Mineral bio-accessibility and intrinsic saccharides in breakfast flakes manufactured from sprouted wheat

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ABSTRACT

The objective was to produce breakfast flakes by extrusion using whole meal from sprouted wheat to increase mineral bio-accessibility and provide intrinsic sweetness without compromising their textural properties. Resting the ingredient mixture containing whole meal from (sprouted) wheat at 50 °C and pH 3.8 for 1 h prior to flake production allowed *in situ* endogenous phytase action. This resulted in end-products that had higher bio-accessibilities of Fe (29–38%) and of Zn (17–32%) than corresponding values for control flakes (8% and 8%, respectively). Further, the sweetness of breakfast flakes can be enhanced as sprouted grains are a source of intrinsic saccharides released by starch degrading enzymes. Substituting control wheat by sprouted wheat in the breakfast flake recipe led to darker flakes with increased density corrected hardness. The latter was due to lower bulk densities and higher starch crystallinity than observed for control flakes. Nevertheless, the flakes still had 70–80% of their initial hardness after soaking for 30 s in semi-skimmed milk. The here developed wheat flakes meet the growing consumer interest in healthy and tasty foods. Further, the presented technology lowers the need for extrinsic micronutrient fortification by making full use of the nutritional potential of wheat grains.

Abbreviations used

$F_{\max}$
maximum force
ICP-MS
inductively coupled plasma mass spectrometry
 N_{sr}
spatial frequency of ruptures
rpm
rotations per minute

1. Introduction

Extrusion cooked breakfast flakes are popular with the consumer because of their texture and taste, shelf life and ease of in home preparation (Brennan, Derbyshire, Tiwari, & Brennan, 2013).

Extruders generally consist of a pre-conditioning system, a feeding system, a screw, a barrel and a die with a cutter (Navale, Swami, & Thakor, 2015). The product shape and quality depend on the ingredi-

ents, their moisture content, the barrel temperature, the speed and configuration of the screw(s) and the type of extruder (*i.e.* single-screw versus twin-screw) (Brennan et al., 2013). During extrusion, the rotating screw(s) propel(s) the cereal material forward at elevated pressure, shear and temperature. At the end of the screw the material is forced through the die. The sudden pressure drop at the interface between die and atmosphere results in evaporation of water and product expansion to a degree which depends on the rheological properties of the material, its composition and moisture content, its temperature and the geometry of the die. The extrudate is usually cut by a rotating knife on the outer side of the die (Brennan et al., 2013; Brennan, Merts, Monro, Woolnough, & Brennan, 2008; Le Corre, 2006; Sacchetti, Pittia, & Pinnavaia, 2005). For breakfast flake production, the extruded pellets are then flaked between two steel rollers and the resulting flakes are toasted at typically 274–329 °C for a short time (*e.g.* 90 s) to reduce their moisture content (Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006). This heat treatment leads to their specific crisp texture, flavour and colour attributes (Le Corre, 2006).

Wheat (*Triticum aestivum* L.) white flour is a common raw material for breakfast flakes. As it contains less dietary fibre and important nutrients such as minerals and vitamins than wheat whole meal, breakfast

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flakes are often fortified with vitamins and minerals (Woods & Walker, 2007).

Using whole meal rather than refined white flour in the extrusion process would lead to breakfast flakes with better nutritional profiles on account of their higher dietary fibre, mineral and vitamin contents. Regrettably, the bio-accessibility of iron (Fe) and zinc (Zn), *i.e.* the amount of these minerals released from the food matrix upon digestion, is only 3–5% as phytic acid, the main storage form of phosphorus, chelates them (De Brier et al., 2016; Lemmens et al., 2018). A further concern is that commercial breakfast flakes often contain high contents of added sugar (Woods & Walker, 2007) and as a result, on a serving portion weight basis, even contain less minerals and vitamins than if less or no sugar is added.

To overcome the above hurdles, it is useful to sprout cereal grains. Indeed, during sprouting, hydrolytic enzymes are activated or *de novo* synthesised to fuel seedling growth (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). Amylase action leads to (partial) hydrolysis of starch for the mobilisation of sugars to the growing embryo and therefore increases the intrinsic saccharide content in cereal grains (Duke, 2009). Further, the increase in endogenous phytase activity as a result of sprouting directly leads to phytate hydrolysis and thus to release of bound minerals and improved mineral bio-accessibility (Greiner, Jany, & Alminger, 2000; Lemmens, De Brier, Goos, Smolders, & Delcour, 2019).

Extruded products have been produced from recipes containing whole meal (30–100%) from sprouted oats (Kince et al., 2017; Kruma et al., 2018), triticale (Kince et al., 2017; Kruma et al., 2018), rye (Heiniö et al., 2003; Kince et al., 2017; Kruma et al., 2018), wheat (Kince et al., 2017; Kruma et al., 2018; Singkhornart, Gu, & Ryu, 2013) and barley (Kince et al., 2017; Kruma et al., 2018; Singkhornart et al., 2013). In the study by Heiniö et al. (2003, pp. 533–545), a trained panel judged sprouted and extruded rye to have a grainy and fresh flavour but hard texture. Singkhornart et al. (2013) found that extruding sprouted wheat decreases its phytic acid content.

To the best of our knowledge, studies on the impact of using whole meal from sprouted wheat in a breakfast flake making process on intrinsic saccharide formation, on mineral bio-accessibility and on texture properties of flakes have not been performed. Against this background, the aim of this study was to manufacture breakfast flakes with high levels of bio-accessible minerals and sweet intrinsic saccharides by using sprouted whole wheat grains as ingredients and allowing *in situ* enzyme action during the production process. We hypothesised that rationally modifying the breakfast flake making process would allow optimal *in situ* endogenous phytase and amylase action and, hence, the release of minerals from its chelates and saccharides from starch, respectively. This can then lead to breakfast flakes with an improved nutritive value, a unique flavour profile and a cleaner label, since less or no mineral fortification and sugar addition is needed.

2. Materials and methods

2.1. Materials

All chemicals and reagents were of at least analytical grade and purchased from Sigma-Aldrich (Bornem, Belgium) unless otherwise specified. Winter wheat [Cellulose, moisture and protein (N x 5.7) content 12.6 g/100 g, and 9.7 g/100 g of dry matter (dm), respectively] was supplied by Limagrain (Avelgem, Belgium). A reference wheat (NIST1567) flour sample with certified element composition was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Salt, sucrose and glucose were food grade.

Kellogg's All-Bran flakes (made from 66 g/100 g whole wheat meal, 21 g/100 g wheat bran and 6 g/100 g oat meal and fortified with vitamins and Fe) were purchased from a local supermarket and used for comparative reasons. Ultra-high temperature treated semi-skimmed milk was also purchased from a local supermarket and stored and used at 23 ± 1 °C.

2.2. Steeping and sprouting of wheat grains

To study their functionality in breakfast flake making, control wheat grains were steeped and sprouted at least in triplicate in a pilot-scale micro malting system (Joe White Malting Systems, Perth, Australia) at 15 °C. The steeping in excess water consisted of three successive wet stages (7, 7 and 3 h at 15 °C) alternated by two intermediate 6 h air rest stages at 15 °C. The imbibed grains were then sprouted for 48 h at 15 °C. The processed wheat grains were flash-frozen using liquid N₂ and freeze-dried (to a moisture content of 5.0 g/100 g). These sprouted wheat grains and control (*i.e.* unsprouted) wheat grains were then milled with a FOSS Tecator (Hillerød, Denmark) cyclotec 1093 sample mill into whole meal (<500 µm) prior to breakfast flake production. In a next step, control flakes and breakfast flakes derived from sprouted wheat were produced in triplicate, both for the standard and modified process (*cfr.* 2.3). In addition, whole meal blends containing 75 g/100 g dm control wheat and 25 g/100 g dm sprouted wheat were used for breakfast flake making in duplicate with both the standard and the modified process (*cfr.* 2.3).

2.3. Production of breakfast flakes

Standard breakfast flakes were made on pilot scale at the Institut für Getreideverarbeitung (IGV, Potsdam, Germany) essentially as in Joye, Lamberts, Brijs, and Delcour (2011) and De Brier et al. (2015) from a recipe consisting of 358 g dm wheat whole meal, 30.0 g sucrose, 10.0 g glucose and 2.0 g salt. The sucrose, glucose and salt were dissolved in the amount of water (160 ml) needed to obtain 30 g/100 g moisture in the ingredient bill. Next, all ingredients were blended for 120 s in a Stephan UMS 5 electronic mixer (Hameln, Germany), mixed by hand and then mixed again for 30 s to ensure proper homogenisation. After a 30 min rest at room temperature, the mixture was extruded with a Brabender (Duisburg, Germany) single screw extruder [65–100 °C, 1–4 bar, screw speed 100 rotations per min (rpm), nozzle diameter 6 mm] to form pellets with a moisture level of 25.0–28.0 g/100 g (Sartorius Moisture Analyser MA30, Göttingen, Germany). These were then flaked at room temperature with a roller mill (Shule, Hamburg, Germany) at a gap setting of 0.25 mm and air-dried overnight. When needed, the resultant flakes were conditioned in a climate chamber (HC0057, Heraeus Vötsch, Hanau, Germany) at 30 °C and 80% relative humidity for 4 h to a moisture content of 12.0–15.0 g/100 g. Finally, they were toasted in a drum roaster (Probat-Werke von Gimborn Maschinenfabrik, Emmerich am Rhein, Germany) for 3 min at 248 °C resulting in a moisture level of 3.0–5.0 g/100 g.

Samples were withdrawn immediately after mixing, resting, extrusion, flaking and roasting. All samples but the roasted flakes were stored at –20 °C and freeze-dried. After freeze-drying, these samples and part of the roasted flakes were ground with an IKA-mill (Staufen, Germany).

For the modified production of breakfast flakes, acid incubation was executed by adding 160 ml 100 mmol/l sodium acetate buffer (pH 3.8) to the ingredients instead of water (160 ml) and resting the ingredient mixture prior to extrusion at 50 °C for 60 min instead of at room temperature for 30 min. Samples were withdrawn, stored, freeze-dried and ground as described above.

2.4. Analysis of breakfast flakes

Moisture contents of ground flakes were determined according the AACC method 44–15.02, in which samples (2.0 g) are dried for 3 h at 130 °C.

The CIELAB colour space of ground flakes was determined in fivefold with a colorimeter (ColorFlex EZ Spectrophotometer, Hunterlab, Murnau, Germany). This model expresses colour as three values: L* for the lightness from black (0) to white (100), a* from green (–) to red (+) and b* from blue (–) to yellow (+) (Ibraheem, Hasan, Khan, & Mishra, 2012).

The bulk density (g/l) of breakfast flakes relates to their degree of expansion, their stacking and thus to their sizes and shapes (De Brier et al., 2015). It was calculated from the average volume of three samples of 100 g of flakes each brought in a 1.0 l graduated cylinder (7.0 cm diameter). Flakes of intermediate bulk density values are desired by the consumer (Oliveira, Schmieles, & Steel, 2017; Wojtowicz et al., 2015).

The length and thickness of flakes was measured with a digital caliper. We here report the averages obtained for 20 flakes.

The texture of flakes was analysed using an Instron 5943 (Norwood, MA, USA) testing machine equipped with a 1 kN load cell as in De Brier et al. (2015). Tests were performed in sixfold in bulk (15.0 g) with a five-blade Kramer shear cell. The blades of the cell forced the breakfast flakes through the open spaces at the bottom of the cell. Samples were compressed at 120 mm/min at room temperature. The maximum force (F_{max} , [N]) was taken as a measure for the hardness of the sample. The spatial frequency of ruptures (N_{sr} , [mm^{–1}]) was calculated from the force-displacement curves using the following equation (Agbisit, Alavi, Cheng, Herald, & Trater, 2007):

$$N_{sr} = \frac{n}{d}$$

with n the number of peaks and d the travel distance of the probe.

A most important quality parameter of breakfast flakes is how well their texture withstands soaking in milk (De Brier et al., 2015). Here, 15.0 g of breakfast flakes were soaked in triplicate during 30 s in 300 ml semi-skimmed milk in a tea sieve at 23 ± 1 °C under continuous stirring (200 rpm) basically as in Sacchetti, Pittia, Biserni, Pinnavaia, and Rosa (2003) and De Brier et al. (2015). The soaked flakes were drained for 30 s to remove excess milk from the surface and texture analysis was performed as described above.

High Performance Anion Exchange Chromatography (HPAEC) with Pulsed Amperometric Detection (PAD) was performed in triplicate with a Dionex ICS5000 system (Dionex, Sunnyvale, CA, USA) to quantify the soluble saccharides formed during (modified) breakfast flake production. Extracts were obtained by adding 10.0 ml deionised water containing 2.0 mg rhamnose (internal standard) to 80–100 mg accurately weighed sample, extracting (30 min, 150 rpm, 7 °C) and centrifuging (10 min, 1500 g, 7 °C). An aliquot (50 µl) of supernatant was then added to 950 µl deionised water and after filtration (0.22 µm), a subsample (12.5 µL) was injected on Carbpac PA-100 guard and PA-100 anion exchange (250 × 4 mm) columns. The mobile phase (1.0 ml/min) during equilibration and the first 5 min of the run was 100 mmol/l NaOH. The sodium acetate concentration in 100 mmol/l NaOH was then increased by 3.6 mmol/l/min over 25 min. Glucose, fructose, sucrose, maltose and maltotriose standards were used to identify and quantify the saccharides (g/100 g of dm). The total soluble saccharide contents (g/100 g of dm) in the different samples were here calculated as the sum of the measured glucose, fructose, sucrose, maltose and maltotriose contents. Based on the obtained soluble saccharide contents of the samples, their relative sweetness values were calculated using the

following equation (Lavic, 2011):

Relative sweetness =

$$\frac{(\text{glucose content}) \cdot 74 + (\text{fructose content}) \cdot 110 + (\text{sucrose content}) \cdot 100 + (\text{maltose content}) \cdot 50 + (\text{maltotriose content}) \cdot 30}{100}$$

with 74, 110, 50 and 30 the relative sweetness factors of glucose, fructose, maltose and maltotriose, respectively, compared to that of sucrose which has a relative sweetness value of 100.

Phytate contents in wheat flakes were determined at least in triplicate after acid extraction of myo-inositol phosphates (K-Phyt assay kit, Megazyme, Bray, Ireland) as described in Lemmens et al. (2018). The concentration of free phosphates in the extracts was subtracted from the phosphate concentration after subsequent breakdown of myo-inositol phosphates by phytase and alkaline phosphatase. Phytate contents were obtained by dividing bound phosphorus contents by 0.282.

2.5. Iron and zinc bio-accessibility

An in vitro digestion procedure was carried out essentially as in Minekus et al. (2014) with slight modifications to make it suited for evaluating mineral bio-accessibility (Lemmens, De Brier et al., 2019; Lemmens et al., 2018). This procedure simulates the digestion process in mouth, stomach and small intestine. An aliquot (3.0 g dm) of ground breakfast flakes suspended in triplicate in 50.0 ml deionised water (pH 7.2) was incubated for 5 min at 37 °C under magnetic stirring (250 rpm). After adjusting the pH to 2.0 with 150 mmol/l HCl and adding 2.0 ml pepsin solution (Sigma P-7012, 2.0 mg/ml), samples were incubated for 120 min at 37 °C under magnetic stirring (250 rpm). Next, 13.4 ml pancreatin (Sigma P-3292) solution (5.0 mg/ml in 150 mmol/l sodium bicarbonate) was added and the pH was adjusted to 6.8 with 1.0 mol/l NaOH. The mixture was then incubated for 3 h at 37 °C under magnetic stirring (250 rpm). Finally, the suspensions were centrifuged (15 min, 9800 g, room temperature) and the obtained supernatants (digested fractions) and residues (undigested fractions) were flash-frozen with liquid N₂ and freeze-dried.

The elemental contents of the (processed) wheat samples and the freeze-dried digested fractions thereof were determined at least in triplicate using inductively coupled plasma mass spectrometry (ICP-MS). Samples (50 mg, < 200 µm) were weighed into glass tubes and digested with 1.0 ml ultrapure 250 ml/l HNO₃ and subsequently with 2.0 ml aqua regia (750 ml/l ultrapure HCl and 250 ml/l ultrapure HNO₃) at 150 °C, followed by dilution with Milli-Q water (18.2 MΩ cm at 25 °C; Milli-Q Plus, Merck Millipore, Darmstadt, Germany) to 10.0 ml. The elements in the different samples and the NIST1567 wheat reference were analysed by ICP-MS with an Agilent 7700x (Santa Clara, CA, USA). The reported elements were recovered within 10% of the certified values.

The elemental bio-accessibility was then calculated as follows:

Mineral bio – accessibility (%)

$$= \frac{\text{mass of element in digested fraction (g dm)}}{\text{total mass of element in wheat (g dm)}} \cdot 100$$

For each element, the difference between the concentration in breakfast flakes and in the weighted average concentration of all corresponding fractions obtained after the *in vitro* digestion assay varied between 7 and 15%.

The molar ratios of phytate to Fe or Zn were calculated as the millimoles of phytate per kg breakfast flakes divided by the millimoles of Fe or Zn per kg breakfast flakes, respectively. This molar ratio is a useful index for predicting the *in vivo* mineral bio-availability (*i.e.* absorption by the human body) (Morris & Ellis, 1989).

2.6. Statistical analyses

Statistical analyses were conducted using the Statistical Analysis System software 14.0 (SAS Institute, Cary, NC, USA). Given the small amount of samples ($n = 2$ or $n = 3$), we first performed two non-parametric tests: the Van der Waerden test and the Kruskal Wallis test. Both tests confirmed that there were significant differences ($P < 0.05$) between responses for all parameters under study (colour, bulk density, phytate contents, etc.). Given that the residuals of ANOVA seemed to be normally distributed (data not shown), we here reported the results of one-way ANOVA with *post hoc* Tukey multiple comparison testing to verify whether mean values of responses under study were significantly ($P < 0.05$) different.

The treatment was the breakfast flake making process using either control wheat, sprouted wheat or a blend of the former. Several chemical and physical parameters were relevant with several technical replicates of the same material. Data of the technical replicates were averages and only the mean values were used for each of the true independent treatment replicates, i.e. $n = 2$ for the blend and $n = 3$ for control and sprouted wheat.

3. Results and discussion

The starting point for modifying the production process was that although there was a substantial increase in phytase activity during sprouting, phytate breakdown was limited to only 15% (Lemmens, De Brier et al., 2019). Lemmens et al. (2018) showed that hydrothermal processing of sprouted (15 °C, 120 h) wheat at 50 °C and pH 3.8 for 24 h reduced phytate content by 95%. We here simulated these optimal conditions for phytase action in the modified process by replacing the water in the ingredient bill by 100 mmol/l sodium acetate buffer (pH 3.8) and implementing a resting period of 1 h at 50 °C prior to extrusion. In addition, such treatment may favour saccharide release from starch as a result of α -amylase action under conditions more close to its optimum (pH 5.0, 68 °C) (K. Singh & Kayastha, 2014). Finally, whole meal blends containing 25 g/100 g sprouted wheat and 75 g/100 g control wheat were made to check whether there were synergistic effects. For example, one of the questions was whether endogenous enzymes present in sprouted wheat act on control wheat in the ingredient mixture.

3.1. Colour characteristics of the breakfast flakes

Kellogg's All-Bran flakes had L^* , a^* and b^* values of 70, 6 and 23, respectively which were comparable to the values obtained for our flakes (Table 1). Indeed, the L^* values of the flakes varied from 70 to 82. Such high values indicate lightness. The a^* values amounted to 3 to 8, indicating redness and the b^* values ranged from 15 to 23 pointing to a more yellowish colour.

Flakes from control wheat had L^* , a^* and b^* values of 82, 3 and 15 respectively. The L^* values of flakes derived from sprouted wheat and the blend produced using the standard process were lower (70–74) than those of the flakes from control wheat, clearly indicating higher darkness. Further, both a^* (6–8) and b^* (21–23) values of these flakes were higher than those of flakes made with control wheat.

Wheat sprouting increases the levels of reducing sugars and amino groups both of which partake in Maillard reactions (Lasekan & Lasekan, 1997; Tian et al., 2010; van Boekel, 2006). The darker colour and the higher redness and yellowness of flakes derived from sprouted wheat and the blend resulted from more intense Maillard reactions during extrusion (S. Singh, Gamlath, & Wakeling, 2007) and roasting (Coghe, Gheeraert, Michiels, & Delvaux, 2006).

Table 1

CIELAB colour space (L^* , a^* and b^*) of the different breakfast flakes produced using the standard or modified (acid incubation) process. Means and standard deviations of two (blend) or three (control or sprouted wheat) independent treatment replicates are given each measured with five technical repeats. Mean values within one colour component differ significantly ($P < 0.05$) when not sharing the same letter.

Breakfast flake type	L^*	a^*	b^*
Kellogg's All-Bran	70.3 $\pm$ 0.1 ^a	6.2 $\pm$ 0.1 ^{ab}	23.0 $\pm$ 0.1 ^a
Control wheat, standard process	81.5 $\pm$ 0.3 ^b	3.3 $\pm$ 0.1 ^c	14.6 $\pm$ 0.4 ^b
Control wheat, modified process	80.0 $\pm$ 0.3 ^{bc}	4.4 $\pm$ 0.1 ^{cd}	17.3 $\pm$ 0.3 ^b
Sprouted wheat, standard process	70.2 $\pm$ 2.4 ^a	7.6 $\pm$ 0.9 ^a	22.9 $\pm$ 1.3 ^a
Sprouted wheat, modified process	78.4 $\pm$ 1.7 ^c	4.5 $\pm$ 0.5 ^{bcd}	19.0 $\pm$ 1.1 ^a
Blend, standard process	74.4 $\pm$ 1.1 ^{ac}	6.4 $\pm$ 0.5 ^{ab}	20.5 $\pm$ 0.7 ^a
Blend, modified process	77.4 $\pm$ 0.4 ^{bc}	5.7 $\pm$ 0.2 ^{bd}	20.3 $\pm$ 0.4 ^a

Using the modified process for flake production did not have a major impact on the colour characteristics of the resulting flakes (Table 1). Breakfast flakes derived from sprouted wheat using the modified process had significantly higher L^* (78) and lower a^* (5) values than flakes derived from sprouted wheat using the standard process, probably because of a decreased extent to which Maillard reactions had occurred. We believe that the acidic pH of 3.8 is at the basis of our observations. Indeed, at low pH amino groups are less available for Maillard reactions (Ajan-douz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001; Cerny & Brifford, 2007; Karseno, Yanto, Setyowati, & Haryanti, 2017).

3.2. Bulk density and thickness of the breakfast flakes

Kellogg's All-Bran flakes had a bulk density of 171 g/l, compared to 399 g/l for flakes derived from control wheat (Table 2). Oliveira et al. (2017) found a bulk density of 178 g/l for spherical air oven-dried extrudates with a diameter of 9 mm derived from 100% wheat whole meal, while De Brier et al. (2015) found values of 240–330 g/l for their wheat bran-rich breakfast flakes. Further, Wojtowicz et al. (2015) obtained values in a 200–250 g/l range for extruded breakfast cereals made from 70 g/100 g wheat whole meal, 10 g/100 g corn grits, 10 g/100 g rice flour, 5 g/100 g sugar and 5 g/100 g cocoa. The bulk density of flakes produced from sprouted wheat was much lower (198 g/l) than that of flakes made from control wheat using the standard production process (399 g/l). This was related to the much higher level of hydrolytic enzymes when sprouted wheat was used (data not shown). Partial hydrolysis of starch and gluten allowed for more product expansion. Substitution of control by sprouted wheat can indeed lower product bulk density readings to values desired by the consumer (Oliveira et al., 2017; Wojtowicz et al., 2015). In addition, flakes from sprouted wheat were thinner (0.6 mm) than those from control wheat (0.9 mm). This is explained by the lower resistance of these pellets to the forces imposed by flaking as a result of starch and gluten hydrolysis and thus, a less strong starch/gluten network in the extrudate.

Surprisingly, the bulk density of flakes from sprouted wheat (335 g/l) made using the modified production process was significantly higher than that of flakes from sprouted wheat using the standard process (198 g/l). Conditions in the former process (50 °C, pH 3.8) may have negatively impacted amylase and/or peptidase action.

Table 2

Bulk density (g/l), thickness (mm), length (mm), hardness (N), spatial frequency of ruptures N_{sr} (mm^{-1}) and density corrected hardness ($\text{N}\cdot\text{cm}^3/\text{g}$) of the breakfast flakes produced using the standard or modified (acid incubation) process. Means and standard deviations of two (blend) or three (control or sprouted wheat) independent treatment replicates are given each measured with six technical repeats. Mean values within one parameter differ significantly ($P < 0.05$) when not sharing the same lower case letter. The hardness values before and after 30 s of soaking differ significantly ($P < 0.05$) when not sharing the same upper case letter.

Breakfast flake type	Bulk density (g/l)	Thickness (mm)	Length (mm)	Hardness (N)	Hardness after 30 s soaking (N)	N_{sr} (mm^{-1})	Density corrected hardness ($\text{N}\cdot\text{cm}^3/\text{g}$)
Kellogg's All-Bran	171 $\pm$ 4 ^a	1.1 $\pm$ 0.1 ^a	28 $\pm$ 5 ^a	277 $\pm$ 9 ^{aA}	269 $\pm$ 6 ^{aA}	0.4 $\pm$ 0.1 ^a	1618 $\pm$ 50 ^a
Control wheat, standard process	399 $\pm$ 22 ^b	0.9 $\pm$ 0.1 ^{ab}	12 $\pm$ 1 ^b	441 $\pm$ 32 ^{bcA}	322 $\pm$ 38 ^{ab}	1.1 $\pm$ 0.2 ^b	1110 $\pm$ 113 ^b
Control wheat, modified process	324 $\pm$ 17 ^b	0.9 $\pm$ 0.1 ^{ab}	15 $\pm$ 1 ^b	438 $\pm$ 18 ^{bcA}	311 $\pm$ 61 ^{ab}	0.7 $\pm$ 0.1 ^{ab}	1353 $\pm$ 30 ^{ab}
Sprouted wheat, standard process	198 $\pm$ 21 ^a	0.6 $\pm$ 0.1 ^c	20 $\pm$ 1 ^c	439 $\pm$ 50 ^{cA}	332 $\pm$ 89 ^{aA}	0.3 $\pm$ 0.1 ^a	2216 $\pm$ 61 ^c
Sprouted wheat, modified process	335 $\pm$ 39 ^b	0.8 $\pm$ 0.1 ^{abc}	16 $\pm$ 2 ^b	451 $\pm$ 13 ^{bcA}	338 $\pm$ 71 ^{ab}	0.7 $\pm$ 0.2 ^{ab}	1368 $\pm$ 176 ^{ab}
Blend, Standard process	340 $\pm$ 1 ^b	0.7 $\pm$ 0.1 ^{bc}	15 $\pm$ 1 ^b	499 $\pm$ 3 ^{bcA}	397 $\pm$ 53 ^{ab}	0.5 $\pm$ 0.1 ^a	1468 $\pm$ 6 ^{ab}
Blend, modified process	366 $\pm$ 11 ^b	0.9 $\pm$ 0.1 ^{ab}	14 $\pm$ 1 ^b	547 $\pm$ 5 ^{bA}	403 $\pm$ 48 ^{ab}	0.5 $\pm$ 0.1 ^a	1498 $\pm$ 30 ^{ab}

3.3. Texture of the breakfast flakes before and after soaking

The breakfast flakes produced here were harder (400–550 N) than the commercial ones (280 N) but less hard than bran-rich breakfast flakes (550–900 N) made by De Brier et al. (2015). High concentrations of dietary fibre in bran-rich breakfast flakes allow less water to evaporate at the die, decrease the expansion and increase the hardness (Brennan et al., 2008). Moreover, dietary fibre can rupture gas cells, thereby reducing the overall expansion and again increasing hardness (Charunuch, Limsangouan, Prasert, & Wongkrajang, 2014; Oliveira, Alencar, & Steel, 2018). Finally, starch-fibre interactions reduce elasticity and weaken the cell structure, leading to harder structures (Oliveira et al., 2017, 2018).

One factor which definitely impacts the hardness of cellular materials is their (bulk) density. When we corrected the obtained hardness values for flake bulk density as done earlier by De Brier et al. (2015), the obtained intrinsic hardness readings for flakes derived from (blends containing) sprouted wheat (1.5–2.2 $\text{kN}\cdot\text{cm}^3/\text{g}$) were higher than those of flakes made from control wheat (1.1–1.4 $\text{kN}\cdot\text{cm}^3/\text{g}$). This may be because of the impact of the earlier action of amylase on starch on its crystallinity after cooling. Much as what happens during bread cooling after baking, amylose crystallised during cooling of the extruded and roasted breakfast flakes. The resulting network together with the thermoset gluten one contributes to the initial hardness of the flakes (Bosmans, Lagrain, Fierens, & Delcour, 2013; Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2013). Amylase action impacts on amylose and causes it to crystallise more effectively during cooling. Such fast formation of a rigid network with prevention of structure collapse may lead to increased hardness (Bosmans, Lagrain, Fierens, & Delcour, 2013; Goesaert, Leman, Bijttebier, & Delcour, 2009; Lagrain, Leman, Goesaert, & Delcour, 2008). Much as observed for the bulk density results (*cf.* 3.2), the density corrected hardness readings for the flakes derived from sprouted wheat produced using the modified process (1.4 kN) were not significantly different from those of flakes made from control wheat (1.1–1.4 kN). This confirms that the conditions used in the modified process (50 °C, pH 3.8) negatively impacted α -amylase action.

Further, no clear differences were observed in N_{sr} , which is a measure for crispness. The higher N_{sr} , the more brittle the sample, which in sensory terms is perceived as more crispy and of better quality (De Brier et al., 2015). However, the here obtained values (0.3–

1.1 mm^{-1}) were rather low when compared to values (0.6–2.0 mm^{-1}) for bran-rich breakfast flakes obtained by De Brier et al. (2015).

The soaking process in milk did not significantly affect the hardness of commercial flakes (Table 2). However, when the control breakfast flakes were soaked for 30 s their hardness already decreased by about 120 N, which corresponded to a loss of 27% of their initial hardness. Similar trends (loss of 20–24%) were observed both for flakes made from sprouted wheat or from the blend with the standard or modified production process. The present results are in line with those of De Brier et al. (2015) who, for flakes containing 40 g/100 g bran or aleurone enriched bran, observed a loss of 12–23% of the initial hardness upon soaking for 30 s. Finally, crispness (measured as N_{sr}) was lost after soaking all flake types in milk for 30 s (data not shown). De Brier et al. (2015) earlier observed that bran-rich flakes lose their crispness already during the first seconds of soaking.

3.4. Soluble saccharide contents in the breakfast flakes

Table 3 represents the saccharide contents of the breakfast flakes produced using either the standard or the modified process and an ingredient bill which included 7.5 g/100 g sucrose and 2.5 g/100 g glucose. The sucrose content of the commercial flakes amounted to about 15 g/100 g of dm, in line with the data on the label (data not shown). The use of sprouted wheat in the standard process increased the levels of maltose and maltotriose obviously as a result of α -amylase action. Kruma et al. (2018) noted a saccharide (mainly sucrose and maltose) content of 1.5 g/100 g of dm when using 40 g/100 g sprouted cereals (triticale, oat and barley), but 2.2 g/100 g of dm when using 50 g/100 g of sprouted cereals (rye, oats and barley) in breakfast flakes produced by manual flaking and subsequent microwave-vacuum drying at temperatures not exceeding 45 ± 5 °C. They attributed the increase in saccharide content to enzymatic hydrolysis of starch. This raises the question whether this amylolytic activity has an impact on the carbohydrate availability and therefore also the glycaemic response of breakfast flakes derived from sprouted wheat. Unfortunately, there is no consensus in literature on the impact of sprouting cereal grains on *in vitro* starch digestibility. We kindly refer to the review paper by Lemmens, De Brier, Goos, Smolders, and Delcour (2019) for further information on this topic. Further, when using a whole meal blend containing only 25 g/100 g of sprouted wheat and 75 g/100 g of control wheat in the standard process the maltose and maltotriose contents in the resulting flakes were similar to those in the flakes derived from 100% sprouted wheat. This indicates that α -amylase from sprouted wheat can

Table 3

Saccharide contents [g/100 g of dry matter (dm)] in breakfast flakes produced using the standard or modified (acid incubation) process. Means and standard deviations of two (blend) or three (control or sprouted wheat) independent treatment replicates are given each measured with at least one technical repeat. Mean values for saccharide contents differ significantly ($P < 0.05$) when not sharing the same letter.

Breakfast flake type	Glucose (g/100 g of dm)	Fructose (g/100 g of dm)	Sucrose (g/100 g of dm)	Maltose (g/100 g of dm)	Maltotriose (g/100 g of dm)
Control wheat, standard process	1.91 ± 0.03 ^a	0.20 ± 0.12 ^{ab}	7.55 ± 0.32 ^{ab}	1.87 ± 0.08 ^{ab}	0.01 ± 0.01 ^a
Control wheat, modified process	2.08 ± 0.21 ^a	0.28 ± 0.07 ^{abc}	7.77 ± 0.04 ^{ab}	1.42 ± 0.07 ^b	0.02 ± 0.01 ^a
Sprouted wheat, standard process	1.57 ± 0.12 ^a	0.42 ± 0.05 ^{acd}	7.20 ± 0.23 ^{ab}	2.92 ± 0.14 ^c	0.54 ± 0.04 ^b
Sprouted wheat, modified process	1.92 ± 0.41 ^a	0.66 ± 0.07 ^d	6.95 ± 0.04 ^b	1.55 ± 0.07 ^b	0.21 ± 0.01 ^c
Blend, Standard process	1.70 ± 0.03 ^a	0.09 ± 0.04 ^b	8.05 ± 0.02 ^a	2.74 ± 0.03 ^c	0.19 ± 0.01 ^c
Blend, modified process	2.02 ± 0.02 ^a	0.56 ± 0.02 ^{cd}	7.17 ± 0.23 ^{ab}	2.50 ± 0.03 ^{ac}	0.21 ± 0.01 ^c

act on starch present in control wheat as well. Finally, when using sprouted wheat in the modified process, the maltose and maltotriose contents in the resulting flakes were lower than when using sprouted wheat in the standard process. This observation again confirms that the conditions used in the modified process (50 °C, pH 3.8) negatively impacted α -amylase action.

The relative sweetness of control breakfast flakes prepared using the standard process amounted to about 10.0, while it was approximately 10.5 for flakes manufactured from sprouted wheat. As the ingredient bill included relatively high levels of added sugars (7.5 g/100 g sucrose and 2.5 g/100 g glucose) the difference in relative sweetness between the two flake types was not significant ($P > 0.05$). However, sprouted wheat containing high levels of maltose and maltotriose can result in higher sweetness of breakfast flakes and thus can be used to reduce the level of sugar addition.

3.5. Phytate contents during the production of breakfast flakes

During the standard production of breakfast flakes, the highest phytate breakdown was observed either during extrusion or during roasting (Fig. 1). However, it was limited to only 15–20% and did not depend on the use of control or (blend containing) sprouted wheat. Still, the lowest phytate level (0.61 g/100 g of dm) was observed in breakfast flakes derived from sprouted wheat.

Reductions in phytate content of 10–45% have been noted when extruding whole meal from (sprouted) wheat and barley (Singkhornart et al., 2013), a mixture of wheat bran, starch and gluten (Andersson, Hedlund, Jonsson, & Svensson, 1981) or a corn starch-based flour recipe containing common bean flour (Anton, Fulcher, & Arntfield, 2009). Heating and/or shear during extrusion or roasting can lead to breakdown/hydrolysis of phytate (Embaby, 2011; Udensi, Ekwu, & Isinguzo, 2007).

The endogenous phytase activity in control wheat is relatively high (Lemmens, De Brier et al., 2019). Here, it decreased the phytate content from 0.84 g/100 g of dm to 0.65 g/100 g of dm in control wheat during the resting step in the modified production process (“after resting”, *cfr.* Fig. 1). After extrusion, the phytate content amounted to only 0.46 g/100 g of dm using this process. Endogenous phytase can remain active at temperatures exceeding 80 °C (Peers, 1953).

When applying the modified production process using sprouted wheat, we again noted a decrease in phytate content from 0.76 g/100 g of dm to 0.54 g/100 g of dm after resting at 50 °C. However, the final phytate content in these breakfast flakes (0.43 g/100 g of dm) was similar to that obtained for control wheat. This indicates that elevated endogenous phytase activity as a result of sprouting did not lead to more extensive phytate hydrolysis during this process. When using whole meal blends containing only 25 g/100 g of sprouted wheat and 75 g/100 g of control wheat in the modified production process the phytate content in the resulting flakes was about 0.50 g/100 g of dm.

Fig. 1. Phytate contents [g/100 g of dry matter (dm)] during production of breakfast flakes using the standard or modified (acid incubation) process. Columns within the same treatment represent phytate contents of different production steps, from left to right: before mixing (■), after mixing (■), after resting (■), after extrusion (■) and the end product (■). Means and standard deviations of two (blend) or three (control or sprouted wheat) independent treatment replicates are given each measured with at least three technical repeats. Mean values for the same production step but different wheat grain treatments differ significantly ($P < 0.05$) when not sharing the same upper case letter. Mean values for the same wheat grain treatment but different production steps differ significantly ($P < 0.05$) when not sharing the same lower case letter.

3.6. Iron and zinc bio-accessibilities in the breakfast flakes

During breakfast flake making with the standard process, 15–20% of phytate was broken down (*cfr.* 3.5). Here, we investigated *in vitro* whether this decrease enhanced Fe and Zn bio-accessibility. The Fe and Zn bio-accessibility values were both 8% in flakes made with control wheat (Table 4). The use of sprouted wheat in the same production process sharply increased Fe bio-accessibility to 28% and improved Zn bio-accessibility to 14%. It is thus of use to substitute control wheat (partly) by sprouted wheat if one aims to increase mineral bio-accessibility in whole meal products.

The isothermal step for 60 min at 50 °C and pH 3.8 as part of the production of breakfast flakes decreased the phytate content by about 50% in both whole meal from control and sprouted wheat and the blend thereof. As a result, the Fe bio-accessibility increased to about 38% and that of Zn to between 17 and 32% (Table 4). The modified process was most effective at reducing phytate content and increasing Fe and Zn bio-accessibility for control wheat, sprouted wheat and the blend made thereof.

The linear relationship between phytate content and Fe ($R^2 = 0.95$) and Zn ($R^2 = 0.62$) bio-accessibility in breakfast flakes (Fig. 2) suggested that lower phytate contents render Fe and Zn more accessible in the human gastro-intestinal tract. The highest Fe bio-accessibility value of 38% was reached at a phytate content of 0.49 g/100 g of dm. A further decrease in phytate content to 0.43 g/100 g of dm was not reflected in a different level of Fe bio-accessibility (Fig. 2). It is likely that a decrease in phytate content of only 0.06 g/100 g of dm was not

Table 4

Iron and zinc bio-accessibilities (%) in the breakfast flakes produced using the standard or modified (acid incubation) process. Means and standard deviations of two (blend) or three (control or sprouted wheat) independent treatment replicates are given each measured with at least two technical repeats. Mean values within one column differ significantly ($P < 0.05$) when not sharing the same letter.

Breakfast flake type	Iron bio-accessibility (%)	Zinc bio-accessibility (%)
Control wheat, standard process	8.1 ± 0.8 ^a	8.1 ± 4.5 ^a
Control wheat, modified process	38.1 ± 3.5 ^b	26.9 ± 1.0 ^{bc}
Sprouted wheat, standard process	27.7 ± 2.3 ^c	13.9 ± 1.6 ^a
Sprouted wheat, modified process	37.7 ± 5.4 ^b	17.1 ± 0.5 ^{ac}
Blend, standard process	14.8 ± 2.6 ^a	10.0 ± 1.6 ^a
Blend, modified process	38.3 ± 0.8 ^b	31.6 ± 5.9 ^b

Fig. 2. Linear correlation between iron (●) and zinc (▲) bio-accessibilities (%) and phytate content [g/100 g of dry matter (dm)] in breakfast flakes. The R^2 value and the equation of both linear regressions are given.

sufficient to increase the Fe bio-accessibility further. Indeed, Lemmens et al. (2018) found a Fe bio-accessibility value of about 37% in whole meal derived from hydrothermally processed wheat with a phytate content of 0.11 g/100 g of dm. In addition, the linear relationship between phytate content and Zn bio-accessibility had a R^2 value of only 0.62 (Fig. 2). The latter can be explained by the fact that no significant differences in Zn bio-accessibility were found between control breakfast flakes prepared using the standard process and breakfast flakes derived from sprouted wheat (Table 4). Given the above, not only phytic acid limits the mineral bio-accessibility, but other factors were in play as well. These can include the presence of plant cell walls which causes minerals to be physically entrapped (Amrein, Gränicherb, Arrigonia, & Amadoa, 2003; Lemmens, De Brier et al., 2019), the formation of (in)soluble complexes with dietary fibres decreasing the absorption of minerals (Baye, Guyot, & Mouquet-Rivier, 2015; Persson, Nyman, Liljeberg, Önnings, & Frölich, 1991) and the restricted solubility of minerals such as Zn (Silva et al., 2020) and Fe (Hungerford & Linder, 1983) at the pH of the gastrointestinal environment.

The question how these *in vitro* mineral bio-accessibility values of breakfast flakes relate to their *in vivo* mineral bio-availability values is a valid one (*cfr.* 2.5). It has been stated that in order to accomplish a *ca.* twofold increase in Fe absorption from food, the molar ratio of phytate to Fe needs to be maximally 1 (Allen, de Benoist, Dary, & Hurrell, 2006; Hurrell, 2004). Here, the phytate to Fe molar ratio in control wheat flakes produced using the standard process amounted to about 20 (data not shown). Earlier, Magallanes-López et al. (2017) reported phytate to Fe molar ratios of 12.1–29.6 in 46 durum wheat varieties. In the present case, the phytate to Fe molar ratio decreased to about 12 with the modified production process for breakfast flakes derived from whole meal of control and sprouted wheat and the blend thereof. Further, the phytate to Zn molar ratio of control wheat flakes produced using the standard process amounted to about 38, decreasing to about 22 in flakes produced using the modified process (data not shown). Comparable phytate to Zn molar ratios of 17–37 in wheat were found by other authors (Hemalatha, Platel, & Srinivasan, 2007; Magallanes-López et al., 2017; Wang, Liu, Pan, Yuan, & Yin, 2015). At high molar ratios (*i.e.* above 15), Zn absorption from food is predicted to be less than 15% (Allen et al., 2006). Based on these results, an even further breakdown of phytate is needed to increase the mineral bio-availability in breakfast flakes. However, an *in vivo* study is needed to confirm this hypothesis.

Larsson, Rossander-Hulthén, Sandström, and Sandberg (1996) showed that Fe and Zn absorption in humans increased from 4 to 6% and 12–18%, respectively, when consuming a breakfast meal containing malted oat porridge (in which 75% of phytate was hydrolysed) rather than a meal containing non-malted oat porridge. Further, Fredlund et al. (2003) noted an increase in Zn absorption from 15 to 23% with a group of ten healthy subjects when serving them breakfast cereals based on malted barley that contained 30% less phytate rather than breakfast cereals of non-processed barley.

4. Conclusions

This study, for the first time, not only showed the feasibility but also the advantages of manufacturing breakfast flakes by extrusion using whole meal from sprouted wheat. The resulting flakes were indeed of good quality with acceptable colour, bulk density and hardness before and after soaking for 30 s in semi-skimmed milk. Further, using sprouted wheat in the standard production process increased maltose and maltotriose contents. This offers opportunities to reduce the level of sugar addition in breakfast flakes. As a result, less empty calories are present which increases the nutritional value of the resulting flakes. Indeed, when substituting sucrose with whole meal derived from sprouted wheat in the recipe, the resultant flakes on a weight basis

have higher dietary fibre, vitamin and mineral contents. These intrinsic sugars deliver sweet notes to the product and serve as flavour precursors in Maillard reactions leading to caramel and toast aromas. Moreover, resting the ingredient mixture at 50 °C and pH 3.8 for 1 h prior to flake production led to a 45–60% phytate breakdown in the flakes. As a result, a fourfold to fivefold increase in Fe bio-accessibility and a twofold to fourfold increase in Zn bio-accessibility was found compared to the standard process. That the final phytate contents in breakfast flakes derived from whole meal of control and sprouted wheat were similar indicates that elevated endogenous phytase activity as a result of sprouting did not lead to more extensive phytate hydrolysis during the process.

To conclude, by fully exploiting the nutritional profile of wheat grains, palatable whole grain breakfast flakes with a clean label (no added sugars or minerals) can be obtained which meet consumer demands and needs.

Declaration of competing interest

The authors declare that they do not have any conflict of interest. No industrial partner was involved in developing the research protocols or in performing the analyses.

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