The 7th International Vahouny Fibre Symposium was held at the Royal College of Physicians, Edinburgh, on 27–30 May 2002

Session: Health effects of whole grains

Process-induced changes on bioactive compounds in whole grain rye

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Manufacturing of healthy wholegrain foods demands knowledge of process-induced changes in macro-, micro- and non-nutrients. The high content of dietary fibre is a challenge in relation to good product texture and sensory quality. The stability and bioavailability of bioactive compounds have a marked influence on the health effects of cereal foods. It was confirmed that sterols, folates, tocopherols and tocotrienols, alkylresorcinols, lignans, phenolic acids and total phenolics are concentrated in the bran layers of the rye grain, and are only present at low levels in the flour endosperm. The levels of folate and easily-extractable phenolic compounds increase in germination and sourdough baking, but there are negligible changes in the levels of sterols, lignans and alk(en)ylresorcinols. The levels of tocopherols and tocotrienols are reduced during the sourdough fermentation. In conclusion, many of the bioactive compounds in wholegrain rye are stable during food processing, and their levels can even be increased with suitable processing.

Whole grains: Rye: Food processing: Dietary fibre

Intake of wholegrain foods is increasingly reported to be associated with health benefits, including improved regulation of blood glucose levels and decreased risk of diabetes, cardiovascular disease and certain cancers (Jacobs *et al.* 1998*a,b*; Liu *et al.* 2000, Pereira *et al.* 2002). In addition to dietary fibre, grains contain a wide range of nutrients and bioactive compounds, which have been suggested to contribute to the positive health effects. These phytochemicals, such as lignans, phenolic acids, phytosterols, tocotrienols and other vitamins, are concentrated in the germ and in the outer layers of the kernel (Hegedüs *et al.* 1985; Nilsson *et al.* 1997*a*; Glitsø & Bach Knudsen, 1999).

Dietary guidelines recommend increasing the intake of wholegrain cereals, but there is a gap between recommendations and consumption (Adams & Engström, 2000). Despite the growing consumer interest in health aspects of food, good sensory properties remain a key priority among consumer choice criteria. Processing is a prerequisite for consumption of whole grains. Processing must first turn the food into a suitable form and give it good sensory properties. With whole grains much work remains to be done in this area, as traditions for whole-grain consumption are limited on a global scale. Processing is also important in terms of both the content and bioavailability of nutrients and non-nutrients (Clydesdale, 1994). Whereas processing may increase the bioavailability of bioactive compounds in grains, a reduction in processing increases their levels, (for review, see Slavin *et al.* 2000). Processing may also be used to modify the bioavailability of carbohydrates, such as delaying the glycaemic responses (Holm *et al.* 1989; Björk *et al.* 1994) or enhancing

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the solubility and fermentability of dietary fibre components (Camire & Flint, 1991; Beer *et al.* 1997; Poutanen, 2001).

Milling and baking are the most common processes in grain processing for human food. If the extraction rate is approximately 100 %, wholemeal flour is obtained. With decreasing extraction rates in milling more and more of the outer grain layers are removed, accompanied by losses in dietary fibre and associated compounds (Hegedüs *et al.* 1985; Nilsson *et al.* 1997*a*; Glitsø & Bach Knudsen, 1999). During sourdough baking particularly, many biochemical changes occur which affect bread texture. Levels of phytate (Frölich *et al.* 1986; Larsson & Sandberg, 1991), alkyl-resorcinols (Verdeal & Lorenz, 1977) and tocopherols (Piironen *et al.* 1987) have been reported to decrease during the sourdough baking process, whereas levels of lignans show no marked change (Nilsson *et al.* 1997*b*).

Other important grain-processing methods include flaking, malting, extrusion and puffing. Process variables that also influence product bioactivity include the presence of enzymes (endogenous or added), the use of starter cultures, the amount of water and the amounts of thermal and mechanical energy used (Table 1). Literature about the influence of grain processing on levels of bioactive compounds is scarce. The aim of the present work was to study the effect of milling fractionation, sourdough fermentation and germination on the levels of bioactive compounds in rye. Rye is an important source of whole grain in the Nordic diet, because its traditional uses as various soft and crisp breads are based on wholemeal flour.

Materials and methods

Rye grains

Two rye cultivars, small-grained Akusti, and large-grained Amilo, harvested in summer 2000 in Finland were used in the present study. Akusti was used in sourdough baking and germination, and Amilo was used in milling fractionation.

Milling fractionation

Grains were milled at a moisture level of 16·5 % with a roller mill (Bühler MLU 202; Bühler, Uzwil, Switzerland) to give four streams: bran; shorts; C flour; B flour. Fraction B was the milling fraction from brake rolls and fraction C the milling fraction from reduction rolls. Part of the bran fraction was further cleaned by roller milling.

Table 1. Methods and important variables in the processing of wholegrain foods

Processing methods	Major variables
Milling	Heat
Flaking	Water content and distribution
Soaking	Shear
Malting	Enzyme activity (endo- and exogenous)
Extruding	Micro-organisms (natural and starter cultures)
Puffing	Presence of cell wall and aleurone particles
Fermenting	and large amount of cell-wall components (dietary fibre)
Baking	Presence of secondary metabolites

Sourdough baking

Rye sourdough was prepared by mixing 3036 g wholemeal rye flour and 5058 g water with baker's yeast (4 g/kg dough), with *Lactobacillus brevis* (4 g/kg dough), and with *Lactobacillus plantarum* (4 g/kg dough; Horsholmen CHr. Hansen a/s, Copenhagen, Denmark). Sourdough was fermented for 22 h at 30°C to obtain a pH value of 3·9 and total titratable acidity value of 15·2 (typical acidity values for industrial sourdough). After fermentation, sourdough (3217 g) was mixed with 3960 g wholemeal rye flour and 1980 g water with baker's yeast (9 g/kg dough), and with table salt (6 g/kg dough). After a floor time of 45 min at 28°C, the dough was divided into 600 g pieces that were moulded by hand and panned before proofing for 75 min at 35°C (relative humidity 70 %). Loaves were baked at 220–240°C for 40 min.

Germination

Germination was performed in commercial malting equipment (Joe White Malting Systems, Melbourne, Australia) at 5, 10 or 25°C. The grains were steeped for 8 h and allowed to stand in air for 16 h, and steeped a second time for 6 h. The samples were then germinated at a grain moisture content of approximately 42–46 %. The total germination time (including steeping) was 6 d. Samples were taken after 1, 2, 4, and 6 d. The samples were frozen and subsequently lyophilized.

Analysis of bioactive compounds

Sterols. Sterols were determined after acid and alkaline hydrolysis by GC (Piironen *et al.* 2002).

Folates. Folates were determined by a microbiological assay method including extraction and trienzyme treatment as described by Kariluoto *et al.* (2001).

Tocopherols and tocotrienols. Tocopherols and tocotrienols were determined after saponification by HPLC (M Ryynänen, AM Lampi, P Salo-Väänänen and V Piironen, unpublished results).

Alk(en)ylresorcinols. Alk(en)ylresorcinols were determined after methanol extraction by HPLC (Hertog *et al.* 1992; Mullin *et al.* 1992).

Phenolic acids. Phenolic acids were determined as non-esterified, esterified, glycosylated and insoluble-bound fractions by HPLC (Hertog *et al.* 1992; Hatcher & Kruger, 1997).

Lignans. Lignans were determined by GC–MS. Samples were pre-treated using a modification of the original lignan method of Mazur *et al.* (1996). A new lignan method will be described in detail in a forthcoming methodological paper (T Nurmi, S-M Heinonen and H Adlercreutz, unpublished results).

Total phenolics and 1,1-diphenyl-2-pikryl hydratsyl radical-scavenging activity. Methanolic extracts of freeze-dried and ground rye samples were obtained using an ultrasonication-assisted extraction procedure. The residue was further extracted in alkaline conditions. The content of total phenolic compounds (gallic acid equivalents) in methanolic and alkaline extracts was determined using the
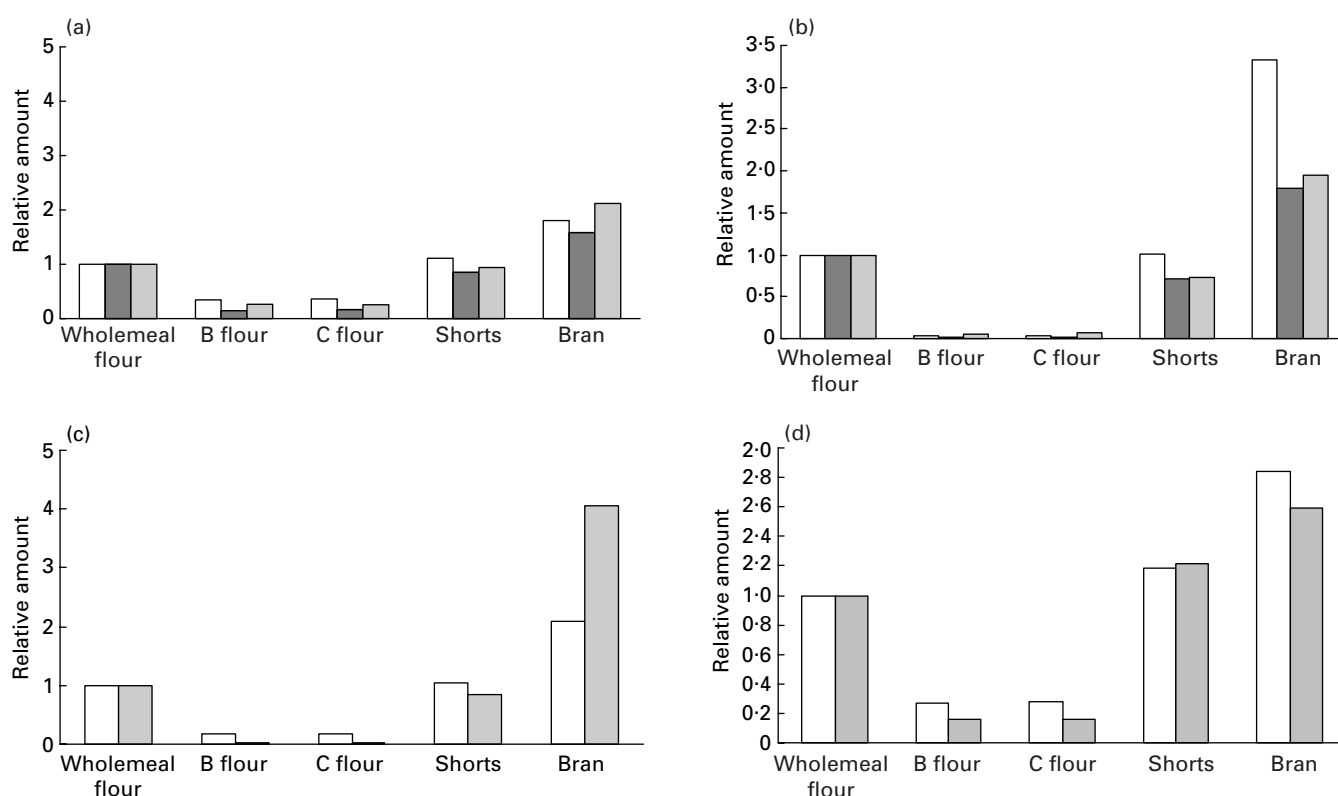
Fig. 1. Distribution of bioactive compounds in milling fractions of rye: (a) sterols (□), folates (▩) and tocopherols and tocotrienols (▩); (b) alk(en)ylresorcinols (□), lignans (▩) and phenolic acids (▩); (c) phenolic compounds after methanol extraction (□) and after alkaline extraction (▩); (d) 1,1-diphenyl-2-pikryl hydratsyl radical-scavenging activity after methanol extraction (□) and after alkaline extraction (▩). B flour, milling fraction from brake rolls; C flour, milling fraction from reduction rolls. For details of milling and analytical procedures, see p. 118.

Folin–Ciocalteau procedure (Singleton & Rossi, 1965). The assessment of the radical-scavenging activity of the samples was based on spectophotometric detection of the absorbance at 517 nm caused by 1,1-diphenyl-2-pikryl hydratsyl radicals, which was decreased or inhibited by antioxidants capable of radical scavenging in the system. This assay was performed using a modification of the method of Goupy et al. (1999).

Results and discussion

Milling fractionation

In the milling of rye the yields of bran, shorts, C flour and B flour were 48, 16, 16 and 19 % respectively. Part of the bran fraction was further cleaned with a roller mill to give two bran fractions comprising 30 and 19 % of the original rye grains. The outer bran fraction contained 3·3–4·0 times more alk(en)ylresorcinols and alkaline-extractable total phenolics and 1·6–2·1 times more sterols, folates, tocopherols and tocotrienols, lignans, phenolic acids and easily-extractable (methanol-extractable) total phenolics than wholemeal rye flour (Fig. 1 (a, b, c)). Previous studies have already established the concentrations of bioactive compounds in the outer layers of rye grain (Verdeal & Lorenz, 1977; Otles et al. 1996; Nilsson et al. 1997a; Andreasen et al. 2000; Lampi et al. 2002; Piironen et al. 2002).

In the shorts fraction, the relative amounts of bioactive compounds were very similar to those of the original

wholemeal rye flour. In the inner flour fractions (B and C) little or none of these compounds were present. The milling fractions of rye also exhibited varying amounts of antioxidant activity, i.e. radical-scavenging activity (Fig. 1 (d)). Bran showed the highest radical-scavenging activity, as also reported by Gray et al. (2000) for oats, whereas flours obtained from the inner parts of the grains exhibited the weakest activity. With the shorts fraction the same radical-scavenging activity was obtained as in wholemeal flour.

The results demonstrate that in addition to dietary fibre, most of the bioactive compounds suggested to play a role in the health benefits are concentrated in the outer layers of the grain, indicating the importance for utilizing the whole grain for human consumption.

Germination

During the 6 d germination of rye at 5, 10 or 25°C, the relative amounts of folates and easily-extractable phenolic compounds increased, whereas only very small changes were detected in the amounts of sterols, tocopherols and tocotrienols, alk(en)ylresorcinols, lignans and alkaline-extractable phenolic compounds (Fig. 2). The highest increase in the amounts of folates (Fig. 2 (a)) and easily-extractable phenolic compounds (Fig. 2 (c)) was observed at the germination temperature of 25°C. Despite the increase in the amount of extractable phenolic compounds, the anti-
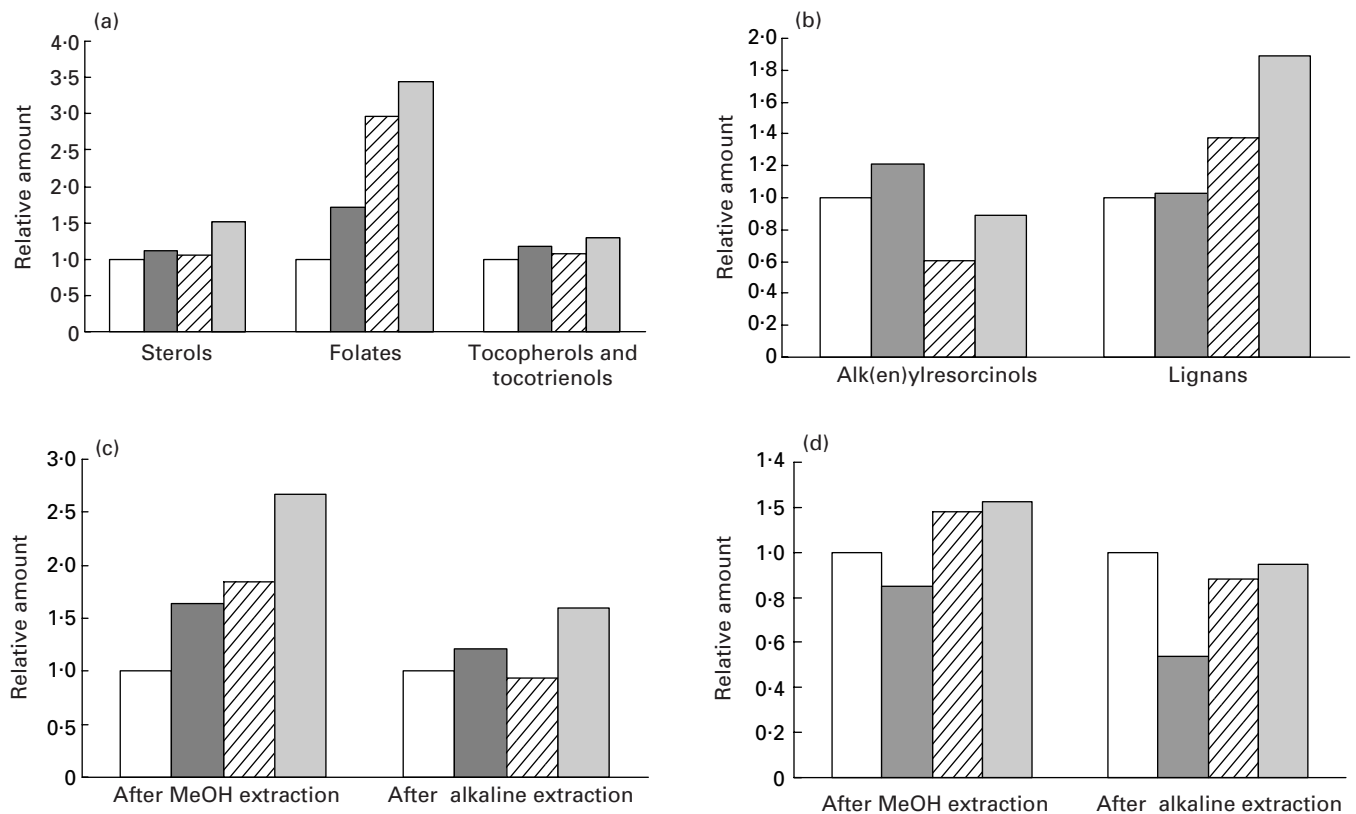
Fig. 2. Effects of 6 d germination of rye on levels of bioactive compounds: (a) sterols, folates and tocopherols and tocotrienols; (b) alk(en)yl-resorcinols and lignans; (c) phenolic compounds after methanol (MeOH) extraction and after alkaline extraction; (d) 1,1-diphenyl-2-pikryl hydratsyl radical-scavenging activity after MeOH extraction and after alkaline extraction. (□), Native grains; (▨), germination at 5°C; (▨), germination at 15°C; (▨), germination at 25°C. For details of germination and analytical procedures, see p. 118.

oxidant activity of germinated rye grains remained very similar to that of the original ungerminated rye grains.

These results indicate that the bioactivity of rye can be increased by germination. In the studies of Walker *et al.* (2002) the folic acid content of green malt was higher than that of the corresponding unmalted barley, but it decreased during kilning. However, the folic acid concentration of kilned malt was still greater than that of unmalted barley. It has also been shown that the amount of phytate can be reduced by germination (Larsson & Sandberg, 1992, 1995). Germination is also an effective pre-treatment for whole grains to produce improved texture and change of flavour, as previously shown for oats (Heiniö *et al.* 2001; RL Heiniö, T Nikkola, K Latva-Kala, A Wilhelmson, K Katina, O Myllymäki, KH Liukkonen and K Poutanen, unpublished results). The major application of germination so far has been in the malting of barley for beer production. However, germination and hydrothermal treatments may also provide attractive processing methods for the production of new healthy and tasty wholegrain foods (Fredlund *et al.* 1997; Wilhelmson *et al.* 2001).

Sourdough baking

The sourdough baking process of rye included: (1) preparation of sourdough by mixing wholemeal rye flour, yeast

and *Lactobacillus* cultures and by fermenting for 22 h at 30°C; (2) addition of 'fresh' wholemeal rye flour; (3) proofing; (4) baking of the breads. The fermentation phase more than doubled the levels of folates and easily-extractable phenolic compounds, but the addition of 'fresh' unfermented wholemeal flour slightly diluted the effect (Fig. 3 (a, b, c)). The levels of tocopherols and tocotrienols decreased during the sourdough fermentation (Fig. 3 (a)), probably due to oxidation (Piironen *et al.* 1988), while the amounts of sterols, alk(en)ylresorcinols, lignans, phenolic acids and alkaline-extractable phenolic compounds changed very little (Fig. 3 (a, b, c)). The fermentation stage also increased the anti-oxidant activity (1,1-diphenyl-2-pikryl hydratsyl radical-scavenging activity) in the methanol-extracted fraction (Fig. 3 (d)), probably due to increased levels of easily-extractable phenolic compounds. Losses of folates, tocopherols and tocotrienols during the baking stage at 220–240°C were surprisingly low.

After baking the amounts of all analysed bioactive compounds of the bread, except those of tocopherols and tocotrienols, were at the same or slightly higher than those of the wholemeal flour (Fig. 3 (a, b, c)). Antioxidant activity (radical-scavenging activity) of the bread was also very similar to that of the wholemeal flour (Fig. 3 (d)). However, the positive effects of the fermentation stage on the bioactivity of rye were mostly lost by 'fresh' flour addition.
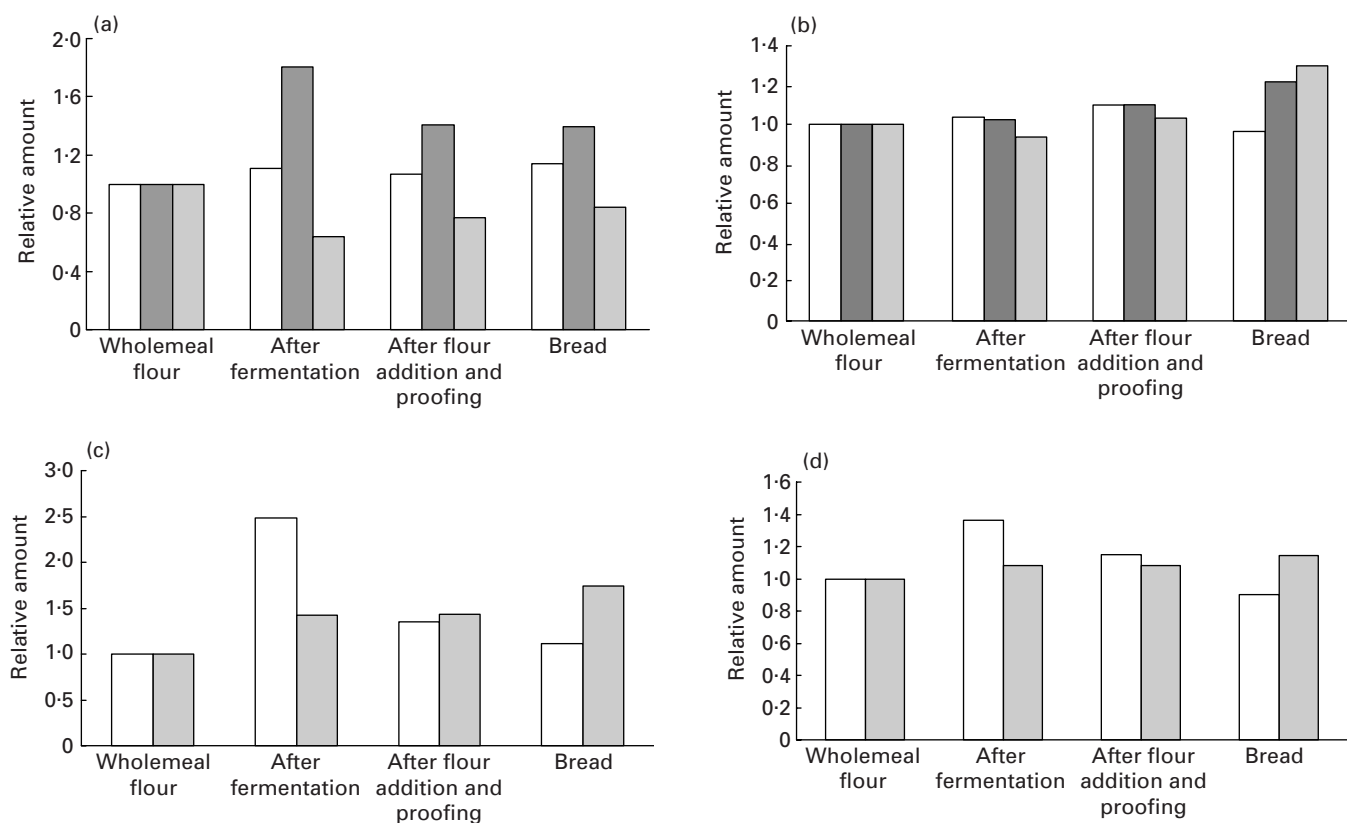
Fig. 3. Effects of sourdough baking of rye on levels of bioactive compounds: (a) sterols (□), folates (▥) and tocopherols and tocotrienols (▤); (b) alk(en)ylresorcinols (□), lignans (▥) and phenolic acids (▤); (c) phenolic compounds after methanol extraction (□) and after alkaline extraction (▤); (d) 1,1-diphenyl–2-pikryl hydratsyl radical-scavenging activity after methanol extraction (▤) and after alkaline extraction (□). For details of baking and analytical procedures, see p. 118.

Conclusions

The profile and concentrations of bioactive compounds in rye can be modulated by milling fractionation, germination and sourdough baking. The use of milling fractionation concentrated the amounts of all the bioactive compounds studied in certain fractions. Germination and sourdough baking increased the amounts of folates and easily-extractable phenolic compounds in wholegrain rye. The amounts of other bioactive compounds, except those of tocopherols and tocotrienols, remained almost unchanged or slightly increased during germination and sourdough baking.

Acknowledgements

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