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Original article

Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread

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Summary The impact of bread fortification with dry spent yeast from brewing industry on physical, chemical and sensorial characteristics of home-made bread was evaluated with the goal of increasing its β-glucan content. A serving of 50 g of bread fortified with dry spent yeast increased β-glucan intake from 65 to 125 mg, which is within the guidelines suggested by European Food Safety Authority. Although this fortification darkened the crumb and increased crumb and crust springiness and had impact on volatile profile, concerning key odours only hexanal presented a significant increase in fortified bread. Two machine types (with 1 or 2 paddles) were tested but had a minor impact on bread characteristics. Descriptive analyses performed by a trained panel showed no significant differences in sensorial attributes. Dry spent yeast can be used as ingredient in home-made bread to increase β-glucan intake, and contribute to valorisation of this brewing by-product.

Keywords Brewing by-products, fibre fortification, home-made bread, key odours, sensory analyses, β-glucans.

Introduction

Nowadays consumers search for producing tasty foods that will contribute to improve or maintain their health, due to the addition of new ingredients that contain bioactive compounds. These new ingredients must comply with nutritional, energy and safety needs of consumers and also with legislation. An additional challenge is to find these innovative ingredients in a cost-effective and sustainable way.

Brewing spent yeast is the second major by-product from the brewing industry. It is low in calories, fat, sodium and carbohydrates, but can be a valuable source of cheap fibre, mainly β-glucans (Aimanianda *et al.*, 2009; Petravić-Tominac *et al.*, 2011), vitamins and chromium (Ferreira *et al.*, 2010), which will result profitable for both the industry and the consumer.

β-Glucans are natural cell wall polysaccharides and are a major structural component of baker's and brewing yeast. They consist of β-(1→3) and β-(1→6) linked glucose polymers (Aimanianda *et al.*, 2009; Ahmad *et al.*, 2012; Kittisuban *et al.*, 2014).

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Long-chain, water-insoluble and nondigestible yeast β-glucans are able to modulate mucosal immunity of the intestinal tract, facilitate bowel motility and can be used in obstipation, among other intestinal problems (Volman *et al.*, 2008). Also, β-(1→3)-glucan has proved to reduce blood serum cholesterol, showing both hypolipidaemic and hypoglycaemic effects in animal and human studies (Naumann *et al.*, 2006; Kapur *et al.*, 2008; Nazare *et al.*, 2009).

The European Food Safety Authority (EFSA) has already approved the use of Saccharomyces β-glucans – referred to as 'yeast beta-glucans' – as a new ingredient and suggests a use ranging between 50 and 200 mg of 'yeast beta-glucans' per serving (EFSA, 2011). However, studies about the impact of this functional ingredient in food quality, namely bread, are scarce (Kittisuban *et al.*, 2014).

Bread is one of the most consumed foods in the world and an important source of nutrients, namely proteins, starch, dietary fibres, vitamins, micronutrients and antioxidants. As bread is consumed in a daily base, there is a growing interest on the incorporation of functional ingredients in bread to reply consumer's demands on healthy nutrition (Gallagher *et al.*, 2004; Paraskevopoulou *et al.*, 2012; Fitzgerald

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et al., 2014). Additionally, interest in new sources of dietary fibre and bioactive compounds has grown rapidly. Fibres in various forms have been previously used in bread making to increase bread nutritional value (Almeida *et al*., 2013; Ktenioudaki *et al*., 2013). Novel sources of dietary fibre to incorporate in bakery products, such as those generated from by-products, received much attention (Ktenioudaki & Gallagher, 2012; Ktenioudaki *et al*., 2012, 2013; Acosta-Estrada *et al*., 2014; Basanta *et al*., 2014; O'Shea *et al*., 2015).

However, the addition of new ingredients to bread can influence its quality. The assessment of bread quality is a complex process usually defined according to sensory parameters, volume, texture, colour and flavour (Zehentbauer & Grosch, 1998; Heenan *et al*., 2008, 2009; Birch *et al*., 2013a,b). The volatiles that contribute to bread aroma are one of the most important parameters influencing consumer acceptance. More than 300 volatile compounds have been identified in bread (Pozo-Bayón *et al*., 2006; Quílez *et al*., 2006; Jensen *et al*., 2011; Birch *et al*., 2013a,b). From the wide variety of chemical compounds that contribute to bread flavour, the most relevant are the aldehydes, alcohols, ketones, esters, acids, pyrazines and pyrrolines, as well as hydrocarbons, furans and lactones (Pozo-Bayón *et al*., 2006; Bianchi *et al*., 2008). Not all these compounds have the same degree of influence on the flavour, but there are key odorants that have a marked influence on both crust and crumb, namely 3-methyl-1-butanol, 3-methyl-butanal, 2-phenylethanol, 2,3-butanedione, hexanal and trans 2-nonenal (Quílez *et al*., 2006; Birch *et al*., 2013a,b).

Concerning bread manufacture, nowadays, domestic bread-making machines incorporating one or two paddles offer an alternative and facilitate the lives of those who like to bake their own bread at home. Flour mixtures added of salt, sugar and yeast are commercially available and can include bioactive ingredients.

The objective of this work was to prepare home-made bread with enhanced nutritional benefits, due to high content of β-glucans, through fortification with dry spent yeast from brewing industry, and investigate the impact on physical parameters, volatile composition and sensorial characteristics. For this purpose, several subgoals were established, namely (i) quantification of β-glucans in dry spent yeast and also in fortified and nonfortified breads; (ii) assessment (prepared in one-paddle and two-paddle domestic machines) of the influence of spent yeast addition in bread volume, texture, colour and impact on volatile profile and quantification of relevant key odour bread compounds in home-made breads; and (iii) effect on sensory characteristics of home-made bread.

Material and methods

Chemicals and standards

All solvents, reagents and standards used in this study were of analytical grade. Methanol was supplied by Merck (Darmstadt, Germany). The standards used for quantification and/or confirmations of the identity of the volatiles were 2-phenylethanol, ethanol, 3-methyl-1-butanol, 2-methyl-1-butanol, hexanal, 3-methylbutanal, *trans*-2-nonenal, 2-methyl-1-propanol, hexanol, octanol, 2-methyl-butanal, heptanal, benzaldehyde, pentane, hexane, 2-propanone, 2-butanone, octanoic acid, methyl acetate, ethyl acetate, methyl butyrate, furfural, furfuryl alcohol, acetylfuran and 2-pentylfuran. In addition, 2-ethylbutiric acid was used as internal standard. Standards were supplied by Sigma-Aldrich (St. Louis MO, USA).

Standard sock solutions of 2-phenylethanol, 3-methyl-1-butanol, hexanal, 3-methylbutanal and *trans*-2-nonenal were prepared by injecting 10 μL of refrigerated standard with a syringe through the septum of a 15-mL HS vial filled with 10 mL of methanol and sealed. The exact weight of methanol and standard was recorded, expressing the concentration in mg mL^{-1} and taking into account the density of methanol. This solution is stable for at least 2 weeks if kept at 4 °C. Diluted standard solutions were prepared in methanol and kept in refrigerator and protected from light.

Preparation of dry spent yeast from brewing industry

The spent yeast biomass used to produce lager beer (*Saccharomyces pastorianus*) was supplied by a local beer industry. It was washed three times with deionised water to remove beer residues, at a ratio of 1:3 (w/v) (yeast biomass:water). Between each wash, it was centrifuged at 10 000 ***g***, 4 °C, 5 min. The resulting yeast cell pellet was slowly dried in an dynamic oven at 30 °C for 48 h and milled with a Knife Mill Grindomix GM200 (Retsch GmbH, Haan, Germany). β-glucan content was quantified using the 'Enzymatic yeast beta-glucan – assay procedure' (Megazyme International Ireland Ltd., Bray, Ireland).

Bread making

White bread-baking mix flour supplied by Cerealis-Nacional (Maia, Portugal) was selected to perform this study. The mix contains wheat flour, dried yeast, dextrose and salt. Control bread samples were prepared as described in product label; thus, 500 g of this mix was added to 320 mL of water and baked using two different types of domestic bread machines: A – Moulinex with 2 paddles and 1650W power (Groupe SEB,

Ecully, France), and B – Clatronic with 1 paddle and 600 W power (Clatronic International BBA GmbH, Germany). The conditions of leaving and baking were the same for the two machines used. Fortified breads were prepared in a similar way as control breads, but 10 g of dry spent yeast was added to 500 g of the commercial mix flour. Total bread-making time was 3 h, and bread was cooled at room temperature 90 min before physical and volatile analyses. Twelve control breads were prepared for the six tests carried out for optimisation of HS-SPME-GC/MS conditions; for this purpose, only machine A was used. With regard to analyses of volume, texture, colour and volatile profile, six breads were prepared in machine A, three control breads (coded as CBA) and three fortified breads with the addition of 10 g of dry spent yeast (coded as FBA). Similarly, six breads were prepared in machine B to obtain CBB and FBB samples. Concerning quantification of selected volatiles and sensorial analyses, another batch of three CBA, three FBA, three CBB and three FBB was prepared. Moreover, samples of control and fortified breads were prepared to perform the panel training.

Evaluation of bread volume, texture and colour

Bread weight and volume were measured 90 min after removal from bread-making machine. The bread-specific volume (SV) was measured using a seed displacement method (Cerealis internal method) and the following formula

$$\mathrm{SV} = S \times 1.35/P,$$

where P (g) is bread weight, S (g) is weight of the displaced seeds, 1.35 ($cm^3\ g^{-1}$) is specific volume of the *Phalaris canariensis* seeds, and SV ($cm^3\ g^{-1}$) is specific volume of the bread.

For texture and colour assays, the bread was cut in half and the measurements were performed in two different zones of the bread, crumb and side crust. Texture analysis was performed using a texture analyser (model TA-XT-2iHR; Stable MicroSystems, Ltd., Surrey, UK) containing 5 kg of load cell. Calibrations were performed with 2 kg of load cell. Exponent software supplied with the instrument was used. Bread crust and crumb were subjected to a 30-mm penetration depth through a two-cycle sequence using a spherical probe (25 mm in diameter) (Cyl. Perspex P/25; Stable MicroSystems, Ltd.) with a cross-head speed of 1 mm s^{-1} at three different points for either crumb or crust. The texture parameters were the following: hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); springiness (m) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required for the first compression and A2 the total energy required for the second compression); and chewiness (J) = the work needed to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness). A Minolta CR-300 colorimeter (Minolta, Ramsey, NJ, USA) with illuminate D65, a 0° standard observer and a 2.5-cm port/viewing area was used for the measurement of colour in the CIELab system – lightness (L^*), redness (a^*) and yellowness (b^*). The colorimeter was standardised with a white tile having the following values: $L^* = 93.5$, $a^* = 1.0$ and $b^* = 0.8$ before measurement in bread crust ($n = 3$) and crumb ($n = 3$).

Volatile compounds analysis by HS-SPME-GC/MS

The first step was optimisation of extraction conditions for HS-SPME-GC/MS analysis of volatiles; thus, a slice (1.5 cm of thickness) of each of the twelve test samples of bread was collected after 90 min of cooling and entirely crushed, including the crust and the crumb. The influence of water/salt addition and SPME extraction time and temperature were evaluated using the method described by Petisca *et al.* (2013); briefly, 2 g of bread was placed in a 50-mL vial. After sealing, the vials were kept at −4 °C for 10 min and in an ultrasonic cleaner (Fungilab, Barcelona, Portugal) for 15 min, favouring the equilibrium between the matrix and the HS (Petisca *et al.*, 2013). Extraction of volatile compounds was performed using a CAR–PDMS SPME fibre (75 μm thickness; Supelco Co., Bellefonte, PA, USA). The fibre was inserted into the sample vial through the septum and exposed to the HS at variable time and temperature. Constant magnetic stirring at 600 rpm was performed at this stage. Thereafter, the SPME fibre was inserted and desorbed for 10 min at 280 °C, in the split-less mode, with 1 mL min^{-1} flow.

Six tests were performed in duplicate: test 1 – SPME extraction at 37 °C for 40 min; test 2 – addition of 3 g of salt and SPME extraction also at 37 °C for 40 min; test 3 – addition of 5 mL of water and 3 g of salt, at 37 °C for 40 min; test 4 – addition of 5 mL of water and 3 g of salt, at 50 °C for 40 min; test 5 – addition of 5 mL of water and 3 g of salt, at 50 °C for 60 min; test 6 – addition of 10 mL of 20% NaCl solution (pH 3 with 0.05 M citric acid), at 50 °C for 60 min.

After selection of HS-SPME-GC/MS analytical conditions (test 6), the next step was the evaluation of impact on volatile profile of spent yeast addition followed by quantification of relevant aroma compounds on control (three CBA and three CBB) and fortified breads (three FBA and three FBB).

GC–MS conditions

Chromatographic analysis was performed using an Agilent 6890 gas chromatograph (Agilent, Avondale, PA, USA) coupled to a mass selective detector (Agilent 5973). Volatiles were separated on a 5% fenil metilpolisiloxano (SPB-5), bonded-phase fused-silica capillary column (Hewlett–Packard, Palo Alto, CA, USA; 60 m–320 lm i.d., film thickness 1 lm), operating at 80 kPa column head pressure, resulting in a flow of 1 mL min^{-1} at 40 °C. The oven temperature program was isothermal for 5 min at 40 °C, raised to 135 °C at a rate of 3 °C min^{-1} and then raised to 220 °C at 20 °C min^{-1}. The transfer line to the mass spectrometer was maintained at 250 °C. Mass spectra were obtained by electronic impact at 70 eV, with a multiplier voltage of 2056 V, collecting data at a rate of 1 scan s^{-1} over the m/z range 30–500. Volatile compounds were identified by comparison with the mass spectrum of standards and/or from Nist 98 data bank (NIST/EPA/NISH Mass Spectral Library, version 1.6, Santa Clara, CA, USA). Compounds were also detected by m/z characteristic ion.

Calibration curves for quantification of key odorants

Five volatile compounds, 2-phenylethanol, 3-methyl-1-butanol, hexanal, 3-methylbutanal and *trans*-2-nonenal, which are described as bread key odorants, were quantified by external calibration curve method. Standard solutions for calibration curves were prepared in methanol. 2-Ethylbutiric acid was used as internal standard. 100 μL of standard solution mixture (concentration range between 0.025 and 2 mg L^{-1} for 2-phenylethanol, 0.1 and 10 mg L^{-1} for 3-methyl-1-butanol, 0.01 and 1 mg L^{-1} for hexanal, 0.1 and 2 mg L^{-1} for 3-methylbutanal, 0.01 and 0.05 mg L^{-1} for *trans*-2-nonenal) and 100 μL of internal standard solution (0.5 mg L^{-1}) were placed in a 50-mL HS vial containing 9.8 mL of a 20% NaCl solution (pH 3 with 0.05 M citric acid). After sealing, the vials were kept at −4 °C for 10 min and in an ultrasonic cleaner for 15 min. HS-SPME extraction was performed at 50 °C for 60 min. The quantity of each volatile compound in bread samples was calculated using the calibration curves constructed (volatile peak area/internal standard peak area vs. volatile concentration).

Bread sensory analysis

The sensory panel was composed of fourteen master students from the University of Porto who had sensory analysis in their curriculum and expressed an interest and disposition to undertake the work. The panel was trained for descriptive analysis according to the guidelines in the ISO 8586 (2012) to evaluate the influence of dry spent yeast addition and machine type on the bread sensory characteristics. In session 1, panellists proposed various attributes to start the panel training and tasted control bread samples and redundant descriptive terms were removed (Bassett *et al.*, 2014). Session 2 was designed to establish ballot anchors for selected attributes that were fitted on an unstructured scale (seven points). To assist panellists, different reference bread samples were used for training each attribute and respective intensity as summarised in Table 1. In session 3, the ballots were tested individually by panellists using unknown representative samples. The panel agreed on sixteen attributes to constitute the breads' descriptive profile. Each assessor received a list of sixteen attributes: 'crust colour', 'crumb colour', 'odour intensity', 'bread odour', 'strange odour', 'elasticity of the crumb (in the fingers)', 'aroma intensity', 'bread aroma', 'strange aroma', 'salty', 'astringent', 'bitter', 'taste persistence', 'crispy crust', 'adhesiveness (mouth) 'and 'overall assessment'. Analysis of variance (Hrušková *et al.*, 2012) was performed on data collected on two more training sessions, and panellists' deviations were assessed to determine whether additional training was

Table 1 Preparation of reference bread samples used for panellist training of attributes and respective scale

Bread attributes	Preparation of reference samples	Range	Scale
Crust colour	Control bread	0 g of oat	3
Crumb colour	added with	50 g of oat	4
	oat flour	100 g of oat	5
Odour intensity	Control bread	10 min	6
Bread odour	with different	90 min	4
Aroma intensity	cooling periods	180 min	3
Bread aroma			
Elasticity of the crumb			
Adhesiveness	Control bread with	10 min	4
(mouth)	different cooling	90 min	2
	periods	180 min	1
Crispy crust	Control bread with	Low	1
	different selection	Medium	3
	of machine baking time	High	5
Strange odour	Control bread with	0 mL of whey	1
Strange aroma	replacement of part	50 mL of whey	3
Astringent	of water by liquid	100 mL of whey	5
Bitter	milk whey		
Taste persistence			
Salty	Control bread with	Mix flour	1
	increased content	2 g of salt	3
	of salt	5 g of salt	6

needed. In evaluation sessions, approximately 50 g of each sample (a slice with 1.5 cm of thickness) including the crust and crumb was presented to assessors in a 3-digit coded glass covered with a glass lid. Assessment was carried out individually under white light at room temperature. Each assessor was provided with filtered water and asked to cleanse their palate between tastings. Control (three CBA and three CBB) and fortified breads (three FBA and three FBB) were analysed over three sessions.

Statistical analysis

The mean averages and standard deviations were calculated for each experimental parameter. The effect of fortification with β-glucans by the addition of dry spent yeast and the effect of machine type were analysed by two-way ANOVA. Statistical analyses were performed with SPSS for Windows, version 20 (SPSS, Chicago, IL, USA).

Results and discussion

β-glucan quantification in dry spent yeast, in fortified and nonfortified breads

The β-glucan contents expressed in (w/w dry weight) was 8% in dry spent yeast, 0.13% in nonfortified bread and 0.25% in bread fortified with 10 g of dry spent yeast. Concerning the β-glucan intake from a serving of bread (50 g), 65 mg was quantified in nonfortified bread and the intake increases to 125 mg in bread fortified with 10 g of dry spent yeast. These contents were within the range suggested by EFSA guidelines of 50–200 mg of *Saccharomyces* β-glucans per serving (EFSA, 2011).

Influence of dry spent yeast addition in bread volume, texture and colour

Mean weight of CBA samples (709 ± 12 g) was similar to mean weight from CBB samples (709 ± 2.9 g). As expected, fortified breads present increased weight, 720 ± 20 and 718 ± 9 g, respectively, for FBA and FBB samples; however, the increase was not statistically significant ($P > 0.05$).

Concerning the bread volume, mean SV of CBA samples (4.6 ± 0.3 $cm^3\ g^{-1}$) was similar to mean SV of FBA (4.5 ± 0.2 $cm^3\ g^{-1}$), whereas the mean SV of CBB was 3.9 ± 0.2 $cm^3\ g^{-1}$ and FBB was 3.9 ± 0.1 $cm^3\ g^{-1}$. Two-way ANOVA indicates that no significant differences were observed due to β-glucan fortification by dry spent yeast addition ($P > 0.05$), but the machine type had a significant effect on the bread volume ($P < 0.05$). The two-paddle machine (A) promoted an increase of bread volume. According to Cauvain (2007), improvement of bread volume is achieved with vigorous mixing and kneading.

Mean and standard deviation of crumb and crust texture and colour of control and fortified breads

Table 2 Mean values and standard deviation (SD) of crumb and crust texture and colour parameters

		Mean* ± SD			
		Crumb		Crust	
	Machine	CB	FB	CB	FB
Texture					
Hardness	A	1.13 ± 0.13	1.60 ± 0.25	3.12 ± 0.62†	3.58 ± 0.36†
	B	2.21 ± 0.30	2.39 ± 0.57	4.57 ± 0.71††	4.85 ± 1.08††
Cohesiveness	A	0.74 ± 0.04	0.76 ± 0.02	0.82 ± 0.02	0.84 ± 0.03
	B	0.76 ± 0.04	0.77 ± 0.03	0.84 ± 0.01	0.86 ± 0.02
Springiness	A	0.67 ± 0.06a	1.68 ± 0.07b	0.781 ± 0.02a	1.56 ± 0.04b
	B	0.69 ± 0.06a	1.54 ± 0.02b	0.81 ± 0.01a	1.43 ± 0.02b
Chewiness	A	0.48 ± 0.14	2.59 ± 0.83	5.24 ± 0.87	14.18 ± 2.68
	B	2.08 ± 1.74	5.55 ± 2.00	12.00 ± 4.34	26.14 ± 11.62
Colour					
*L**	A	70.21 ± 2.33a	72.41 ± 1.19b	50.89 ± 9.32	46.64 ± 4.73
	B	69.39 ± 1.00a	72.11 ± 1.68b	51.39 ± 9.39	51.37 ± 7.64
*a**	A	1.08 ± 1.52	19.63 ± 1.67	0.87 ± 1.75	28.78 ± 5.75
	B	19.87 ± 0.85	21.50 ± 0.88	32.10 ± 2.02	30.31 ± 4.08
*b**	A	0.26 ± 0.32	0.06 ± 0.37	12.78 ± 3.84	15.09 ± 1.54
	B	0.28 ± 0.20	0.20 ± 0.33	15.24 ± 1.40	14.46 ± 5.8

*a** – redness; *b** – yellowness; *L** – lightness. Differences were tested according to two-way ANOVA. In a column for each parameter, different numbers of symbol † and †† indicate significant differences ($P < 0.05$) due to machine type. In a line, different letters (a and b) indicate significant differences ($P < 0.05$) in crumb or crust due to β-glucan fortification by dry spent yeast addition.

Table 3 Optimisation of extraction conditions for HS-SPME

Test	Water volume (Gallagher *et al.*,.)	Salt (g)	20% NaCl solution (pH 3)	Temp	Time (min.)	Peak area $\times 10^{9a}$	Compound number[b]
1	–	–	–	37	40	9.01	41
2	–	3	–	37	40	10.01	37
3	5	3	–	37	40	11.56	49
4	5	3	–	50	40	11.88	53
5	5	3	–	50	60	11.01	56
6	–	–	**10**	**50**	**60**	**12.74**	**61**

[a]Expressed as arbitrary units of area, mean value of duplicate analyses.
[b]Number of compounds identified in the chromatograms.
Bold values highlight the selected conditions.

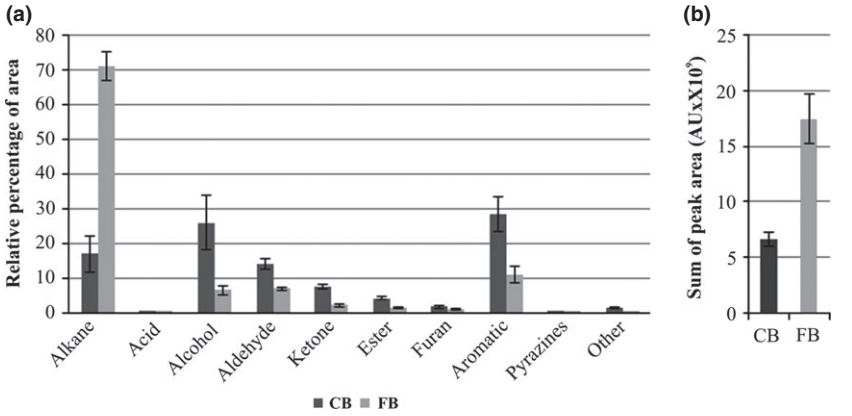

Figure 1 (a) Relative percentage of area volatile compounds grouped by chemical classes extracted from control and fortified breads; (b) mean total area of volatiles in control breads (BD) and fortified breads (FB).

from two different types of machine are summarised in Table 2. Two-way ANOVA performed to evaluate the effect of β-glucan fortification by dry spent yeast addition and machine type revealed that in general no significant effects were observed on texture and colour parameters. Regarding the texture of control and fortified breads, only springiness had significant differences ($P < 0.05$) in the crumb and crust. With regard to colour, the L^* values of crumb were significantly different in control and fortified breads ($P < 0.05$). Concerning the effect of machine type, significant differences were observed in crust hardness ($P < 0.05$). In conclusion, fortification with β-glucans darkened the crumb and increased crumb and crust springiness, which are characteristics appreciated by consumers.

Changes in volatile profile and quantification of relevant aroma compounds

Concerning optimisation of SPME parameters, the response evaluated during all experiments was the total sum of peak areas, obtained in the GC-MS analysis and the number of identified compounds. Conditions adopted were those that gave greater total peak area and higher number of volatile compounds. Table 3 presents mean results obtained in the six tests. The SPME process is influenced by temperature because the partition coefficients are temperature dependent. In general, the number of compounds extracted increased with temperature and with the extraction time. According to results from Table 3, the HS-SPME conditions selected were 2 g of bread in a 50-mL vial containing 10 mL of a 20% NaCl solution (pH 3 with citric acid 0.05 M), and extraction with CAR/PDMS fibre for 60 min at 50 °C under constant agitation (600 rpm).

The selected HS-SPME conditions were applied to the analyses of control and fortified breads. Figure 1a shows the relative percentage of area volatile compounds grouped by chemical classes extracted from control and fortified breads. Tool bars present mean values obtained for six bread samples (three from machine A and three from machine B). The bread volatile profile when expressed as relative percentage of area shows that in control breads, the major volatiles were alcohols, aldehydes, alkanes and aromatic compounds, whereas in fortified breads, alkanes and aromatics were the predominant chemical classes. However, as shown in Fig. 1b, the total area of volatiles in CB breads is significantly lower when compared with the total area of volatiles in FB ($P < 0.05$,

Table 4 Mean values (μg kg^{-1}) and standard deviation (SD) of the volatile compounds quantified

Compound	Ion *m/z*	Mean ± SD				ys*mach *P*-value	Two-way ANOVA	
		CBA	FBA	CBB	FBB		ys (*P*-value)	Mach (*P*-value)
3-Methylbutanal	44	12.1 ± 3.3	10.4 ± 1.0	9.7 ± 6.5	12.7 ± 1.7	ns	ns	ns
3-Methyl-1-butanol	55	563 ± 22	477 ± 125	813 ± 140	430 ± 14	ns	ns	ns
2-Phenylethanol	91	8.1 ± 0.2	8.8 ± 0.5	10.8 ± 0.5	13.1 ± 1.7	ns	ns	0.021
Hexanal	44	8.9 ± 0.1	13.6 ± 0.5	7.9 ± 1.6	13.9 ± 2.0	ns	0.014	ns
trans-2-Nonenal	43	0.04 ± n.d.	0.04 ± 0.01	0.04 ± 0.01	0.09 ± 0.02	ns	ns	ns

If a significant interaction effect was found, the two-way ANOVA is not performed on the main effects and one-way ANOVA is used instead, yeast amount and machine type separately, and hence, the interaction effect is then the most important effect.

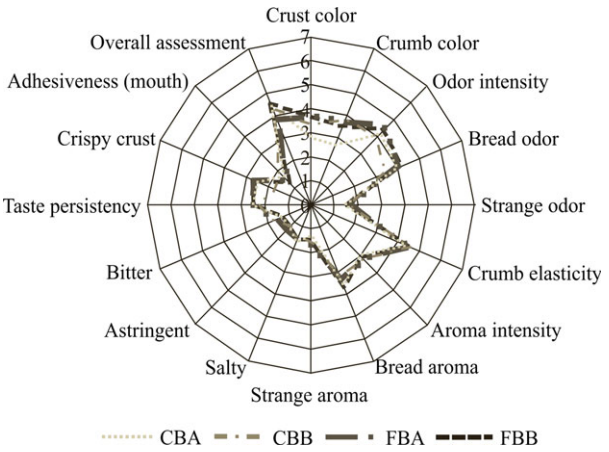

Figure 2 Spider chart representation of bread sensory characteristics.

t-test). Thus, a detailed analysis of volatile profile was performed using individual peak area.

Mean values of peak area of the volatile compounds identified in control (fifty-six volatiles) and fortified breads (fifty-seven volatiles) are shown in supplementary Table S1. Two-way ANOVA was performed using peak area of volatiles to evaluate the effect of β-glucan fortification by dry spent yeast addition and the effect of machine type. However, for 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-octen-3-ol, cis-4-decen-1-ol, 2-octanone, 2,6,8-trimethyl-4-nonanone, methyl acetate and styrene, the significant interaction between yeast fortification and machine type was the most important effect; thus, two-way ANOVA was not performed on the main effects. Concerning the effect of fortification, the peak area of hexanal and benzaldehyde increased in fortified breads from the two machines, although it was not statistically significant. Almost all alkanes as well as pentyl propanoate, ethyl hexanoate, ethyl decanoate, acetylfuran and all pyrazines increased significantly the peak area in fortified breads. Only 4-methyl-2-pentanone decreased significantly in fortified breads. In fortified breads, 2-ethyl-1-hexanol, 1-octanol, 3-pentanone and ethyl acetate were not detected although they were present in control breads, whereas 2-methyl-1-hepten-6-one was only detected in fortified breads. The effect of bread machine was less prominent, as significant differences were observed only in 2-phenylethanol, *trans*-2-nonenal, octanoic acid, ethyl octanoate, furfural, acetylfuran and 2,6-dimethylpyrazine.

Mean and standard deviation values of the quantification of key odour compounds from bread are presented in Table 4. For each compound, the ion with greatest abundance was used for quantification purposes. The most abundant key odour was 3-mehyl-1-butanol, and *trans*-2-nonenal was the key odour found in lower quantity. In general, the results from quantification by external standard method using an internal standard and expressed as μg kg^{-1} are in agreement with peak area quantification of volatile profile, although the quantification with calibration curves is more reliable as described by Petisca *et al.* (2014). Two-way ANOVA indicates that bread fortification increased hexanal content, while the type of machine influenced 2-phenylethanol content. Quantification of crumb volatiles in bread samples prepared with different yeast concentration and fermentation temperature (Birch *et al.*, 2013a,b) and also in bread prepared with different types of baker's yeast (Birch *et al.*, 2013a,b) was performed by dynamic headspace. The most abundant volatile was 3-mehyl-1-butanol, which is in agreement with our results; however, quantitative comparison of this and other key volatiles with literature is difficult because only crumb was analysed and not the whole bread and also due to the high variability of results, depending on bread making and experimental analytical conditions and time between bread preparation and volatile analyses.

Analyses of volatile profile of control and fortified breads and quantification of key odour compounds indicate that β-glucan fortification by dry spent yeast addition has an impact on volatile profile, although concerning key odours, only hexanal presented a significant increase in fortified bread. However, hexanal

is an aroma-active compound that is often characterised as off-flavour (Birch *et al*., 2013a,b).

Impact of β-glucan fortification by dry spent yeast addition on sensory characteristics of bread

Figure 2 summarises results from sensory analyses in a spider chart. The general profile of control and fortified breads from the two machines is similar. Two-way ANOVA was performed to evaluate the effect of β-glucan fortification by dry spent yeast addition and the effect of machine type. For crumb colour, bread odour, bread aroma and crispy crust, the significant interaction between yeast fortification and machine type was the most important effect; thus, two-way ANOVA was not performed on the main effects. CBA samples presented significantly lower crumb colour; CBB presented significantly lower bread odour and crispy crust. No significant differences were found on bread aroma. Main effects of β-glucan fortification indicate significant differences on crust colour and odour intensity, although no significant differences were observed in the other attributes, namely for overall assessment.

Conclusions

A serving of bread fortified with dry spent yeast increases β-glucan intake from 65 to 125 mg, a content within the guidelines suggested by EFSA. Although this fortification darkened the crumb, increased crumb and crust springiness and had impact on volatile profile, concerning key odours (3-methylbutanal, 3-methyl-1-butanol, 2-phenylethanol, hexanal and *trans* 2-nonenal), only hexanal presented a significant increase in fortified bread. Descriptive analyses performed by a trained panel corroborate differences on crust colour and odour intensity, although no significant differences were observed in the other attributes, namely for overall assessment. Additionally, the machine type (with 1 or 2 paddles) has a minor impact on bread characteristics. Thus, dry spent yeast can be used as ingredient in bread to increase β-glucan intake without compromising the sensory characteristics, contributing to valorisation of by-products from the brewing industry. Moreover, the brewing yeast is recognised as a good source of chromium and B vitamins; thus, further studies to quantify the increase of these bioactive compounds in fortified bread are pertinent.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Means values of peak area of volatiles in control and fortified breads. The results of two-way ANOVA are shown for the two factors, yeast amount (ys) and machine type (mach), and their possible interaction (ys*mach).