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Original article

Use of grape by-product as a source of dietary fibre and phenolic compounds in sourdough mixed rye bread

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Summary The study evaluated the effect of grape by-products (GP) on the chemical composition, soluble (SDF) and insoluble (IDF) dietary fibre, phenolic compounds and antioxidant activity (AA) and organoleptic characteristics of sourdough mixed rye bread. The following samples of sourdough mixed rye bread were prepared: control bread (BC) and breads with GP at four different levels: 4%, 6%, 8% and 10%. Addition of GP significantly improves dietary fraction contents, as bread with a 10% addition of GP accounts for 39% and 37% higher contents of IDF and SDF than BC. The assay of radical-scavenging activity and reducing ability showed that GP addition greatly enhanced antioxidant properties of mixed rye breads. Profiles of phenolic compounds of supplemented breads were dominated by procyanidin B1 and B2, catechin, epicatechin, caffeic acid and myricetin. With an increase in the level of GP, the hardness and gumminess of the bread significantly increase. Although both BC and supplemented breads showed common volatile compound profiles, there were slight differences in the concentrations of those components. Sensory evaluation of GP-enhanced breads revealed that a maximum of 6% GP could be incorporated to prepare acceptable products.

Keywords Dietary fibre, grape by-products, phenolic compounds, sourdough mixed rye bread, texture analysis, volatile compounds.

Introduction

As the role of diet in the prevention of human diseases such as cancer, atherosclerosis, heart disease, osteoporosis or obesity has become more evident, many consumers increasingly often search for functional food to improve their diets. Because bakery products have been, and still are, a central constituent in the diets of most populations for thousands of years, the use of these products supplemented with various nutritious, protective and ballast substances may be appropriate.

Recent studies suggest that plant food phytochemicals, including polyphenols, might have potential beneficial health effects, mainly related to antioxidant capacity (Llobera & Cañellas, 2007, 2008). A large number of epidemiological investigations have established an association between a diet rich in polyphenolic compounds and a decrease in the risk of suffering from many civilisation-related diseases (Rice-Evans & Packer, 1998).

There is also a trend to search for new sources of dietary fibre (DF) as functional components for the food industry. Dietary fibre promotes beneficial physiological effects, including gastrointestinal function, moderation of postprandial insulin response and reduction in total and LDL cholesterol content (Davidson & McDdonald, 1998). Generally, fruit DF has better nutritive value than those derived from cereals, because there are also known to contain significant amounts of bioactive compounds such as polyphenols and carotenoids. Grape by-products, even after contact with the fermenting wine, still contain a huge amount of phenolic compounds, depending on the type of grape, the part of the tissue as well as processing conditions. They are good sources of flavanols, flavonols, anthocyanins and phenolic acids (Lafka *et al.*, 2007; Mildner-Szkudlarz *et al.*, 2010). Moreover, according to Llobera & Cañellas (2007), grape pomace and steam are valuable sources of SDF and Klason lignin. The main components of SDF in the pomace sample were pectins, accounting for more than 60% of SDF, while Klason lignin had important amounts of condensed tannins and resistant protein.

There have been many investigations on enhancing the nutritive value of cereal-based products, realised by supplementation of white bread with minerals, DF or polyphenols (Vergara-Valencia *et al.*, 2007;

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Mildner-Szkudlarz *et al.*, 2009; Bajerska *et al.*, 2010). So far, there are no reports on the use of grape by-products as the source of both DF and polyphenols. Moreover, to the best of our knowledge, there are no reports on the use of sourdough mixed rye bread as a matrix for bioactive compounds. Sourdough fermentation has a generally accepted role in improving both flavour and structure of rye and wheat breads and thus might be helpful in masking eventually negative effects of physiologically active plant ingredients on bread quality. Furthermore, fermentation provides additional benefits for humans, such as stabilisation or increase in the levels of various bioactive compounds, increase in the solubility of pentosans, retardation of starch bioavailability and improved mineral bioavailability.

The aim of this study was to evaluate the influence of grape by-product (GP) on sourdough mixed rye bread quality. Moreover, contents of functional ingredients as a result of supplementation, polyphenol profiles characterised by HPLC analyses of samples and AA were also estimated.

Materials and methods

Standards and reagents

The fallowing standards were used: gallic acid, γ-resorcylic acid, *p*-coumaric acid, *trans*-ferulic acid, caffeic acid, sinapic acid, catechin, epicatechin, quercetin-3-beta-D-glucoside, quercetin and myricetin, all from Sigma-Aldrich (Steinheim, Germany). Procyanidin B1 and B2 were from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich (Steinheim, Germany).

Bread-making process

Red grape by-product was provided by a wine-making factory from Poland from the vintage of 2008. The material was lyophilised up to a moisture content of approximately 2–4%, and the skins separated from the seeds with the aid of the sieve were milled to obtain a fine powder GP. Chemical characteristics of GP are given in Table 1.

Table 1 Chemical composition of GP

Properties	GP
Ash (g per 100 g d.m.)	6.40 ± 0.09
TDF (g per 100 g d.m.)	50.93 ± 0.52
SDF (g per 100 g d.m.)	4.53 ± 0.06
IDF (g per 100 g d.m.)	46.39 ± 0.46
TPC (mg GAE per g d.m.)	58.95 ± 1.50
FRAP (mM Fe per g d.m.)	548.97 ± 15.85
DPPH (mM TRE per g d.m.)	471.23 ± 9.75

Values are means of three determinations ± SD.
GP, grape by-products; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TPC, total phenol contents; GAE, equivalent of gallic acid; FRAP, ferric reducing ability of plasma; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; TRE, Trolox equivalents.

The starter culture of *Lactobacillus brevis* (VTT E-95612) was obtained from the VTT Technical Research Centre of Finland. The LAB strain was grown on MRS medium at 30 °C under anaerobic conditions. Cells were harvested by centrifugation, washed with bidistilled sterile water and again centrifuged.

Sourdough was prepared by mixing 750 g of tap water, 500 g of rye flour (ash content 2.0 g per 100 g) and the inoculum of LAB in a large beaker and covered with aluminium foil. Fresh cells were added to rye flour at 10^7 cfu per gram sourdough. The mixture was allowed to ferment at 32 °C for 20 h without agitation. Ready sourdough showed pH value of 3.75. Sourdoughs were used in the subsequent baking at the level of 20 g per 100 g of bread dough.

Control mixed rye breads (BC) were prepared without any addition of GP. The recipe for BC was as follows: wheat flour with an ash content of 0.6 g per 100 g (200 g), rye flour with an ash content of 0.7 g per 100 g (150 g), rye flour with an ash content of 2.0 g per 100 g (82 g), sourdough (170 g), fresh yeast (15 g), salt (7.5 g) and water (221 mL). The other variations were prepared by adding GP at four different levels: 4%, 6%, 8% and 10% (flour basis), and the samples were marked as B4%, B6%, B8% and B10%. Thus, the amount of flour or flour with GP and water was the same in the control mixed bread and in the supplemented breads. Breads were prepared as described earlier (Bajerska *et al.*, 2010).

Nutritional characteristics

Dietary fibre and ash content analyses

Total, soluble (SDF) and insoluble (IDF) dietary fibre contents were determined by a method described by Asp *et al.* (1983). Ash (AOAC, method 930.22, 1995) was evaluated using analytical methods recommended by AOAC.

Phenol content and antioxidant activity determination

Phenolic compounds were extracted using a method described by Wang & Zhou (2004). The total phenol contents (TPC) were determined according to Singleton & Rossi (1965). The results were expressed as an equivalent of gallic acid (GAE) in mg GAE per g of dry starting material. The antiradical efficiency of was evaluated according to Bandoniene *et al.* (2002). Results were expressed as Trolox equivalents (TRE) in mM TRE per g of dry starting material. The ferric reducing ability of plasma (FRAP) assay was estimated according to Benzie & Strain (1996). Results were expressed as Fe^{II} equivalents (Fe^{II}) in mM Fe^{II} per g of dry starting material.

HPLC analysis

The HPLC analyses were performed on a Waters Alliance HPLC System 2695 (Milford, MA, USA) equipped with an X-Terra RP18, 5-μm column according to the method described by Andrade *et al.* (2001). Phenolic quantification was obtained from absorbances recorded in the chromatograms relative to external standards of phenolics, with detection at 280 and 360 nm.

Sensory and physical characteristics

Texture analysis

The texture property of the crumb was measured in a TAXT2 Texture Analyzer using an aluminium plunger with 36 mm diameter (AACC, method 74-09, 1998). Hardness, springiness, cohesiveness, gumminess and resilience of the crumb were measured based on the force–time curve.

Sensory evaluation

A 10-member panel performed the odour profiling of samples in three sessions. Samples from the same lots were presented to panel members three times within 8 h. Five attributes, i.e. volume, porosity, hardness, overall acceptance and aroma (flavour and taste) attributes, i.e. typical aroma of freshly baked breads, acidic, floury, malty, fruity, alcoholic and sharp, were selected according to a list of standardised lexicon of terms for bread evaluation (Meilgaard *et al.*, 1999). Panel members assigned the intensity of each odour descriptor on a linear graphic scale ranging from 0 to 10. Results from linear scales were converted into numerical values for data analysis. Mean, variance and standard deviations were calculated for all attributes of each sample for each session separately and across all the three sessions.

Volatile compound analyses

Volatile compounds of the crusts were isolated by HS-SPME sampling and analysed by GC/MS. The amount of 1/2 g of ground samples, placed into a 20-mL headspace vial, was mixed with 10 mL of bidistilled water and fitted with a Teflon-lined septum. Volatiles were sampled for 30 min at 50 °C from the headspace of the vial using CAR/PDMS fibre (Carboxene/Polydimethylsiloxane, Supelco, Bellefonte, PA, USA). An Agilent Technologies 7890A gas chromatograph coupled to an Agilent Technologies 5975C (Triple-Axis Detector) mass spectrometer was used. The chromatograph was equipped with a Supelcowax 10 (30 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte, Pa., USA) column. Operating conditions for GC/MS were as follows: helium flow 0.6 mL min^{-1}, initial oven temperature 45 °C (2 min), then 0.5 °C min^{-1} to 51 °C, 8 °C min^{-1} to 170 °C and 18 °C min^{-1} to 230 °C (8 min). Volatile compounds were identified by a comparison of their retention indices (RI) and mass spectra with standards or in some cases tentatively only by NIST MS Search 2.0 mass spectra library search and Kováts RI.

Statistical analysis

All analytical values represent means of three analyses performed in at least two different experiments. Data were analysed by the one-way ANOVA ($P < 0.05$), to estimate the differences between values of the compounds tested. If a significant F ratio was obtained, Tukey's HSD was used to locate differences between means. The STATISTICA 8.0 software (StatSoft, Krakow, Poland) was used for analysis.

Results and discussion

Nutritional characteristics of mixed rye breads

The effect of GP addition on the chemical composition of breads is given in Table 2.

Ash content of GP was about 6.40 g per 100 g d.m. and was between the value described by Bravo & Saura-Calixto (1998) and Valiente *et al.* (1995) for red and white grape by-products (5.7–9.2 g per 100 g d.m.), whereas it was significantly higher than both mango DF (2.8 g per 100 g d.m.) (Vergara-Valencia *et al.*, 2007). The appreciably higher ash content of GP results in a significant increase in its level in all supplemented mixed rye breads (Table 2). Bread with a 10% addition of GP had about 41% greater content of ash than control mixed rye bread.

Total dietary fibre (TDF) content in GP was high and amounted to approximately 50.93 g per 100 g d.m. From the nutritional point of view, DF preparations should have equal levels of SDF and IDF fractions. In case of GP, the insoluble fraction was about tenfold

Table 2 Chemical composition (g per 100 g d.m.) of sourdough mixed rye breads with and without GP addition

	Ash	TDF	SDF	IDF
BC	1.29 ± 0.00^a	13.21 ± 0.07^a	2.67 ± 0.01^a	10.54 ± 0.05^a
B4%	1.54 ± 0.00^b	14.52 ± 0.06^b	2.95 ± 0.01^b	11.58 ± 0.05^b
B6%	1.66 ± 0.00^c	15.05 ± 0.01^c	3.15 ± 0.04^c	11.90 ± 0.02^c
B8%	1.73 ± 0.01^d	16.90 ± 0.12^d	3.38 ± 0.06^d	13.52 ± 0.07^d
B10%	1.82 ± 0.01^e	18.41 ± 0.05^e	3.67 ± 0.09^e	14.74 ± 0.04^e

Values are means of three determinations ± SD. Mean with the same superscript letters are not significantly different ($P < 0.05$).

GP, grape by-products; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre.

greater than SDF. Likewise, a similar ratio SDF/IDF was obtained by Bravo & Saura-Calixto (1998) for red grape skins, by Ubando-Rivera *et al*. (2005) for Mexican lime peel and by Jiménez-Escrig *et al*. (2001) for guava fibre. Among the fruit fibre preparations, only mango DF contained equal levels of the two fibre types, with 13.8 g per 100 g d.m. IDF and 14.3 g per 100 g d.m. SDF (Vergara-Valencia *et al*., 2007). As it could have been expected, the high amount of DF in GP resulted in a significantly greater content of dietary fractions in supplemented mixed rye breads (Table 2). An addition of GP significantly improves dietary fraction contents, as the B10% accounts for about 39% and 37% higher contents of IDF and SDF than BC. Breads with the smallest addition of GP were also characterised by significantly higher, on average by 10%, TDF level than BC. In mango DF–supplemented breads, TDF increased on average by 16% (Vergara-Valencia *et al*., 2007), while in breads prepared from wheat and barley flour on average by 10% (Dhingra & Jood, 2001), as compared with control samples. According to Llobera & Cañellas (2007), the main constituents of SDF in grape by-products are pectins, while in IDF, neutral sugars provided mainly from celluloses and hemicelluloses. According to Pérez-Jiménez *et al*. (2008), grape antioxidant DF significantly reduced the lipid profile and blood pressure, the effects being significantly bigger than the ones caused by other DFs, such as oat fibre or psyllium, probably due to the combined effect of DF and antioxidants. Thus, a novel formulation of sourdough mixed rye bread produced wit GP may be an alternative source of DF in the diets.

Table 3 shows the contents of TPC and particular phenolic compounds, quantified by HPLC in control and supplemented breads. Contents of particular phenolic compounds increased gradually and significantly with the increasing GP level. Control bread was characterised by a slight level of phenolic compounds with gallic acid being predominant. In supplemented samples, the most abundant compounds were phenols recorded at 280 nm, whose contents were about threefold greater as compared with compounds recorded at 360 nm. Profiles of phenolic compounds in supplemented mixed rye breads were dominated by procyanidin B1, B2, catechin and epicatechin. Catechin and procyanidins have been shown to be powerful *in vitro* inhibitors of LDL oxidation and platelet aggregation (Hayek *et al*., 1997). Furthermore, catechins have many other positive effects on human health, including their antitumorgenic, antimutagenic, antipathogenic and antioxidative properties (Buettner, 2004). Among compounds recorded at 360 nm, predominant phenols were caffeic acid and myricetin, which contributed to nearly 12% of the total amount of phenolic compounds in mixed rye breads enriched with GP. According to García *et al*. (1998), some phenolic acids such as *p*-coumaric, caffeic and ferulic acids show cytostatic activity against carcinoma cells as well as anti-inflammatory activity. In addition, considerable amounts of quercetin and quercetin-3-beta-D-glucoside were determined. Several biological actions of quercetin, including protection of LDL cholesterol against oxidation and promotion of endothelial vasorelaxation (Mayer *et al*., 1998), have been reported. Furthermore, quercetin and

Table 3 Phenolic compound contents (mg per 100 g d.m.) of sourdough mixed rye breads with and without GP addition

Component	BC	B4%	B6%	B8%	B10%
TPC*	153.27 ± 3.22[a]	334.32 ± 1.92[b]	480.54 ± 7.50[c]	550.05 ± 10.70[d]	613.77 ± 1.57[e]
Detection at 280 nm					
Gallic acid	3.04 ± 0.03[a]	5.32 ± 0.04[b]	5.65 ± 0.06[c]	5.91 ± 0.08[d]	7.34 ± 0.06[e]
γ-resorcylic acid	nd[a]	3.36 ± 0.17[b]	4.02 ± 0.17[c]	5.91 ± 0.01[d]	6.37 ± 0.03[e]
Catechin	2.39 ± 0.11[a]	5.34 ± 0.00[b]	8.23 ± 0.63[c]	10.00 ± 0.44[d]	12.32 ± 0.24[e]
Procyanidin B1	nd[a]	30.16 ± 1.68[b]	49.65 ± 1.32[c]	60.53 ± 1.81[d]	81.77 ± 1.54[e]
Procyanidin B2	nd[a]	2.10 ± 0.14[b]	6.23 ± 0.32[c]	10.02 ± 0.19[d]	13.65 ± 0.36[e]
Epicatechin	nd[a]	12.69 ± 0.36[b]	18.42 ± 0.59[c]	23.06 ± 0.73[d]	35.33 ± 0.53[e]
p-coumaric acid	1.08 ± 0.07[a]	3.27 ± 0.02[b]	3.55 ± 0.06[c]	3.83 ± 0.06[d]	4.88 ± 0.09[e]
trans-ferulic acid	1.07 ± 0.04[a]	2.25 ± 0.07[b]	2.64 ± 0.07[c]	3.06 ± 0.08[d]	3.43 ± 0.03[e]
Detection at 360 nm					
Caffeic acid	Nd[a]	7.79 ± 0.18[b]	10.27 ± 0.11[c]	11.44 ± 0.27[d]	13.55 ± 0.20[e]
Sinapic acid	nd[a]	5.21 ± 0.09[b]	5.95 ± 0.09[c]	6.61 ± 0.09[d]	7.25 ± 0.08[e]
Quercetin-3-beta-D-glucoside	nd[a]	3.18 ± 0.09[b]	3.48 ± 0.08[c]	3.74 ± 0.03[d]	4.13 ± 0.07[e]
Myricetin	nd[a]	9.79 ± 0.08[b]	10.37 ± 0.06[c]	10.99 ± 0.09[d]	11.71 ± 0.05[e]
Quercetin	nd[a]	4.76 ± 0.06[b]	5.47 ± 0.08[c]	6.21 ± 0.09[d]	6.92 ± 0.06[e]

Values are means of three determinations ± SD. Mean with the same superscript letters are not significantly different ($P < 0.05$).
*GAE equivalents.
n. d., not detected; GP, grape by-products; TPC, total phenol contents.

other polyphenols have been associated with a reduced risk of cancer (Wattenburg, 1990).

The AA values of five mixed rye breads with or without GP addition are shown in Fig. 1. The assay of DPPH· radical-scavenging activity and reducing ability showed that GP is a very good source of active compounds, and adding them greatly enhanced antioxidant properties of breads. DPPH· in five types of mixed rye breads ranged from 5.55 to 15.02 mM TRE per g d.m. Definitely greater differences were observed for FRAP value (from 4.44 to 48.14 mM Fe^{II} per g d.m.). Likewise, antioxidant properties expressed as DPPH· radical-scavenging ability and reducing ability were recorded in the descending order: B10% > B8% > B6% > B4% > BC. Presumably, the improved antioxidant properties of GP-enriched breads were attributable to the incorporation of phenolic compounds, mainly catechins and procyanidins, which had been shown to possess strong AA. It is well known that there is a strong relationship between the structure and activity of different compounds by the DPPH· method. For flavonoids, three structural groups are important for determining their antioxidative capacity: the o-dihydroxy (catechol) structure in the B-ring, which confers greater stability to aroxyl radicals; the 2,3 double bond conjugated with a 4-oxo function, responsible for electron delocation from the B-ring; and the presence of both 3- and 5-hydroxyl groups for maximal radical-scavenging capacity and strongest radical absorption (Lafka *et al.*, 2007).

Sensory and physical characteristics of mixed rye breads

For the bioactive ingredients added to food for enhancing their nutritive value, it is necessary to study their impact on quality attributes. Texture is a very important characteristic of breads and is commonly used as an index to determine bread quality (Wang *et al.*, 2007). Thus, the effect of GP on mixed rye bread texture was investigated (Table 4). The hardness and gumminess of breads significantly increased with an increasing level of GP. The hardness of mixed rye bread with an addition of 10% GP was about 1.9-fold greater than that for control bread. As bread matrix is a complex system, the mechanism responsible for the increase in bread hardness with an addition of ingredients rich in phenolic compounds and DF still remains unknown. The increase in hardness might be attributable to a higher water absorption of fibre-rich incorporated doughs. This observation is explained by an interaction between water and hydroxyl groups of polysaccharides through hydrogen bonding. The varied hardness in

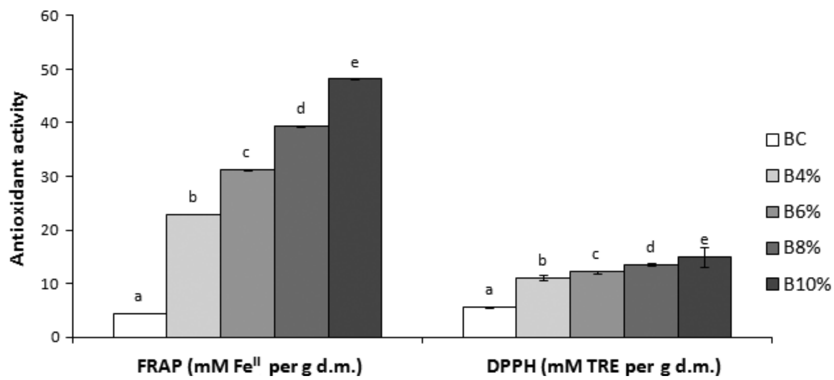

Figure 1 Antioxidant activities measured by 2,2-Diphenyl-1-picrylhydrazyl and ferric reducing ability of plasma assays of sourdough mixed rye breads with and without grape by-products addition Values are means of three determinations ± SD. Means with the same superscript letters are not significantly different ($P < 0.05$).

Table 4 Textural analysis of sourdough mixed rye breads with and without grape by-products addition

	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g*mm)	Resilience
BC	220 ± 2.72^{a}	0.83 ± 0.02^{a}	0.63 ± 0.08^{a}	154 ± 3.25^{a}	106 ± 5.26^{a}	0.27 ± 0.03^{a}
B4%	309 ± 3.89^{b}	0.77 ± 0.01^{a,b}	0.55 ± 0.02^{a,b}	177 ± 9.00^{b}	128 ± 8.12^{a,b}	0.25 ± 0.02^{a,b}
B6%	349 ± 3.88^{c}	0.77 ± 0.03^{a,b}	0.54 ± 0.01^{a,b}	184 ± 5.88^{b}	133 ± 0.56^{b}	0.24 ± 0.01^{a,b}
B8%	359 ± 5.30^{c}	0.76 ± 0.00^{a,b}	0.53 ± 0.01^{b}	191 ± 6.53^{b}	140 ± 9.11^{b}	0.22 ± 0.01^{b}
B10%	416 ± 3.57^{d}	0.71 ± 0.02^{b}	0.50 ± 0.01^{b}	211 ± 9.08^{c}	148 ± 6.36^{b}	0.21 ± 0.01^{b}

Values are means of three determinations ± SD. Mean with the same superscript letters are not significantly different ($P < 0.05$).

supplemented with phenols breads might be also explained by the affected enzyme activity and yeast activity (Wang *et al*., 2007). Zhang & Kashket (1998) suggest that the activities of amylases in dough might be restricted by the phenolic compounds, leading to inadequate maltose for yeast activity during proofing. Moreover, Turchetti *et al*. (2005) reported that catechins were able to inhibit the activity of yeast, causing a poorer gassing power.

Nevertheless, cohesiveness and resilience did not change significantly up to 6% GP addition, chewiness up to 4%, while springiness did not change till 8% of GP added. In our opinion, sourdough fermentation might slightly improve texture of fibre-rich breads. Without sourdough, fibre-rich breads or breads with functional additives are very difficult to process. For example, Wang *et al*. (2007) found that the more green tea extract added to wheat flour, the harder the produced bread. Moreover, Masoodi & Chauhan (1998) and Sudha *et al*. (2007) found that increasing levels of apple pomace conduced to a harder structure of bread and cookies. In bread with an 11% addition of apple pomace, texture was about four times harder than that of control bread (Masoodi & Chauhan, 1998).

Attractive aroma, which includes both taste and flavour, and overall consumer acceptance are also very important because this ensures marketing success of novel food. The results of sensory evaluation of sourdough mixed rye bread with and without GP added are given in Fig. 2. Sensory evaluation of bread samples showed that as the level of GP increased, the bread volume, porosity and overall acceptance decreased. However, no significant differences were observed up to 6% of GP. Moreover, differences were found in aroma attributes between control bread and bread with GP. The dominating descriptors, for which the higher differences were observed, were alcoholic and sharp notes. Breads with greater GP levels were described by a higher insensitivity of these descriptors. Additionally, floury attribute decreased, while fruity attribute increased with higher levels of GP. Typical aroma of freshly baked breads also decreased with increasing GP levels. Scharbert & Hofmann (2005) reported that catechins are responsible for astringent (sharp) note. The lowest threshold concentration for the astringency in liquids exhibits epigallocatechin gallate, while the rest of catechins are also responsible for the astringency but at relatively higher threshold concentrations. On the

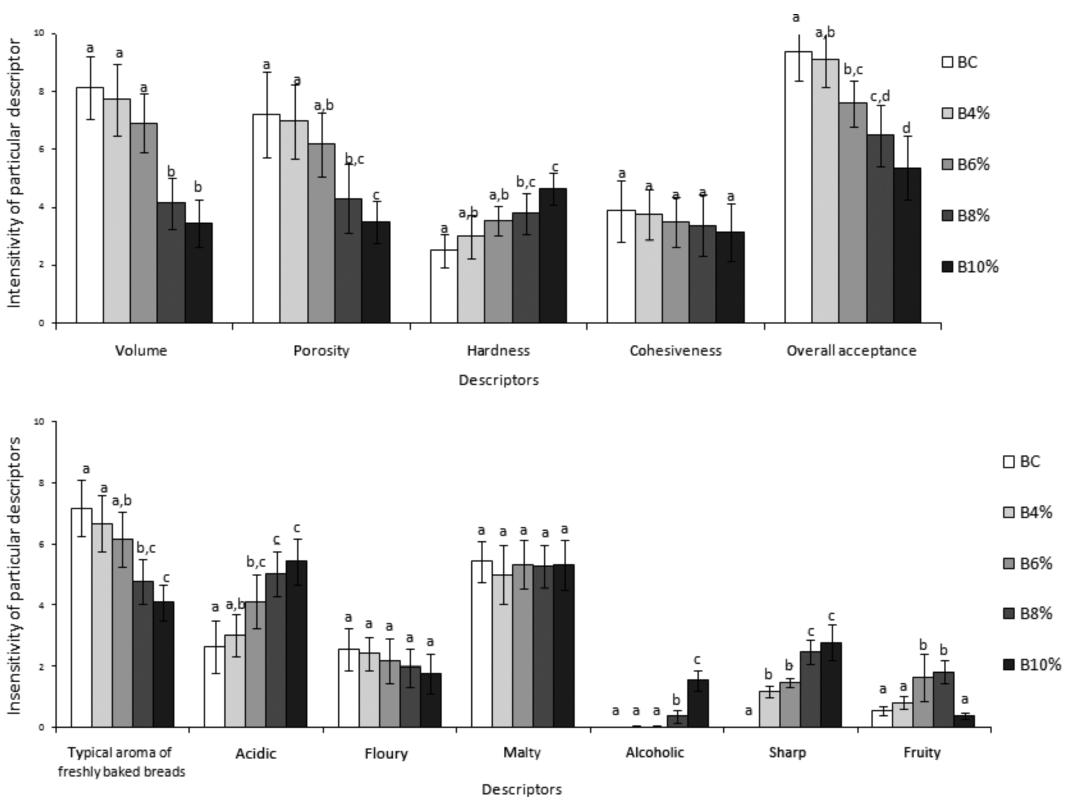

Figure 2 Histograms of sourdough mixed rye breads with and without the grape by-products addition Values are means of three determinations ± SD. Means with the same superscript letters are not significantly different ($P < 0.05$).

other hand, Robichaud & Noble (1990) found that catechin and gallic acid were more bitter than astringent. From the aforementioned results, it could be concluded that mixed rye breads with up to a 6% GP level could be baked with satisfactory performance. In our opinion, sourdough fermentation might slightly masked the inferior flavour connected with GP addition. Anil (2007) and Masoodi & Chauhan (1998) found that 5% of hazelnut testa and apple pomace did not influence organoleptic properties of wheat breads, while Gawlik-Dziki *et al.* (2009) established that only a 2.5% buckwheat preparation added to wheat bread was accepted.

Several volatile components that contribute to the aroma profile of control mixed rye bread (BC) and breads supplemented with GP using SPME-GC/MS are listed in Table 5. Most of these components represented groups of characteristic volatiles produced through enzymatic and thermal reactions during the sourdough fermentation and baking process. Although all mixed rye breads showed a common volatiles profile, there are little differences in the concentrations of those components. Generally, contents of some volatiles increased gradually with the increasing level of GP, while in turn for others they decreased with an increasing level of GP. The largest differences were observed for esters such as ethyl octanoate, 3-methylbutyl acetate and ethyl acetate. All those volatiles observed in control samples showed a significant increase in case of B10%. Ethyl acetate and ethyl octanoate presented in rye sourdoughs fermented with heterofermentative LAB and yeasts are also the most abundant esters in wine, responsible for their aroma profiles (Swiegers *et al.*, 2005). Moreover, significant differences were observed in carbonyl compound profiles, which could be derived from either an enzymatic oxidation or an autoxidation of the unsaturated fatty acids present in the flour. Volatile markers of baking process, furfural and 5-methyl-2-furfural, i.e. sugar degradation products that originate from pentose and hexose, respectively, are also found in wine (Chuenchomrat *et al.*, 2008).

Conclusion

A novel formulation was developed for sourdough mixed rye bread produced with GP as an alternative source of DF, ash and polyphenols in the diet. The chemical composition of GP indicated high levels of fibre, ash and phenolic compounds with powerful AA. GP-enhanced sourdough mixed rye breads showed considerably higher TDF contents than in the control, and it was characterised by a significantly higher AA associated with their phenolic compound content. However, it was found that GP was significantly involved in the quality of bread including hardness, gumminess and sharpness. The texture analysis showed that the hardness and gumminess of breads significantly

Table 5 Volatile compounds identified in sourdough mixed rye breads with and without GP addition using SPME-GC/MS

	Peak area (TIC*10^5)				
Volatile compound	**BC**	**B4%**	**B6%**	**B8%**	**B10%**
Ethyl acetate	220.89 ± 24.81	329.95 ± 38.39	578.32 ± 30.29	636.26 ± 38.61	688.55 ± 33.05
Pentanal	8.05 ± 0.23	2.13 ± 0.34	3.30 ± 0.64	4.43 ± 0.45	6.05 ± 0.01
Hexanal	117.75 ± 9.34	31.31 ± 1.32	49.00 ± 0.54	51.53 ± 3.93	58.23 ± 2.10
3-methylbutyl acetate	1.47 ± 0.38	1.89 ± 0.37	2.68 ± 0.04	3.86 ± 0.16	4.84 ± 0.30
Heptanal	0	3.46 ± 0.01	5.93 ± 0.01	11.65 ± 0.5	12.02 ± 1.28
1-pentanol	39.67 ± 2.06	32.46 ± 1.81	31.14 ± 1.05	36.87 ± 4.82	37.79 ± 0.94
Furan-2-pentyl	12.36 ± 0.15	13.74 ± 0.01	15.72 ± 1.03	17.51 ± 0.67	21.37 ± 3.78
Pyrazine methyl	12.02 ± 2.34	11.31 ± 0.5	13.54 ± 0.13	14.32 ± 1.92	15.14 ± 1.16
Hexyl acetate	3.89 ± 0.64	5.16 ± 0.25	5.86 ± 0.26	6.92 ± 0.28	7.38 ± 0.85
Octanal	15.21 ± 1.85	11.15 ± 1.85	14.06 ± 0.14	15.72 ± 0.31	16.96 ± 1.61
(E)-2-heptenal	2.17 ± 0.17	2.53 ± 0.05	4.21 ± 0.05	5.63 ± 0.01	6.82 ± 0.05
1-hexanol	20.38 ± 0.84	18.74 ± 1.75	26.26 ± 0.02	28.24 ± 0.37	33.78 ± 0.89
2-nonanone	0.53 ± 0.02	0.62 ± 0.01	0.85 ± 0.01	0.95 ± 0.01	1.13 ± 0.1
Nonanal	11.50 ± 0.24	7.63 ± 0.05	10.74 ± 0.01	11.46 ± 1.13	12.70 ± 1.00
Ethyl octanoate	7.36 ± 0.38	13.54 ± 0.37	22.55 ± 1.79	29.13 ± 3.18	33.81 ± 1.38
Furfural	2.82 ± 0.07	2.86 ± 0.36	7.67 ± 0.28	12.59 ± 0.67	15.94 ± 0.47
1-hexanol-2-ethyl	5.36 ± 0.26	1.35 ± 0.57	1.84 ± 0.04	1.93 ± 0.05	2.48 ± 0.14
Furan, 2-ethyl-5-methyl-	2.18 ± 0.01	1.45 ± 0.17	2.06 ± 0.21	2.21 ± 0.24	2.76 ± 0.46
Benzaldehyde	2.83 ± 0.01	2.23 ± 0.11	2.75 ± 0.16	3.05 ± 0.1	3.23 ± 0.12
(E)-2-nonenal	1.14 ± 0.02	0.82 ± 0.03	0.86 ± 0.02	0.93 ± 0.01	1.16 ± 0.03
5-methyl-2-furfural	0	0	0.76 ± 0.01	1.58 ± 0.35	1.70 ± 0.35

Values are means of three determinations ± SD.
GP, grape by-products.

increase with an increasing level of GP, whereas cohesiveness and resilience did not change significantly up to 6%, while springiness till 8% of GP. There were also slight differences between BC and supplemented breads in the concentrations of volatiles compound profiles. Based on sensory and physical characteristics, it may be concluded that GP could be incorporated at a level of up to 6% in order to prepare acceptable products.

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