland. Incubations were carried out in a mixture of 10 g of gamma-sterilised (10 kGy) or non-sterile rye flour and 40 ml sterile water. Some other lactic acid bacteria were individually screened for their folate production/consumption. The inocula were 0.5 ml of each yeast culture grown in 50 ml of YPD overnight, or all the cells collected from 50 ml of each bacterial culture. Tubes were incubated at 30 °C for 19 h with shaking at 180 rpm. The samples were kept at −20 °C before analysis for total folate.

Three strains isolated from non-sterilised rye flour were identified using Gram staining, API 20 E or API 50 CHL biochemical test strips (BioMerieux SA, Marcy l'Etoile, France) based on carbohydrate fermentation patterns, and PCR followed by sequencing from both ends of the amplified DNA fragments according to the manufacturer's instructions (ABI Prism 310 Genetic Analyzer, Applied Biosystems-Applera Corporation, Foster City, CA, USA). The 16S rRNA primers used were pA and pE described by Edwards et al. (1989). DNA was amplified using an Eppendorf Mastercycler Personal machine (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). The PCR programme for 16S rDNA was denaturation at 94 °C for 5 min, followed by 29 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension with Dynazyme II DNA polymerase (Finnzymes, Espoo, Finland) at 72 °C for 1.5 min, and final extension at 72 °C for 2 min after which the sample was cooled to 4 °C. The PCR programme for sequencing reactions was: denaturation at 96 °C for 2 min, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s, and extension at 60 °C for 4 min, and finally cooled to 4 °C. The PCR products were purified