



Functionalisation of wheat and oat bran using single-strain fermentation and its impact on techno-functional and nutritional properties of biscuits

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Abstract

The adequate intake of dietary fibre is linked to several health benefits, for example, reducing the risk of non-communicable diseases, such as cardiovascular disease and diabetes. However, the population's intake of dietary fibre is below the dosage recommended by the World Health Organisation. The incorporation of fibre ingredients, such as bran, in cereal based products affects the techno-functional and sensory properties, resulting in inferior product quality. To compensate quality loss, wheat bran (WB) and oat bran (OB) were fermented using the lactic acid bacterium strain *Leuconostoc citreum* TR116 prior to the application in a biscuit system. Two types of fermentation, one without any addition of sugars (FB) and one with the supplementation of 5% fructose and 5% sucrose to trigger the production of mannitol (FB+), were conducted and sugar and acid profiles as well as pH and total titratable acids (TTA) were evaluated. Fermented WB showed a higher TTA (+58%) compared to fermented OB. Furthermore, FOB+ resulted in higher microbial cell count and higher residual sugars after 48 h of fermentation. The application of fermented brans in a biscuit system showed a decrease in dough stickiness (−41.7%) and an increase in dough hardness (+32%). The type of bran (WB and OB) as well as the type of fermentation (FB or FB+) influenced the results of biscuit dough and biscuit quality ($p < 0.005$). Fermentation increased biscuit spreading, influenced biscuit snap force, enhanced crunchiness and colour formation, and lowered the predicted glycaemic index. Fermented OB (FOB+) resulted in a sensory profile comparable to the control.

Keywords Fibre-enriched biscuits · Bran fermentation · In vitro starch digestibility · *Leuconostoc citreum* TR116

Introduction

Cereals are a staple food and provide about 50% of the total dietary fibre intake in the Western World [1]. Dietary fibre, such as cellulose, hemicellulose, pectin, hydrocolloids, resistant oligosaccharides, resistant starch and lignin [2], is resistant to enzymatic digestion in the human small intestine [3]. Even though the intake of dietary fibre has been linked to several beneficial effects on colonic function, blood

cholesterol and blood glucose levels, the population's average intake is below the recommended dosage of 25 g per day and 35 g per day for women and men, respectively [4]. Due to a high consumers' demand for fibre-rich food products, the fortification of processed foods with fibre ingredients is a topic of high interest to research and food industry. Cereal brans, such as wheat bran (WB) and oat bran (OB), are a side-stream of the refinement process and are usually used to produce wholemeal flour [1]. However, brans are highly nutritious and contain large amounts of macronutrients, such as dietary fibre and protein. WB, for example, includes 37–53% dietary fibre, of which 95% is insoluble fibre, mainly arabinoxylans, cellulose and lignin [5]. OB contains about 15–20% dietary fibre, of which 61% is soluble fibre including β -glucan (5–20%) [6]. Several studies revealed the impact of WB and/or OB on bread and pasta quality [7–10], and resulted in an inferior product quality compared to controls. The incorporation of bran in biscuits changes dough properties [11], restricts biscuit spreading

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and increases biscuit hardness [12]. It has to be noted that WB affects techno-functional and sensory characteristics to a higher extent than OB, leading to a lower maximum addition level of WB (20%) compared to OB (30%) in biscuits [13]. In addition, several studies revealed the impact of bran particle size on biscuits, and showed a more compact biscuit structure when fine particle sizes were applied, which led to an increased biscuit hardness [11].

Even though the effect of bran fortification in biscuits has been investigated in depth, studies about overcoming quality loss due to bran addition are scarce. Fermentation technology represents an efficient tool to ameliorate sensory properties and improve techno-functional characteristics. *Leuconostoc citreum* TR116 showed in several studies its ability to compensate quality losses in cereal based food products, such as sugar reduced burger buns, cakes and biscuits [14–16]. TR116 possesses the gene to synthesise the enzyme mannitol-dehydrogenase to convert fructose into mannitol [14, 17, 18]. Naturally produced mannitol together with organic acids, produced during fermentation, compensate techno-functional losses and increases flavour in biscuits caused by changes in product formulation [15].

This study reveals the functionalisation of wheat and oat bran by controlled single-strain fermentation using *Leuconostoc citreum* TR116. Changes in bran characteristics, such as sugar and acid profiles, pH, and total titratable acids, during fermentation, and the effect of the incorporation of fermented brans on biscuit dough and biscuit properties was investigated. Furthermore, the impact of fermented brans compared to unfermented brans on in vitro starch digestibility and sensory characteristics was evaluated.

Materials and methods

Raw materials

Bran fermentation was performed using two different types of bran, wheat bran (WB) (Odlums, Dublin, Ireland) and

oat bran (OB) (ABS FOOD, Peraga di Vigonza, Italy) originated from untreated oats, sterile tap water, fructose (Sigma Aldrich, Gillingham, UK) and sucrose (Sigma-Aldrich, Gillingham, UK).

For biscuit production, biscuits flour (BF) (Odlums, Dublin, Ireland) and wholemeal flour (WF) (Odlums, Dublin, Ireland) were used as controls. Fibre-enriched biscuits were prepared by replacing BF by unfermented or fermented bran. The composition of the raw ingredients (BF, WF, WB, OB) was provided by the suppliers and is listed in Table 1. Furthermore, salt (Glacia British Salt Limited, Middlewich, UK), sugar (Siúcra, Dublin, Ireland), sodium stearoyl lactylate (Danisco, Copenhagen, Denmark), shortening (Stork, London, UK), baking powder (Odlums, Dublin, Ireland) and tap water were used.

Bran fermentation

For the single-strain fermentation, LAB strain *Leuconostoc citreum* TR116, which was isolated from yellow pea sourdough, was chosen. The strain is part of the culture collection of the Department of Biological Sciences, Cork Institute of Technology and the inoculum and the cell harvest for the bran fermentation was conducted as reported previously by Sahin et al. (2019) [14].

Two different type of brans, WB and OB, were used as raw ingredients. The dough yield (DY) used for the fermentation of those two different brans had to be adjusted (data not shown) to ensure the dominance of TR116 and to depress the microbial contaminant commonly present in bran. Using a high DY increases the water activity of the dough and provides more free water for microbial growth. A DY of 300 in OB however caused mould growth throughout the sample, determined on agar plates. Hence, WB was fermented choosing a dough yield (DY) of 300, while the DY of OB fermentation was 250.

The fermentation of brans (FWB = fermented wheat bran; FOB = fermented oat bran) was conducted by mixing bran, sterile tap water and the starter culture TR116 with

Table 1 Compositional information of biscuit flour, wholemeal flour, wheat bran and oat bran

	Biscuit flour (BF)	Wholemeal flour (WF)	Wheat bran (WB)	Oat bran (OB)
Energy [kcal/100 g]	355	336	206	350
Protein [g/100 g]	9.8	10	14.1	18
Carbohydrates [g/100 g]	80.9	64	26.8	33
Of which sugars [g/100 g]	0.6	2.1	n.l	1.5
Fat [g/100 g]	1.4	2.2	5.5	9
Of which saturated [g/100 g]	0.4	0.3	n.l	1.5
Fibre [g/100 g]	4	9	36.4	25
Sodium [g/100 g]	0.002	0.003	n.l	0.05

The abbreviation ‘n.l.’ indicates that these values were not listed on the specification sheet

an inoculation level of 10^7 colony forming units (CFU) per gram dough. In addition, 10% of the bran was replaced by 5% sucrose and 5% fructose (FB +), to trigger the production of functional metabolites, such as mannitol and acetic acid, to improve techno-functional, nutritional and sensory properties of biscuits. The ingredients were mixed in sterile stomacher bags and homogenised using Stomacher 400 (Seward Ltd, Worthing, UK) at highest speed twice for 60 s. Samples for analysis were taken at time point 0 h and 48 h.

Characterisation of fermented brans

The pH, TTA and microbial cell count of all fermented brans were determined at time point 0 h and 48 h as described by Sahin et al. (2019) [14]. The analyses were performed in duplicate per bran batch. The extraction of sugars (glucose, fructose, sucrose/maltose), polyols (mannitol) and acids (lactic acid, acetic acid) from freeze-dried fermented brans was conducted as described by Sahin et al. (2019) [15].

Biscuit dough preparation

For the preparation of the biscuit dough, the procedure described by Sahin et al. (2019) [15] was followed and the formulations illustrated in Table 2 were prepared. As controls BF (C1) and WF (C2) were used. The fibre fortification was performed by replacing BF with WB or OB in concentrations needed to achieve 3 g of dietary fibre content in 100 g of biscuit. This would allow to claim the biscuits as ‘source of fibre’ according to European regulation [19].

Biscuit dough characterisation

Dough characteristics give a deeper insight into the interactions of the compounds in the complex matrix and explain differences in biscuit quality. Thus, dough hardness, dough stickiness and the rheological behaviour during oscillation were investigated.

Dough hardness and stickiness

Biscuit dough hardness and dough stickiness were determined using a Texture Profile Analyser (Stable Micro Systems, Godalming, UK), as previously reported by Sahin et al. (2019) [15].

Viscoelastic properties

Oscillation measurements using a rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany) were conducted to investigate changes in viscoelastic properties of the biscuit doughs. To evaluate the damping factor, a frequency sweep with a constant strain of 0.01% and a frequency range from 0.1 to 100 Hz was performed at a constant temperature of 20 °C. Each dough was analysed in a triplicate.

Biscuit baking procedure

The biscuits were prepared by firstly dough mixing and resting, as explained before, followed by dough sheeting, cutting

Table 2 Recipes of the control as well as the fibre-enriched biscuits including the level of replacement of biscuits flour by fibre ingredients

	Control biscuits		Addition of unfermented bran		Addition of fermented bran	
	Biscuit flour	Wholemeal flour	Wheat bran	Oat bran	FWB/FWB +	FOB/FOB +
Biscuit flour	100.00	–	92.00	88.00	92.00	88.00
Wholemeal flour	–	100.00	–	–	–	–
WB/FWB/FWB +	–	–	8.00	–	24.00	–
Of which solids	–	–	–	–	8.00	–
Of which water	–	–	–	–	16.00	–
OB/FOB/FOB +	–	–	–	12.00	–	30.00
Of which solids	–	–	–	–	–	12.00
Of which water	–	–	–	–	–	18.00
Sugar	40.44	40.44	40.44	40.44	40.44	40.44
Shortening	39.97	39.97	39.97	39.97	39.97	39.97
Salt	0.69	0.69	0.69	0.69	0.69	0.69
Sodium stearoyl lactylate	0.49	0.49	0.49	0.49	0.49	0.49
Baking powder	0.49	0.49	0.49	0.49	0.49	0.49
Water	20.04	20.04	20.04	20.04	4.04	2.04

WB, FWB and FWB + represent wheat bran, fermented wheat bran without sucrose and fructose addition and fermented wheat bran with the addition of sucrose and fructose, respectively. OB, FOB and FOB + are the abbreviations for oat bran, fermented oat bran without sugar addition and fermented oat bran with sugar addition. The amounts are given in % based on flour + bran

and baking as previously described by Sahin et al. (2019) [15].

Techno-functional properties and colour of biscuits

The incorporation of unfermented and fermented brans influences the properties of biscuits. Hence, techno-functional properties of biscuits, such as biscuit spreading during baking, snap force, colour, and water activity were investigated.

Biscuit spreading

Biscuit spreading was evaluated by measuring the biscuit diameter using a calliper and comparing it to the diameter of the cutter (70 mm). Ten biscuits of each batch were analysed.

Biscuit snap force

The biscuit snap force reveals the hardness of the biscuit while breaking it in half. The snap force was determined using a Texture Profile Analyser (Stable Micro Systems, Godalming, UK) equipped with a three-point bend rig. The settings used are reported by Sahin et al. (2019) [15]. Ten biscuits of each batch were measured after the cooling time. In addition, the biscuit snap force after 7 days was determined to evaluate the degree of softening during storage. Biscuits were stored in sealed plastic bags at room temperature.

Colour

Colour measurements were conducted using Colorimeter CR-400 (Konica Minolta, Osaka, Japan) and the differences in colour compared to the controls C1 and C2 were determined using the Scofield equation: $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$.

Water activity

Biscuits were first ground using a food processor (Kenwood Ltd, New Hampshire, UK) at speed 1 for 30 s. The water activity of 8 g of the ground biscuits was measured using a water activity meter (HygroLab, Rotronic, Bassersdorf, Switzerland).

Nutritional value of fibre-enriched biscuits

The replacement of flour by bran affects the final composition of the biscuits and thus can have an impact on the nutritional value. The total starch as well as the sugar, polyol and acid profiles of the biscuits was determined, and the total starch content was measured. Furthermore, an in vitro starch

digestibility was performed to evaluate the impact of fibre fortification on the predicted glycaemic index of the biscuits.

The sugar, polyol and acid profiles of the biscuits were determined by HPLC analysis using the same methodology as for the analysis of fermented brans as mentioned before.

Based on the method described by Hager et al. (2013) [20], the in vitro starch digestibility of biscuits was conducted with slight modifications as reported by Sahin et al. (2019) [15]. For the determination of the pGI, the concentration of total starch in the biscuits was required and, thus, determined using the total starch enzyme test kit K-TSTA-100A (Megazyme, Bray, Ireland).

Sensory profile of fibre-enriched biscuits

The sensory characteristics of fibre-enriched biscuits were determined by evaluation the intensity of defined descriptors. First, a sensory panel of 12 people (six females and six males, age: 24–32) was trained 6 h per week over 6 months prior the tasting. The descriptors for the sensory analysis involved taste (sweetness, sourness, bitterness), texture (adhesiveness, crunchiness) and flavour (cereal grain, butter).

To obtain an individual sensory profile of each sample, one sample at a time was presented to the panellists to avoid comparison. The intensity scale ranged from 0 (not intense at all) to 10 (very intense). The training of the panel as well as the sensory analysis of the samples was conducted in a sensory room with a temperature of 21 ± 1 °C. The sensory analysis was performed twice on two different days.

Sensory profile of fibre-enriched biscuits

All trials (fermentation, biscuit dough preparation/analysis, biscuit analysis) were conducted in a triplicate. The number of measurements for each test differed and is mentioned in the individual methods. Variance analysis (one-way ANOVA, $p \leq 0.05$, Tukey test) was performed using Minitab 17 and a correlation analysis was conducted using Microsoft Excel 2010. Furthermore, two-way ANOVA was used to investigate the influencing factor (type of bran or type of fermentation) on the dough and biscuit quality.

Results

Qualitative comparison of nutritional profile of raw ingredients and sugar profile of brans

The raw ingredients, in particular their composition, have a major impact on the metabolism of the LAB strain. In general, compared to the flours, both brans were higher in protein, fat and fibre and showed lower levels of carbohydrates

according to the suppliers' data. The data provided by the supplier are in accordance to quantities reported in literature [6, 21]. Comparing the brans with each other, OB showed a significantly higher amount of protein and fat, but a lower concentration of fibre. Table 3 shows the sugar profile of unfermented brans. Interestingly, OB only contained sucrose/maltose, while in WB, all analysed sugars were present.

Characterisation of fermented brans

The results of pH, TTA and microbial cell count of TR116 are illustrated in Table 3.

WB as well as OB showed the same pH before fermentation. After fermentation, fermented WB resulted in lower pH values compared to fermented OB. The supplementation of sugars showed a significant effect on the performance of TR116 in OB but did not impact WB fermentation. A significant lower pH in FOB+, compared to FOB occurred (FOB: 5.60 ± 0.29 ; FOB+: 4.90 ± 0.22).

The TTA of time point 0 h was significant lower in OB (2.28 ± 0.15 ml 0.1 N NaOH) compared to WB (3.45 ± 0.11 ml 0.1 N NaOH), and even after fermentation, WB showed the highest TTA values amongst all samples (FWB: 21.10 ± 0.45 ; FWB+: 23.14 ± 0.75). The increase in TTA of fermented OB was relatively low resulting in 5.92 ± 0.33 and 9.53 ± 0.36 ml 0.1 N NaOH for FOB and FOB+, respectively.

After fermentation, all brans showed an increased cell count by tenfold. Brans supplemented with sugars resulted

in a higher microbial cell counts than brans without sugar addition.

Unfermented WB contained in average 3.82 g/100 g sugars based on dry matter (boDM), including 2.16 ± 0.13 g/100 g sucrose/maltose, 0.75 ± 0.21 g/100 g glucose and 0.91 ± 0.27 g/100 g fructose. Fermentation of WB by *Leuconostoc citreum* TR116 revealed a decrease in all sugars, resulting in a final average sugar content of 2.01 g/100 g boDM of which more than 50% was sucrose/maltose (1.35 ± 0.18 g/100 g). Mannitol was produced during both fermentations FWB and FWB+, with FWB+ resulting in the highest overall mannitol concentration. During fermentation, TR116 produced more lactic acid than acetic acid, yet the acetic acid production increased, when sucrose and fructose were added. Thus, the lactic/acetic acid ratio decreased from 1.82 in FWB to 1.04 in FWB+ (= - 0.78).

OB as a raw ingredient contained in average 1.69 g/100 g boDM of sucrose/maltose; no other mono- or disaccharides were present. The total sugar content of FOB was 0.13 ± 0.03 g/100 g boDM consisting of only glucose. FOB+ resulted in a total sugar content of 8.43 g/100 g boDM, consisting of 5.20 g/100 g sucrose/maltose and 3.23 g/100 g fructose. Mannitol was detected in FOB as well as in FOB+, yet in significantly lower amounts compared to fermented WB. The presence of 5% sucrose and 5% fructose (FOB+) increased the production of mannitol by 15-fold. As observed in WB, the concentrations of lactic acid were higher than acetic acid in both fermentations. The incorporation of sucrose and fructose increased the production of acetic acid from 0.16 ± 0.02 g/100 g to 0.38 ± 0.02 g/100 g boDM, which caused a decrease in lactic/acetic acid ratio

Table 3 Sugar, mannitol, and acid profiles as well as pH, total titratable acids (TTA) and microbial cell count of wheat bran and oat bran before and after 48 h of controlled single-strain fermentation using *Leuconostoc citreum* TR116

	Unfermented raw ingredients		Fermented brans FB		Fermented brans FB+	
	Wheat bran	Oat bran	Wheat bran 48 h	Oat bran 48 h	Wheat bran 48 h	Oat bran 48 h
Sucrose/Maltose cDM [g/100 g]	2.16 ± 0.13 (b)	1.69 ± 0.08 (c)	1.35 ± 0.18 (d)	–	2.72 ± 0.54 (b)	5.20 ± 0.42 (a)
Glucose cDM [g/100 g]	0.75 ± 0.21 (a)	–	0.19 ± 0.03 (c)	0.13 ± 0.03 (c)	0.42 ± 0.05 (b)	–
Fructose cDM [g/100 g]	0.91 ± 0.27 (b)	–	0.47 ± 0.08 (c)	–	0.72 ± 0.06 (b)	3.23 ± 0.31 (a)
Total sugars (sum) [g/100 g]	3.82	1.69	2.01	0.13	3.86	8.43
Mannitol cDM [g/100 g]	<0.04	–	0.97 ± 0.10 (c)	0.10 ± 0.02 (d)	4.54 ± 0.31 (a)	1.51 ± 0.21 (b)
Lactic acid cDM [g/100 g]	–	–	1.31 ± 0.16 (a)	0.41 ± 0.01 (c)	1.01 ± 0.07 (a)	0.53 ± 0.02 (b)
Acetic acid cDM [g/100 g]	–	–	0.72 ± 0.20 (a)	0.16 ± 0.02 (c)	0.97 ± 0.07 (a)	0.38 ± 0.02 (b)
Total acids (sum) [g/100 g]	–	–	2.03	0.57	1.98	0.91
pH	6.70 ± 0.38 *(a)	6.78 ± 0.12 *(a)	4.37 ± 0.22 (c)	5.60 ± 0.29 (b)	4.35 ± 0.31 (c)	4.90 ± 0.22 (bc)
TTA [ml 0.1 N NaOH]	3.45 ± 0.11 *(d)	2.28 ± 0.15 *(e)	21.1 ± 0.45 (a)	5.92 ± 0.33 (c)	23.14 ± 0.75 (a)	9.53 ± 0.36 (b)
Microbial cell count TR116 [$\times 10^7$ CFU/g]	3.5 ± 0.4 *(e)	1.9 ± 0.5 *(f)	25.4 ± 3.2 (c)	12.3 ± 1.5 (d)	32.3 ± 6.4 (bc)	88.1 ± 9.0 (a)

FB represents the fermentation of brans without any addition of sugars; In FB+ 10% of the brans was replaced by 5% sucrose and 5% fructose. The values represent the average concentration based on dry matter (cDM) per 100 g bran \pm standard deviation. Values in the same row with the same lower-case letter showed no significant difference. *Represents values which were measured at time point 0 h of fermentation

from 2.56 in FOB to 1.39 in FOB+. A total acid amount of 0.57 g/100 g and 0.91 g/100 g was produced in FOB and FOB+, respectively.

Biscuit dough characterisation

Dough hardness

The substitution of BF with brans led to changes in biscuit dough hardness (Fig. 1). Among all samples, C1 showed the lowest dough hardness (2.25 ± 0.26 N), followed by the C2 (2.46 ± 0.25 N). The replacement of flour by bran resulted in an increase in dough hardness with OB representing the highest value (2.93 ± 0.24 N).

The incorporation of fermented bran into the biscuit dough system revealed a significant decrease in dough hardness. The type of fermentation, with or without sugar addition, did not affect the impact on biscuit dough hardness when WB was used. However, the hardness of biscuit dough including fermented OB was significantly influenced by the type of fermented OB used. Fermented OB without sugar addition showed no significant difference on dough hardness compared to unfermented OB. On the contrary, the addition of FOB+ to the biscuit dough system decreased the dough hardness significantly (2.58 ± 0.10 N). Two factor statistical analysis revealed that the type of bran influenced the dough hardness significantly ($p=0.0001$).

Dough stickiness

The results of dough stickiness are illustrated in Fig. 1. The highest dough stickiness was detected in C1 (0.36 ± 0.03 N). C2 resulted in a significant lower stickiness value (0.26 ± 0.03 N). The incorporation of bran, regardless in which form (unfermented or fermented) caused the same biscuit dough stickiness as C2. However, the average values revealed that OB decreased the dough stickiness to a higher extent than WB. The addition of FOB resulted in the lowest biscuit dough stickiness (0.21 ± 0.01 N). Statistical evaluation showed significant influence of type of bran ($p=0.000$) as well as type of fermentation ($p=0.002$).

Viscoelastic properties

Oscillation measurements are represented by the damping factor (Fig. 1). C1 resulted in a higher damping factor (0.41 ± 0.01) than C2 (0.37 ± 0.03). The substitution of flour by WB or OB did not change the proportions of viscous and elastic parts of the biscuit dough. Furthermore, fermentation of the brans also did not influence the damping factor significantly.

Techno-functional properties and colour of biscuits

Biscuit diameter

The biscuit diameter indicates the degree of spreading or shrinkage of the biscuit during baking and the results are illustrated in Table 4.

C1 resulted in the smallest diameter (68.6 ± 2.0 mm), while C2 showed the largest diameter (70.8 ± 2.0 mm). The incorporation of WB enhanced biscuits spreading during baking, whereas OB decreased the biscuit diameter slightly (69.5 ± 1.1).

The incorporation of FWB+ and FOB+ contributed to biscuit spreading resulting in biscuit diameters of 70.4 ± 1.3 mm and 70.3 ± 1.5 mm, respectively. The investigation of the influencing factors using two-way ANOVA revealed that the type of fermentation influenced the diameter significantly ($p=0.026$) and to a higher extent than the type of bran used ($p=0.035$).

Biscuit snap force

The snap force of biscuits represents the biscuit hardness and is shown in Table 4. C1 had a snap force of 34.57 ± 8.32 N, while C2 was the softest biscuit among all samples (12.39 ± 2.93 N).

The addition of fermented WB caused a softening of the biscuits compared to C1, in particular FWB+ (26.36 ± 5.48 N). On the contrary, the addition of fermented OB increased the hardness of the biscuits, with FOB+ showing the highest snap force among all samples

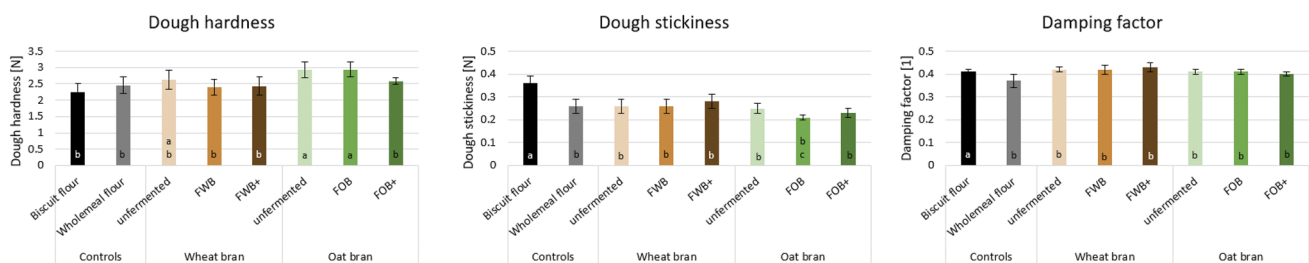


Fig. 1 Quality characteristics of biscuit doughs, including dough hardness [N], dough stickiness [N] and damping factor [I]. Biscuits including unfermented wheat bran or unfermented oat bran are presented as WB and OB, respectively

Table 4 Biscuit quality characteristics

	Control biscuits		Biscuits including wheat bran			Biscuit including oat bran		
	Biscuit flour	Wholemeal flour	WB	FWB	FWB +	OB	FOB	FOB +
Biscuit diameter [mm]	68.6 ± 2.0(c)	70.8 ± 2.0(a)	70.2 ± 1.80(ab)	69.9 ± 1.4(abc)	70.4 ± 1.3(ab)	69.5 ± 1.1(abc)	69.2 ± 2.2(bc)	70.3 ± 1.5(ab)
Biscuit snap force [N]	34.57 ± 8.32(b)	12.39 ± 2.93(d)	36.09 ± 6.56(b)	35.12 ± 4.54(b)	28.69 ± 3.71(c)	38.72 ± 6.22(ab)	41.96 ± 6.05(a)	35.19 ± 6.02(b)
Biscuit snap force day 7 [N]	18.15 ± 8.67(c)	15.14 ± 2.67(c)	27.33 ± 5.33(b)	27.40 ± 4.39(b)	26.36 ± 5.48(b)	30.67 ± 6.93(b)	30.45 ± 6.62(b)	38.82 ± 5.88(a)
ΔSnap force [N]	− 16.42	+ 2.75	− 8.76	− 7.72	− 2.33	− 8.05	− 11.51	+ 3.63
ΔE (biscuit flour)			7.05 ± 1.59(a)	7.62 ± 1.64(a)	6.83 ± 1.60(a)	4.59 ± 1.75(b)	2.59 ± 0.91(c)	4.31 ± 1.86(b)
ΔE (whole-meal flour)			14.75 ± 1.56(d)	13.87 ± 1.32(e)	14.58 ± 1.49(de)	21.63 ± 1.30(a)	18.85 ± 0.81(b)	18.05 ± 1.14(c)
Water activity	0.25 ± 0.05(d)	0.15 ± 0.02(e)	0.28 ± 0.04(cd)	0.33 ± 0.03(bc)	0.37 ± 0.03(b)	0.46 ± 0.03(a)	0.38 ± 0.05(ab)	0.45 ± 0.03(a)

WB and OB represent biscuits including unfermented wheat bran or unfermented oat bran, respectively. Biscuits with fermented wheat bran are presented as FWB (fermentation without sugar addition) and FWB + (fermentation with sugar addition), while the results of biscuits including fermented oat bran are illustrated as FOB (fermentation without sugar addition) and FOB + (fermentation with sugar addition). ΔSnap force represents the difference in hardness between biscuits analysed after baking and biscuits measured after seven days of storage. ΔE refers to the difference in colour considering L*, a* and b* values compared to the controls. The results are given as average values ± standard deviation. The same lower-case letter in one row indicates no significant differences

(41.96 ± 6.05 N). The addition of sucrose and fructose only influenced the snap force of biscuits with WB. Thus, FOB + resulted in the same biscuit hardness as C1 or biscuits with unfermented OB.

In addition, the snap force after 7 days was evaluated to investigate the degree of biscuit softening or hardening during storage. C1 showed significant softening during storage with a reduction in snap force by 16.42 N. C2 resulted in a slight increase in snap force (+ 2.75 N). The replacement of BF by unfermented bran caused softer biscuits after storage. However, the degree of softening is by 50% lower compared to C1. Fermentation of WB, in particular FWB +, counteracted this softening affect resulting in the lowest difference in snap force among all samples during storage. FOB + caused an increase in hardness by 3.63 N, whereas FOB caused a significant biscuit softening (− 11.51 N).

Colour

The changes in colour are visually noticeable in Fig. 2 and the results of ΔE are presented in Table 4. Biscuits including bran showed higher differences compared to C2 than compared to C1. However, biscuits with WB showed a more similar colour profile to C2 than biscuits with OB. The comparisons of the colour to C1 showed the changes due to bran incorporation since BF was the base in bran-enriched biscuits. Compared to C1, the incorporation of WB caused a higher difference in colour than the addition of OB.

Water activity

The water activity indicates the degree of free water in the biscuits and the results are illustrated in Table 4.

Both controls, C1 and C2, showed the lowest values, 0.25 ± 0.05 and 0.15 ± 0.02, respectively. The incorporation of unfermented brans led to an increase in water activity. The addition of WB resulted in a water activity, which was not significantly different from C1, while OB caused a significant increase in water activity (0.46 ± 0.03).

The fermentation of WB resulted in a further increase in free water in the system compared to biscuits with unfermented WB (FWB: 0.33 ± 0.03; FWB +: 0.37 ± 0.003), while the water activity of biscuits including unfermented or fermented OB did not differ significantly from each other. However, the addition of fermented OB led to higher water activity values compared to the controls.

Nutritional value of fibre-enriched biscuits

The concentrations of dietary fibre, protein and fat are based on calculation considering the composition of the raw ingredients. The results are presented in Table 5. Since those data are only predicted based on suppliers' information, the values give a qualitative information rather than a quantitative one.

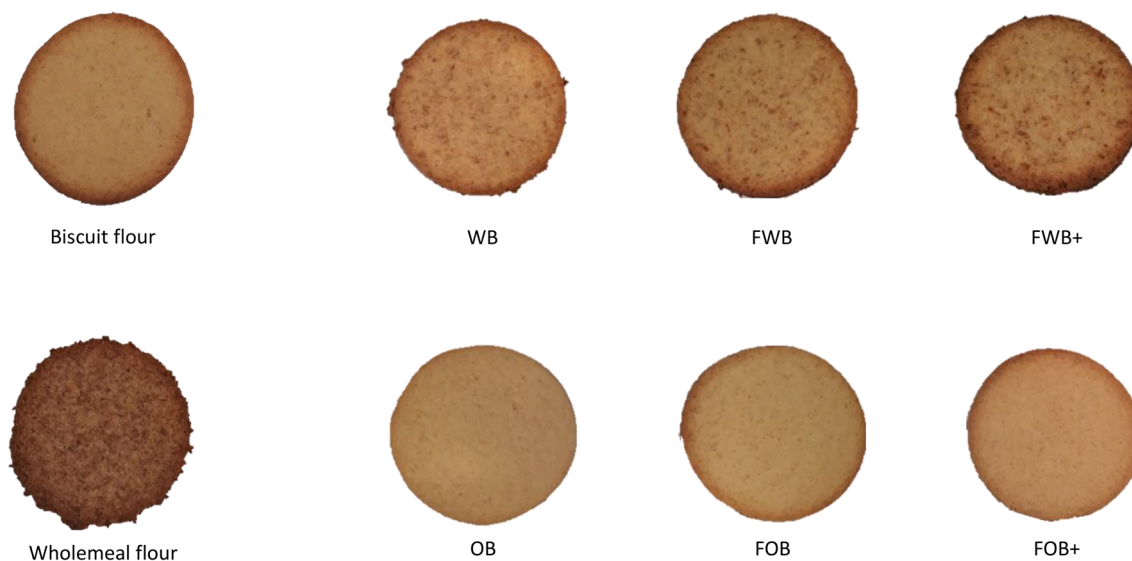


Fig. 2 Appearance of biscuit samples. Biscuit flour and wholemeal flour represent the controls. WB and OB show biscuits including unfermented wheat bran or oat bran, by u. FWB (fermented wheat bran) and FOB (fermented oat bran) present biscuits including fer-

mented brans without sugar addition at the beginning of fermentation, while biscuits with FWB+ (fermented wheat bran) and FOB+ (fermented oat bran) include brans which were fermented with the addition of sugars

Table 5 Composition of biscuits including controls (biscuit flour, wholemeal flour), biscuits including unfermented wheat bran (WB), wheat bran fermented without (FWB) and with addition of sugar

(FWB+), unfermented oat bran (OB) and oat bran fermented without (FOB) and with (FOB+) sugar addition

	Control biscuits		Biscuits including wheat bran			Biscuit including oat bran		
	Biscuit flour	Wholemeal flour	WB	FWB	FWB+	OB	FOB	FOB+
Fibre [g/100 g]*	1.98	4.46	3.26	3.26	3.13	3.23	3.23	3.10
Protein [g/100 g]*	4.85	4.95	5.02	5.02	5.00	5.43	5.43	5.39
Fat [g/100 g]*	15.52	15.92	15.69			15.98		
Total starch cDM [g/100 g]	39.41 ± 0.49(a)	34.49 ± 0.24(b)	39.36 ± 0.98(a)	39.53 ± 0.26(a)	39.72 ± 0.78(a)	39.03 ± 0.99(a)	39.95 ± 0.59(a)	40.45 ± 0.77(a)
Sucrose/Maltose cDM [g/100 g]	26.55 ± 1.45(a)	27.68 ± 1.24(a)	26.47 ± 1.91(a)	22.16 ± 2.06(b)	23.72 ± 0.96(ab)	24.36 ± 2.12(ab)	22.34 ± 2.81(b)	24.14 ± 2.05(ab)
Glucose cDM [g/100 g]	0.06 ± 0.01(d)	0.06 ± 0.03(d)	< 0.04	0.23 ± 0.02(c)	0.92 ± 0.18(a)	< 0.04	0.38 ± 0.08(b)	0.29 ± 0.06(bc)
Fructose cDM [g/100 g]	0.04 ± 0.01(d)	0.04 ± 0.02(d)	< 0.04	0.24 ± 0.02(c)	0.88 ± 0.17(a)	< 0.04	0.35 ± 0.07(b)	0.45 ± 0.07(b)
Mannitol cDM [g/100 g]	n.d.	< 0.04	< 0.04	0.06 ± 0.02(c)	0.20 ± 0.03(a)	< 0.04	< 0.04	0.12 ± 0.00(b)
Lactic acid cDM [g/100 g]	< 0.03	< 0.03	< 0.03	0.10 ± 0.01(a)	0.08 ± 0.01(a)	< 0.03	0.05 ± 0.00(b)	0.06 ± 0.00(b)
Acetic acid cDM [g/100 g]	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.00(a)	n.d.	< 0.02	0.02 ± 0.00(b)
Predicted glycaemic index (pGI)	100.0 ± 0.5(a)	104.4 ± 9.0(a)	97.8 ± 8.1(a)	96.6 ± 1.9(a)	93.8 ± 0.3(a)	87.9 ± 5.8(a)	91.7 ± 0.8(a)	92.2 ± 5.8(a)

Values are given in averages ± standard deviation. Values in the same row with the same lower-case letter showed no significant difference

Sugars, mannitol, and acid

The replacement of BF by bran, in particular fermented bran, changes the concentrations of metabolites, sugars, polyols and acids in biscuits, as illustrated in Table 5.

The sucrose/maltose concentrations showed the highest values in the control biscuits (C1: 26.55 ± 1.45 g/100 g boDM; C2: 27.68 ± 1.24 g/100 g boDM), followed by biscuits with unfermented brans. The lowest sucrose/maltose values were detected in biscuits with fermented brans FWB and FOB.

C1 and C2 included glucose in relatively low amounts (0.06 g/100 g boDM). The replacement of BF by brans resulted in glucose concentrations lower than 0.04 g/100 g, regardless the type of bran.

The addition of fermented WB showed higher concentrations for biscuits including FWB + than FWB. Furthermore, in biscuits with fermented OB, a higher concentration in glucose was detected compared to the biscuits with unfermented OB. The type of fermentation did not influence the glucose level significantly.

Both control biscuits showed low fructose concentrations. The replacement of BF by unfermented brans resulted in fructose levels below 0.04 g/100 g boDM. An increase in fructose resulted in 0.24 ± 0.02 g/100 g in biscuits with FWB and 0.88 ± 0.17 g/100 g in biscuits with FWB +, which had the highest overall fructose concentration. In biscuits including fermented OB, the type of fermentation did not influence the final fructose concentration, resulting in 0.35 ± 0.07 g/100 g (FOB) and 0.45 ± 0.07 g/100 g (FOB +) fructose.

Mannitol was detected in all biscuit samples except in C1. However, the concentrations of mannitol detected in C2, in biscuits with unfermented brans as well as in biscuits with

FOB were below the quantification limit of 0.04 g/100 g boDM. The highest level of mannitol was determined in biscuits including FWB +, followed by FOB +.

Lactic acid was detected in all biscuit samples. Yet, the concentration of lactic acid could only be quantified in biscuits including fermented brans. Fermented WB caused a higher lactic acid concentration compared to fermented OB.

Acetic acid was only determined in biscuits with fermented bran which was spiked with sucrose and fructose before fermentation (FWB + and FOB +), while biscuits with FWB + (0.04 ± 0.00 g/100 g) were higher in acetic acid compared to biscuits with FOB + (0.02 ± 0.00 g/100 g).

Total starch and predicted glycaemic index (pGI)

Total starch concentration as well as pGI values is presented in Table 5.

All biscuits with BF as a base did not differ significantly in starch concentration. C2 showed the lowest total starch content (34.49 ± 0.24 g/100 g).

The substitution of BF by brans led to a lower average pGI-value, with unfermented OB showing the lowest value (87.9 ± 5.8), a reduction by 12.1 compared to C1. The fermentation of WB caused a decrease in average pGI-value in biscuits compared to unfermented WB. On the contrary, fermentation of OB led to an increase in average pGI values compared to unfermented OB.

Sensory profile of fibre-enriched biscuits

Figure 3 demonstrates the individual sensory profiling of all biscuit samples.

C1 showed pronounced intensity in sweet taste, hardness, and adhesiveness, as well as in buttery aroma, while bitter

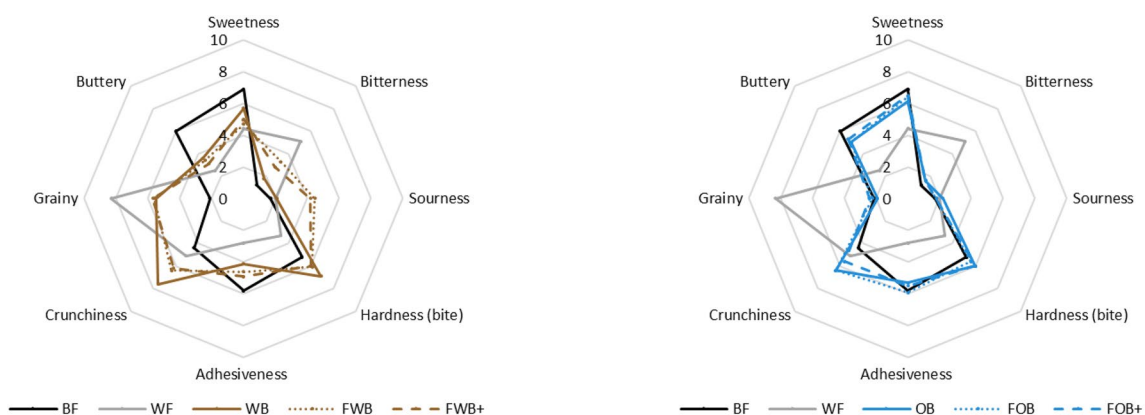


Fig. 3 Sensory profile of biscuits including unfermented wheat bran (WB) and oat bran (OB) in comparison to biscuit control (BF) and wholemeal flour control (WF). Biscuit including fermented brans are labelled with FWB (fermented wheat bran without sugar addition)/FOB (fermented oat bran without sugar addition) and

FWB + (fermented wheat bran with sugar addition)/FOB + (fermented oat bran with sugar addition). The sensory profiles show intensity of each descriptor from a scale from 0 (not detected) to 10 (highest intensity). The intensity values are average values with a confidential interval of ≤ 0.97 ($\alpha = 0.05$; $n = 10$)

and sour tastes as well as grain-like aroma were significantly low. C2 was evaluated as lower in sweetness, hardness and buttery aroma compared to C1, but showed a higher intensity in bitterness, crunchiness, and grain-like aroma.

The substitution of BF by WB led to a significant change in the overall sensory profile. Unfermented WB resulted in intensities between C1 and C2 for most of the attributes. Only crunchiness and hardness increased compared to both controls. The incorporation of fermented WB increased bitterness and sourness, regardless which type of fermentation was applied.

The sensory profile of biscuits with OB showed the same pattern as C1 with a slight increase in crunchiness. Fermentation of OB did not influence the sensory profile of the biscuits, especially sourness in these biscuits was not perceived by the trained sensory panel.

Discussion

Fermented wheat and oat brans were characterised by analysing pH, TTA, microbial cell count as well as changes in metabolites, such as sugars, polyols, lactic acid, and acetic acid. During the fermentation, the pH of the medium decreased steadily due to the production of acids as metabolites. The TTA value strongly correlated with the production of acetic acid ($r=0.98$; $p=0.02$). During fermentation, TR116 produced lactic acid and acetic acid. Lactic acid possesses buffering potential in solutions which pH ranges from 2.86 to 4.86, while the buffering capacity of acetic acid occurs in pH values between 3.75 and 5.75 [22]. The higher amount of acetic acid produced in WB is due to the presence of fructose in the raw ingredient. Furthermore, the TTA is influenced by the buffering capacity of the system, which increases with increasing amount of minerals [23]. WB contains a significantly higher amount of minerals compared to OB [6]. Several minerals are positively charged ions, which can bond to the hydroxy group of NaOH added during titration. This results in remaining free acids in solution which requires higher amounts of NaOH to achieve an increase in pH, reflected by the higher TTA value.

Leuconostoc citreum TR116 is a heterofermentative lactic acid bacteria strain, which produces acetic acid, while fructose is enzymatically reduced to mannitol [14, 17]. The link between acetic acid and mannitol production is supported by a positive correlation between mannitol and acetic acid concentrations ($r=0.83$; $p=0.17$). Additionally, the production of acetic acid is linked to the generation of an extra ATP [24], providing the LAB with extra energy, which contributed to higher microbial cell count in brans supplemented with fructose. The sum of fructose and mannitol in FWB is higher than in unfermented WB. While oat contains lower levels of fructans (0.6–1.0 g/100 g), WB is naturally higher

in fructooligosaccharides (1.5–2.0 g/100 g) [6], which can be cleaved during fermentation resulting in single fructose [25]. This causes an increase in total fructose and mannitol concentration in fermented WB.

The difference in microbial growth between FWB and FOB occurred due to the lower amounts of fermentable sugars in OB compared to WB, which also caused a lower yield in metabolite.

Although higher amounts of metabolites, such as acids, were detected in FWB+, a higher microbial cell count was determined in FOB+ after fermentation. There are two potential explanations for this finding. First, the microbial cell count was not monitored over time, only determined at time point 0 h and time point 48 h. Hence, information about at which stage of microbial growth TR116 is in both substrates after 48 h is missing. Putatively, in WB, TR116 is in the death phase, while OBTR116 is in the stationary phase. This hypothesis is supported by the significant different buffering capacities of the brans with WB having a higher buffering capacity than OB, as previously mentioned. The fermentation of WB resulted in a higher production of metabolites, such as acids, which, most likely, led to a quicker drop in pH and thus a sooner occurrence of stress for the strain compared to oat bran fermentation. Hence, TR116 putatively showed an extended log-phase in OB fermentation with a higher cell count after 48 h compared to fermented WB. Second, in OB, 61% of the dietary fibres are soluble, mainly β -glucan [6]. The soluble fibre in combination with the added sugars in FOB+ increased the osmotic pressure in the system. Consequently, TR116 reduced its metabolic activity [26]. Furthermore, the lower dough yield applied in OB fermentation most likely contributed to an increased osmotic stress for the strain due to dehydration. The production of mannitol was maintained since mannitol counteracts the osmotic stress in the cells [26, 27].

The addition of fibre-rich ingredients, or the use of WF instead of BF, increases the water absorption capacity of the system, and, thus, decreases the stickiness of the dough due to less free water in the system [11, 28]. Soluble fibre has a higher water absorption capacity than insoluble fibre [29], and thus influences dough stickiness to a higher extend. Biscuit doughs including OB showed a higher dough hardness than WB doughs due to higher amounts of soluble fibre in OB [30]. Furthermore, the additional level of OB to the biscuit system was higher than of WB, which enhances the water absorption capacity of the dough system.

Fermentation of brans decreased the dough hardness most likely due to acidification of the dough. A decrease in pH causes unfolding of proteins and cleaves amorphous regions of the starch. This results in a looser starch and protein network and, consequently, in a softer dough [31, 32]. As mentioned before, OB contains a high amount of soluble fibre. During fermentation, soluble fibre, such as β -glucan

in oats, can be enzymatically cleaved creating shorter chain polysaccharides and increase the number of water binding sites [33]. This, in turn, increases the viscosity of the system [3, 34] and causes a lower dough stickiness. Furthermore, the increase in viscosity could enhanced moisture retention during baking and, hence, biscuits with OB showed a higher water activity compared to all other biscuits. The decrease in dough hardness favoured biscuit spreading and caused a lower biscuit snap force. In addition, the changes in protein and starch structure due to acidification putatively enhanced the entrapment of free water in the system and led to an increase in water activity of the final biscuits including fermented bran.

Biscuit softening/hardening over time is influenced by the composition and the effect of the components on starch retrogradation. A reduced softening effect was determined in biscuits which showed higher amounts of monosaccharides. These recrystallize on the biscuit surface after baking and prevent from moisture absorption during storage [35, 36]. Especially biscuits including fermented brans showed generally a lower biscuit softening over time. Acidification accelerates starch retrogradation which counteracts the softening due to moisture absorption over time [32].

The changes in biscuit colour due to the addition of bran are first influenced by the colour of the bran. WB has a dark brown colour, while OB has a light creamy colour. Compared to C2, the addition of WB led to smaller changes in colour than OB. Furthermore, colour changes occur due to changes in the presence of Maillard reactants, such as reducing sugars, which were higher in fermented brans with fructose and sucrose addition resulted in higher ΔE -value.

The degree of starch digestion is influenced by the composition of the food products, especially the amounts of carbohydrates, fat, and protein, but also by the type of fibre and the production process, such as the implementation of fermentation. Generally, the pGI-lowering effect of OB was higher than WB due to the presence of higher amounts of soluble fibre, which are known to have a GI lowering effect by increasing the viscosity and, thus, impede enzymatic starch hydrolysis [34]. Furthermore, since the carbohydrates and fat of all biscuits are not significantly different, the protein content played a major role in reducing the pGI. During the biscuit production process, proteins partially cover the starch granules and prevent them from enzymatic degradation during digestion [37]. Biscuits with OB showed the highest protein content and the lowest pGI value. During fermentation, the decrease in pH initiates the unfolding of proteins which enhances their flexibility and, in turn, their ability to cover starch granules [31, 37].

Sensory analysis showed different sensory profiles comparing C1 and C2. The increased bitterness and grainy flavour in C2 are due to the presence of phenolic compounds and alkylresorcinols [38], while the crunchiness

was enhanced by the higher extend of biscuit spreading resulting in thinner biscuits. The replacement of BF by WB increased the crunchiness due to the incorporation of insoluble fibre and the grainy flavour due to higher amounts of phenolics and alkylresorcinols. Fermentation of WB increased sourness due to the incorporation of acids produced during fermentation, and enhanced the bitterness, which is known to be intensified by acidity [38]. Due to a lower TTA of the fermented OB compared to fermented WB, the sensory panel did not perceive sourness in those biscuits. Furthermore, the increased biscuit spreading resulted in a higher crunchiness.

Conclusion

The incorporation of brans or fibre ingredients in biscuits is extensively studied. However, research about compensating quality loss caused by bran fortification in biscuits is scarce. The application of bran fermented by *Leuconostoc citreum* TR116 improved biscuit dough quality, such as the reduction in dough stickiness and compensated biscuit quality loss due to bran addition. In addition, single-strain fermentation with TR116 led to improved biscuit spreading and colour formation during baking. Furthermore, fermentation influenced the sensory characteristics and increased crunchiness, for example. The fermentation medium, with or without addition of sucrose and fructose, highly affected biscuit softening during storage as well as sensory properties. Thus, lactic acid bacterial fermentation represents a useful tool to accommodate bran fortification in biscuits to increase the nutritional value and, simultaneously, improve techno-functional and sensory characteristics. Furthermore, the nutritional value of the biscuits increased reflected by the reduced pGI-value.

Another aspect to mention is the adjustment of the DY. A DY of 300 in OB fermentation resulted in the growth of mould, which could be avoided by reducing the DY to 250. Bran is known to be a reservoir of ubiquitous and/or potential spoilage microorganisms. Thus, the selection of competitive LAB starter culture and the adjustment of the DY significantly influence the possibility to (bio)valorise such functional side streams widening their potential application in different food systems.

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Declarations

Conflict of interest There are no conflicts to declare.

Compliance with ethics requirements All sensory trials involving human participants were performed in compliance with the relevant national laws in Ireland and the institutional guidelines of University College Cork.

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