Food Chemistry 264 (2018) 34–40

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of sourdough addition and storage time on *in vitro* starch digestibility and estimated glycemic index of tef bread

Habtu Shumoy[a,⁎], Filip Van Bockstaele[b], Dilara Devecioglu[a], Katleen Raes[a]

[a] Laboratory of Food Microbiology and Biotechnology, Department of Food Technology Safety and Health, Faculty of Bioscience Engineering, Ghent University - Campus Kortrijk Belgium, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium

[b] Research Unit of Cereal and Feed Technology, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Campus Schoonmeersen Valentin Vaerwyckweg 1, 9000 Gent, Belgium

ARTICLE INFO

Keywords:
Gluten free bread
Glycemic index
Rapidly digestible starch
Resistant starch
Slowly digestible starch

ABSTRACT

The effect of sourdough amount and storage time on starch digestibility and estimated glycemic index (eGI) of tef bread was investigated. The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of 0–30% sourdough fresh tef breads ranged from 49 to 58, 16 to 29 and 20 to 26 g/100 g starch, respectively. Storage of tef breads up to 5 days decreased the RDS by more than 2-fold while SDS and RS increased by 2 and 3 fold, respectively. The eGI for fresh and stored breads ranged from 39 to 89. Addition of sourdough increased the eGI of fresh breads while no uniform pattern was seen in the stored breads. As the storage time increased, all the breads showed a decrease in eGI. *In vivo* study is necessary to further investigate the effect of sourdough on GI of tef bread.

1. Introduction

The global prevalence of diabetes among adults will increase to 8.8% in the year 2035 affecting as much as 592 million adults compared to 8.3% (382 million adults) in the year 2013 (Guariguata et al., 2014). Long term frequent intake of high glycemic index food products produce greater insulin resistance than the low glycemic-index carbohydrates. Lifestyle modification, involving diet and enhanced physical activity, are known to effectively prevent type 2 diabetes mellitus. Owing to this, there is a global shift of consumers from refined white flours to a minimally refined flour or whole meal as consumption of high fiber containing flours are increasingly associated with a lower risk of weight gain, cardiovascular disease and other chronic diseases (Patel, Chandra, Alexander, Soble, & Williams, 2017).

Tef [*Eragrostis tef* (Zucc.) Trotter], an ancient gluten free cereal, is processed into a whole flour and contains high fiber and minerals. This cereal is becoming popular among consumers in Western countries as it is increasingly considered as a healthy and nutritious food. In Ethiopia where tef is highly cultivated, this cereal is used to produce traditional food products mainly *injera* (a fermented flat bread) and thick porridge. Shumoy and Raes (2017a)) reported that the freshly prepared *injera* and porridge food products from different tef varieties exhibited a high GI in the range of 94–137 and 79–99, respectively. Furthermore, Wolter, Hager, Zannini, and Arendt (2013) showed a GI of 74 for a frozen conventional tef bread. The use of tef alone or mixed with wheat flour to prepare bread is becoming more and more popular among Western consumers. There is scarcity of information on GI of conventional tef bread. Tef is gluten free and the manufacture of bread without gluten causes major technological problems for bakers. Indeed, gluten-free breads available on the market are often of poor quality, showing low volume, poor colour and crumbling crumb and mostly with low protein and high fat contents (Segura & Rosell, 2011). However, it has been shown that sourdough could improve the sensory and physical qualities of gluten free breads, *i.e.* among others it can increase the specific volume and lower crumb hardness (Rinaldi, Paciulli, Caligiani, Scazzina, & Chiavaro, 2017). As tef contains high protein content with high digestibility (Shumoy, Pattyn, & Raes, 2018), it could be a good alternative to manufacture a high protein gluten free bread. However, literature regarding tef and the effect of sourdough on the resulting physical quality and starch digestibility of tef bread is scarce. In general, breads, be it at home or in supermarkets, could stay fresh for variable storage times, information pertaining to the freshness level, particularly of tef bread and associated GI is lacking. Therefore, this study was designed to investigate the effect of sourdough addition (10, 20 and 30%) and storage time (1, 2 and 5 days) and fresh breads as a control on *in vitro* starch digestibility and glycemic index of tef bread.

⁎ Corresponding author.
E-mail addresses: habtu.shumoy@ugent.be (H. Shumoy), filip.vanbockstaele@ugent.be (F. Van Bockstaele), dilara.devecioglu@ugent.be (D. Devecioglu), katleen.raes@ugent.be (K. Raes).

https://doi.org/10.1016/j.foodchem.2018.05.019
Received 5 December 2017; Received in revised form 13 April 2018; Accepted 2 May 2018
Available online 04 May 2018
0308-8146/

2. Materials and methods

2.1. Bread production

In this study, flour of unknown varieties of mixed white and brown tef grains were used, as these are commercially available as such on the Ethiopian market. Tef grains of brown and white, 5 kg each, were purchased at a market in Mekelle, Ethiopia and carefully cleaned for impurities by sifting, sieving and winnowing. They were milled at a local disc attrition milling (Mekelle, Ethiopia), packed in polythene pouches, transported to Belgium and stored at −20 °C until further analysis.

Sourdough was prepared according to Lappi et al. (2010) using a commercial starter *Lactobacillus fermentum* (Florapan LA4K; kindly provided by Lallemand, France). Briefly, 1% LA4K starter (based on flour weight), tef flour and 62.6% water (based on dough weight) were mixed manually and fermented in a fermentation cabinet (30 °C, 85% relative humidity (RH)) for 19.5 h until the pH was 3.9–4.1. The titratable acidity was determined by potentiometric titration using 0.1 M NaOH to pH of 8.5 endpoint (Wolter, Hager, Zannini, & Arendt, 2014).

Tef bread in triplicates were baked as described in Hager, Wolter, Czerny et al. (2012) with slight modifications. Tef bread dough was prepared by mixing tef flour, sourdough in different proportions (0, 10, 20 and 30%), 3% yeast, 2% HPMC (hydroxypropylmethylcellulose), 2% salt, 2% sugar and 139% water, all based on dry matter flour weight. The dough was then immediately divided and put into baking pans and allowed to ferment or proof for 45 min in a fermentation cabinet (30 °C, 85% RH) followed by baking (190 °C, 45 min) in a preheated baking oven (MIWE condo, Arnstein, Germany). After cooling the breads for one hour, they were stored in a closed plastic bag and stored at ambient temperature for up to 5 days. Fresh bread (2h after baking) was used as a control. Fresh (2h after baking) white wheat bread was used as a reference material.

2.2. Physicochemical properties of tef flour

Flour particle size distribution was measured by a laser diffraction particle size distribution analyzer (Beckman coulter, LS 13 320 Series, USA) based on the instrument manual.

The falling number (FN) was determined according to AACC (1999) method No. 56-81b using 7 g flour sample and 25 ml distilled water.

The pasting property of tef flour was determined using a Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria.) according to Hellemans et al. (2017) with slight modification, flour in water suspension 14% (2.8 g of flour in 20 ml of water). The pasting temperature (PTem), peak temperature (PeakT), peak time (PT), initial viscosity (IV), peak viscosity (PV), holding viscosity (HV), final viscosity (FV), breakdown (BD), setback (SV) were recorded. The viscosity was expressed in mPa.s.

Protein content of tef flour was determined by Kjeldahl method (AOAC, 1995) with 5.4 as nitrogen to protein conversion factor.

Apparent amylose content of tef flour was determined by using Megazyme kit K-AMYL and the amylose (%) was calculated according to:

$$\text{Amylose (\%)} = \frac{\text{Absorbance(Con A supernatant)}}{\text{Absorbance(total starch aliquot)}} \times \frac{6.15}{9.2} \times 100$$

where: 6.15 and 9.2 are dilution factors for Con A supernatant and total starch aliquot respectively.

2.3. Bread physical properties

Specific volume (SV) of the breads was measured using a 3D Volscan Profiler (Stable Micro Systems Volscan Profiler 600, UK) following instrument manual. The crumb texture (hardness, springiness, cohesiveness, chewiness and resilience) was measured using a texture analyzer (TA.XTplus, Stable Micro Systems) on uniform slices of 25-mm thickness according to Debonne et al. (2018).

2.4. Free glucose, and starch fractions of tef flour and bread

Free glucose (FG) was measured according to Englyst, Kingman, and Cummings (1992) using an assay kit GOPOD-format K-GLUC 09/14 (Megazyme International Ireland Ltd) and the glucose % was calculated as:

$$\%\text{glucose} = \frac{\text{At} \times \text{Vt} \times \text{C} \times \text{D}}{\text{As} \times \text{Wt}} \times 100$$

where:

At: absorbance of test solutions, **Vt**: total volume of test solutions (Vt = 25.2 plus 1 ml per gram wet weight of samples used), **C**: concentration (C = 0.394 mg glucose/ml) of standard, which may be corrected for moisture content, **D**: dilution factor = 18.

The TS, RDS, SDS and RS contents were determined according to Englyst et al. (1992) using an assay kit GOPOD-format K-GLUC 09/14 (Megazyme International Ireland Ltd) and were calculated as:

*TS = (TG - FG) × 0.9

**RDS = (G20−FG) × 0.9

**SDS = (G120−G20) × 0.9

**RS = TS - (SDS + RDS)

Values were expressed as g/100 g dm of (flour)* and (starch)**.
Where; G120: Glucose content after 120 min of digestion.
G20: Glucose content after 20 min of digestion.
TG: Total glucose.
0.9: Glucose to starch conversion factor.

2.5. In vitro glycemic index of tef bread

The rate of *in vitro* starch hydrolysis was analyzed following the method recommended by Goni, Garcia-Alonso and Saura-Calixto (1997). The area under the hydrolysis curve (AUC) was calculated using the equation:

$$\text{AUC} = C\infty(t\infty - to) - (C\infty/k)[1 - \exp[-k(t\infty - to)]]$$

where C∞ corresponds to the equilibrium percentage of starch hydrolyzed after 180 min, **t∞** is the final time (180 min), to is the initial time (0 min) and k is the kinetic constant.

The hydrolysis index (HI) was calculated as AUC of a sample as percentage of the corresponding AUC of fresh white bread (Goni et al., 1997; Granfeldt, Bjorck, Drews, & Tovar, 1992). The white bread used as reference had a dry matter content of 56 g/100 g and a total starch content of 68 g/100 g dm. Bread crumb, taken from the center of the bread was sampled. The estimated glycemic index (eGI) was calculated according to equations suggested by both:

Goni et al. (1997): $eGI_G = (0.549 \times HI) + 39.71$, and
Granfeldt et al. (1992): $eGI_{Gr} = (0.862 \times HI) + 8.198$

2.6. In vitro protein digestibility of tef flour and bread

The *in vitro* protein digestibility (IVPD) was analysed according to Hsu, Satterlee, and Miller (1977). The IVPD was calculated as:

$$\text{IVPD (\%)} = 65.66 + 18.1\Delta pH_{10\ min}$$

where $\Delta pH_{10\ min}$ is the pH difference of initial and after 10 min digestion in bread suspensions.

2.7. Statistical analysis

To assess differences among tef varieties and fermentation times, two-way analysis of variance (ANOVA) was performed. If ANOVA showed significant ($p < 0.05$) interaction between the main factors,

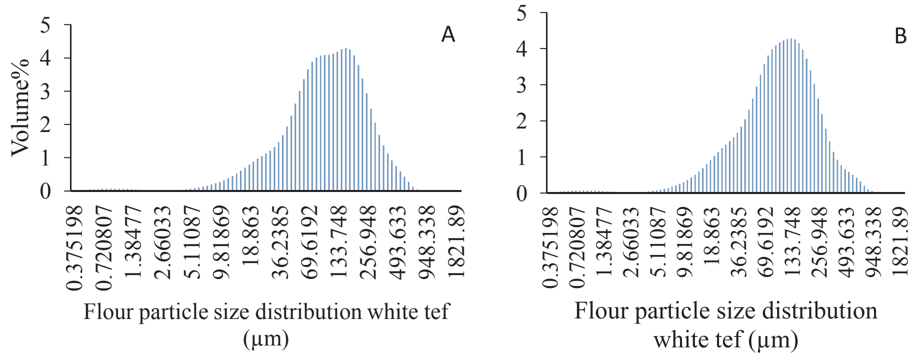

Fig. 1. Particle size distribution of white and brown tef flours milled by disc attrition at a local whole cereal flour miller, in Ethiopia.

data were further subjected to one-way ANOVA. Multiple mean comparison was then done by Tukey's Honestly Significant Differences at $p < 0.05$. All statistical analyses were performed using SPSS version 24 (SPSS Inc., Chicago, IL, USA). All analyses were carried out in triplicate and results were reported on a dry matter (dm) basis.

3. Results and discussions

3.1. General

Two way ANOVA was used to check if there was a significant interaction between tef types (brown and white) and the measured parameters, *i.e.* bread physical features (volume and texture), free glucose, starch fractions and estimated glycemic index. As the interaction was not significant, the results of the white and brown tef were combined as one mean and called tef instead of brown or white tef.

3.2. Characterization of tef flour and sourdough

The average particle size distribution of the brown and white tef flour is given in Fig. 1. Both flours exhibited a similar particle size distribution in that 60% of flour particles had a size of below 150 μm, 300 μm < 90% and 600 μm < 100%. The effect of the flour particle size on the resulting bread quality seems to depend on the type of flour. Wheat flour with a particle size of 75–118 μm showed better sensorial and textural attributes than their coarser flour (> 150 μm) counterparts (Sakhare, Inamdar, & Soumya, 2004). On the other hand, combination of high water content (90–110%) with coarse rice flour (132–200 μm) resulted in a larger bread specific volume than their fine flours (< 132 μm) (De La Hera, Rosell, & Gomez, 2014). In our preliminary baking experiments, combination of high water content (139%) with HPMC (2%) showed highest specific volume. However, speculation on the effect of flour particle size in our study is not possible since the flours had similar particle size distribution.

The FN (Table 1) of the white and brown types were 360 and 368 *sec.*, respectively. FN is mostly used to grade wheat grain *i.e.* wheats with FN < 200 are graded as low quality or with severe sprout damage, 300 > FN > 200, moderately sprout damaged and FN > 300 no sprout damage and/or sound cereal (Kweon, 2010). Based on this, the tef samples used in this study could be graded as sound.

Pasting temperature shows the gelatinization temperature, pasting viscosity indicates the thickening ability and water holding capacity, final and setback viscosities predict the degree of gelation and the gradual retrogradation tendencies on cooling and storage of the pasted system. Pasting properties (Table 1), such as PTem, PeakT, PT and IV, did not show significant differences while PV (1371–1942), HV (741–949), FV (1586–2057), BV (630–960) and SB (845–1100) (in mPa.s) showed significant differences ($p < 0.05$) between the brown and white tef flours. Relatively higher PTem (68–76 °C), HV (1050–1570), FV (2033–2920), but lower BV (105–320) and similar SB (837–1317) (mPa.s) were reported for other tef varieties (Bultosa, 2007). For this study it was important to know if differences in starch pasting behaviour exist between the tef samples as this could explain differences in texture or starch digestibility.

The titratable acidity of the sourdough of the brown and white tef types were 2.3 and 2.1 ml of 0.1 M NaOH/g sourdough, respectively with both showing equal pH of 3.9 after fermentation for 19.5 h. However, the titratable acidy content in this study is lower than the tef sourdough in Wolter et al. (2014). The difference could be attributed to the difference in the source of tef, the starter used and the water to flour proportion used to make the sourdough. Obligate heterofermentative strains *Weissella cibaria* and facultative heterofermentative *Lactobacillus plantarum* were used in Wolter et al. (2014) while a mixture of lactic acid bacteria and yeast was used as a starter in this study. Also 62.6% water on dough weight basis was used in this study while 50% in Wolter et al. (2014). Nevertheless, spontaneously fermented pearl and finger millet sourdoughs showed titratable acidity which ranged from 1.1 to 3.6 ml/g sourdough (Akinola, 2017).

3.3. Bread physical properties

The specific volume (SV) of tef breads containing different sourdough proportions are shown Table 3. The SV of 0–30% sourdough breads ranged narrowly from 1.8 to 1.9 ml/g. The SV of breads in this

Table 1
Protein content (g/100 g dm flour), falling number (second) and pasting properties of tef flour.

Tef	Protein	FN	Tef flour pasting properties								
			PTem	PeakT	PT	IV	PV	HV	FV	BD	SV
Brown	11 ± 0.6	368 ± 6	66 ± 0.3	92 ± 0	9.4 ± 0	17 ± 0.3[b]	1371 ± 27[a]	741 ± 8[a]	1586 ± 15[a]	630 ± 19[a]	845 ± 6[a]
White	9.0 ± 1.1	360 ± 1	65 ± 0.6	92 ± 0	9.4 ± 0	16 ± 0.1[a]	1942 ± 30[b]	949 ± 4[b]	2057 ± 10[b]	960 ± 11[b]	1100 ± 4[b]
p	0.107	0.192	0.125	0.312	–	0.019	0.002	0.001	0.001	0.002	< 0.001

[a,b]Values within a column with different small superscript letters are significantly different ($p < 0.05$). FN: falling number in seconds, PTem: Pasting temperature (°C), PeakT: Peak temperature (°C), PT: Peak time (min), IV: Initial viscosity (mPa.s), PV: Peak viscosity (mPa.s), HV: Holding viscosity (mPa.s), FV: Final viscosity (mPa.s), BD: Breakdown viscosity (mPa.s), SV: Setback viscosity (mPa.s). (n = 3).

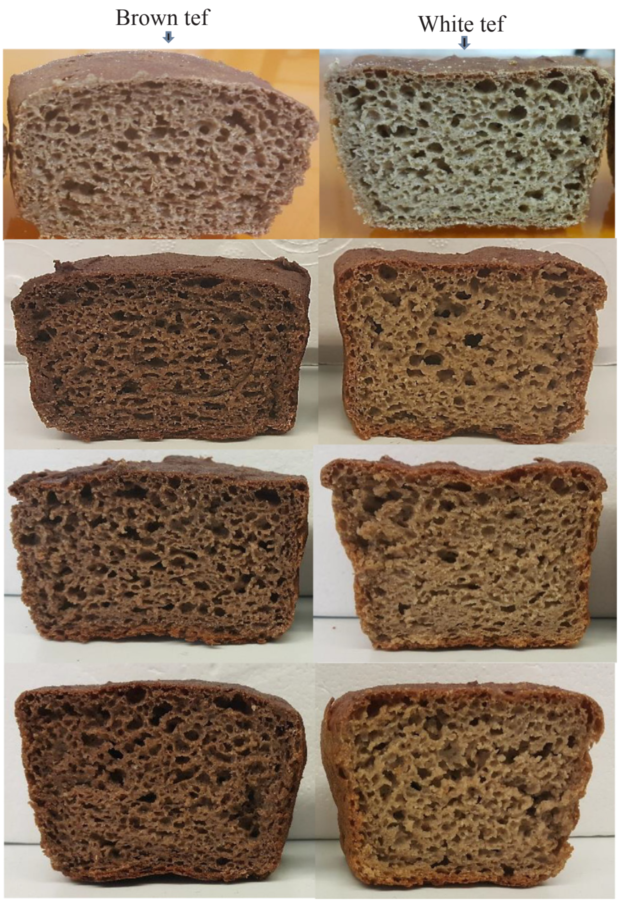

Fig. 2. Visual appearance of bread slices 0% (A), 10% (B), 20% (C) and 30% (D) sourdough tef breads.

study are much higher than previous reports of conventional tef breads with specific volumes in the range of 1.3–1.6 ml/g (Hager, Wolter, Jacob, Zannini, & Arendt, 2012; Marti et al., 2017). Indeed the SV of breads in this study are higher and/or similar compared to other gluten free breads (maize, buckwheat, quinoa, sorghum and rice) and whole wheat with SV ranging from 1.33 to 1.85 but lower than oat breads (2.4) ml/g (Hager, Wolter, Czerny et al., 2012). The higher specific volumes of the tef breads in our study could be attributed to the difference in the formulations of the ingredients in that the breads in our study contained HPMC and higher water levels.

The texture of bread from both the brown and white tef is given in Table 3. The crumb hardness of the breads ranged from 7.7 to 10.5 N. Incorporation of sourdough (20–30%) showed a significant decrease ($p < 0.05$) in bread hardness while 10% sourdough did not show an effect on this. In previous studies, tef bread which contained both HPMC and xanthan hydrocolloids, resulted in a relatively harder texture (24 N) compared to our breads (Hager & Arendt, 2013), while white wheat bread showed a hardness of 8.8 N (Hager, Wolter, Czerny et al., 2012). The fluffiness (desired quality) of breads in the present study (Fig. 2) could be attributed to the combined effect of the addition of sourdough, HPMC and its higher water level (139% based on flour) and longer fermentation time (45 min), with the latter two optimized in our preliminary experiments. Springiness, cohesiveness, chewiness and resilience of the breads ranged from 0.87 to 0.93, 0.56 to 0.58, 3.83 to 5.56 (J) and 0.27 to 0.30, respectively. Springiness and chewiness of the breads significantly decreased ($p < 0.05$) with increased proportion of sourdough while cohesiveness and resilience did not show any significant difference. During sourdough fermentation, the lactic acid bacteria (LAB) can produce a number of metabolites, such as organic acids, exopolysaccharides (can replace hydrocolloid) and/or enzymes, which have a positive effect on the texture and bread staling (Arendt, Ryan, & Bello, 2007). Hydrocolloid can increase water binding ability, viscosity of the system, and create, during mixing, non-gluten networks stabilized by inter- and intra-protein bonds able to mimic gluten properties to increase crumb softness (Cappa et al., 2016). Tef breads without hydrocolloids showed similar springiness (0.942) but higher chewiness (31.9 J) (Hager, Wolter, Czerny et al., 2012) than breads in the present study. Chewiness is tenderness and toughness of a solid food and is affected by hardness, cohesiveness and springiness. Indeed, it simultaneously decreased with the decrease of bread hardness and springiness regardless of the cohesiveness of the bread, as the amount of added sourdough increased.

3.4. Free glucose and starch properties of tef flour and bread

Free glucose, apparent amylose, total starch, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents of the brown and white tef flours are given in Table 2. The brown and white tef grains showed similar free glucose (0.5 g/100 g flour dm) and apparent amylose content (24%). The mean RDS, SDS and RS contents of the flours were 26, 33 and 41 g/100 g starch, respectively. Previously, higher apparent amylose contents (29–32%) were reported for different tef varieties (Shumoy & Raes, 2017a). The RDS and SDS contents of this study are in agreement while the RS is relatively higher than previous reports (Shumoy & Raes, 2017a).

Starch fractions (RDS, SDS and RS) sourdough (0%, 10%, 20% and 30%) tef breads and stored for 1, 2 and 5 days are shown in Table 4. There was no clear influence of sourdough proportion on the RDS and SDS. However, RS showed an increasing pattern with increased sourdough. Similarly, fermentation of slurries of breadfruit and sweet potato with amylolytic *Lactobacillus plantarum* and *Lactobacillus fermentum* increased RS due to the formation of limit dextrins by the action of α-amylase on the amylopectin (Haydersah et al., 2012). Contrary to results in this study, a decrease in RS was revealed in sourdough frozen tef breads (Wolter et al., 2014). The contradiction could be in part attributed to the difference in the starter cultures used and duration of fermentation but also the freshness level of the samples. In this study, a longer fermentation time (45 min) was used. This could have enabled

Table 2
Free glucose and starch properties of brown and white tef flours.

Types	Free glucose*	Amylose (%)	Total starch*	Starch fraction (g/100 g starch)		
				RDS[a]	SDS[b]	RS[c]
Brown	0.5 ± 0.09	23.9 ± 0.62	74.6 ± 2.60	26.1 ± 0.16	32.4 ± 2.53	41.5 ± 2.64
White	0.5 ± 0.02	23.8 ± 0.48	76.7 ± 1.96	26.3 ± 0.68	33.1 ± 3.40	40.6 ± 3.33
p	0.507	0.895	0.319	0.668	0.785	0.732

* (g/100 g dm flour), p: p value.
[a] Rapidly digestible starch.
[b] Slowly digestible starch.
[c] Resistant starch. (n = 3).

Table 3
Physical features of sourdough tef breads: specific volume and texture.

SD%	SV (ml/g)	Hardness (N)	Springiness	Cohesiveness	Chewiness (J)	Resilience
0%	1.9 ± 0.02^{b}	10.4 ± 105^{c}	0.92 ± 0.03^{b}	0.57 ± 0.03	527 ± 49bc	0.27 ± 0.02
10%	1.9 ± 0.01^{ab}	10.5 ± 26^{c}	0.93 ± 0.03^{b}	0.57 ± 0.02	567 ± 31c	0.27 ± 0.01
20%	1.8 ± 0.05^{a}	9.2 ± 64^{b}	0.87 ± 0.02^{a}	0.58 ± 0.04	492 ± 63b	0.30 ± 0.03
30%	1.9 ± 0.01^{b}	7.7 ± 46^{a}	0.87 ± 0.01^{a}	0.56 ± 0.03	391 ± 32a	0.28 ± 0.02
p	0.003	< 0.001	< 0.001	0.543	< 0.001	0.125

^{a,b,c}Values within a column with different small superscript letters are significantly different ($p < 0.05$). SD: Sourdough; SV: specific volume; N: newton; J: joules. (n = 8).

the α-amylase to act on the amylopectin resulting a formation of more limit dextrins which in turn increase the RS proportion. Freezing/cooling of breads could also increase RS content due to retrogradation of starch and this was well demonstrated in all the bread of this study.

As the breads get older, the RDS fractions demonstrated a decreasing order, while SDS and RS contents increased (Table 4). All breads showed significant differences ($p < 0.001$) in RDS with the highest and lowest contents exhibited by the fresh and 5 days old breads, respectively. The highest and lowest SDS and RS contents were exhibited by the 5 days old and fresh breads, respectively. Similarly, cooked and stored rice varieties showed a decrease in RDS while their SDS and RS increased (Rachel, Lu, Chang, & Chiang, 2015). Unlike breads in this study which exhibited low RDS and high SDS and RS, corn and potato based low moisture commercial gluten free breads revealed significantly higher RDS and low SDS and RS in the range of 75–93, 2–21 and 1–3 g/100 g starch, respectively (Segura & Rosell, 2011). The RDS content of the fresh breads (49–58%) in this study (Table 4) showed nearly a 2- fold increase while RS showed a 2-fold decrease compared to the flours (Table 2). However, tef breads still retain high amount of SDS and RS after baking compared to the corn and potato breads in which their RDS accounted for 93 g/100 g starch (Segura & Rosell, 2011). The increase in the RDS during baking could be principally attributed to the starch gelatinization.

During ageing of the breads, retrogradation could be undergone which can be evidenced in this study by the successive decrease of RDS while increase in both the SDS and RS contents. Storage of starch gels at temperatures 4–30 °C induces retrogradation (Wang, Li, Copeland, Niu, & Wang, 2015). The decrease of RDS and increase of SDS and RS of the breads during storage was also accompanied by loss of water. The dry matter contents of the breads (Table 5) ranged from 44% in fresh to 55% after 5 days. Sourdough could slow down retrogradation in that non-sourdough breads showed highest dry matter while the 30% sourdough bread exhibited the lowest dry matter. Sourdough fermentation postpones the starch retrogradation and staling of gluten-free bread (Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). The incorporation of sourdough and storage days did not affect significantly the FG content of the breads (Table 4). Nonetheless, the FG of the breads showed 2–5 folds increase compared to their flours (Table 2). This increase could be due to the starch hydrolysis during the fermentation process.

3.5. Estimated glycemic index (eGI) tef bread

The combined mean estimated glycemic index (eGI) of breads from brown and white tef flours are given in Table 5. The eGI was calculated using models of Goni et al. (1997) (eGI_G) and Granfeldt et al. (1992) (eGI_{Gr}), as these models are being used interchangeably they may result in different eGI (Shumoy & Raes, 2017a). As the amount of sourdough increased from 0 to 30%, the eGI of the fresh breads increased as expressed in eGI_G and eGI_{Gr} in the range of 75–89 and 72–86, respectively. Upon storage, most of these breads also showed an increase in eGI in the range of 70–74, 66–74, 57–67 expressed in eGI_G while 58–67, 51–62, 39–52 in eGI_{Gr}, parallel to the amount of sourdough they contain (10, 20 and 30% respectively). Sourdough or non-sourdough breads of buckwheat, quinoa and sorghum showed eGI that ranged from 68 to 103 (Wolter et al., 2014). Breads with higher sourdough proportion retrogrades slowly which could cause fast hydrolysis (Fardet et al., 2006) resulting in high eGI. Similarly, sourdough breads of quinoa and buckwheat showed higher eGI than their non-sourdough breads while those of tef and sorghum showed lower eGI than their non-sourdough counterparts (Wolter et al., 2014). Sourdough fermentation resulted in a soft bread crumb (Wolter et al., 2014). Indeed, as seen in Table 3, the hardness of the breads reduced when the amount of the sourdough increased which could explain for the increased eGI. Organic acids, such as lactic, acetic and propionic acids, could slow down the gastric emptying (Liljeberg & Bjorck, 1996) resulting in a lower GI.

Table 4
Starch fractions and free glucose contents of sourdough tef breads of different storage time (day).

Storage	Sourdough %				p	Sourdough %				p
	0	10	20	30		0	10	20	30	
	RDS (g/100 g starch)					SDS (g/100 g starch)				
Fresh	52 ± 3.4^{bA}	49 ± 2.4^{cA}	51 ± 1.6^{dA}	58 ± 1.6^{dB}	< 0.001	29 ± 2.6^{aB}	29 ± 1.9aB	28 ± 2.1^{aB}	16 ± 1.5^{aA}	< 0.001
1	40 ± 2.3^{aAB}	44 ± 2.7^{bB}	40 ± 4.5^{cAB}	35 ± 0.7^{bA}	0.001	39 ± 2.9^{bB}	33 ± 2.3^{bA}	33 ± 0.76^{bA}	35 ± 1.8^{bA}	0.008
2	39 ± 2.1^{aB}	38 ± 1.9^{aAB}	35 ± 1.6^{bA}	38 ± 0.97^{cAB}	0.015	37 ± 3.0^{bAB}	33 ± 1.9^{bA}	40 ± 0.21^{cB}	33 ± 2.1^{bA}	0.006
5	35 ± 3.3^{aB}	37 ± 1.3^{aB}	28 ± 2.7^{aA}	26 ± 1.0^{aA}	< 0.001	35 ± 1.9^{bAB}	32 ± 2.1^{bA}	38 ± 2.0^{cBC}	39 ± 3.1^{cC}	0.002
p	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	
	RS (g/100 g starch)					FG (g/100 g dm flour)				
Fresh	20 ± 1.3^{aA}	22 ± 1.2^{aA}	23 ± 2.6^{aAB}	26 ± 2.0^{aB}	0.005	1.2 ± 0.11^{aA}	1.4 ± 0.38^{A}	1.8 ± 0.18^{B}	1.4 ± 0.22^{aA}	0.002
1	23 ± 2.0^{abA}	25 ± 1.7^{bAB}	30 ± 4.8^{bcB}	30 ± 2.5^{bB}	0.005	1.7 ± 0.26^{bA}	1.6 ± 0.26^{A}	1.7 ± 0.20^{A}	2.7 ± 0.07^{cB}	< 0.001
2	24 ± 1.5^{bA}	29 ± 1.0^{cBC}	27 ± 2.7^{abB}	31 ± 1.5^{bC}	< 0.001	2.0 ± 0.10^{cB}	1.5 ± 0.28^{A}	1.7 ± 0.16^{AB}	1.9 ± 0.41^{bB}	0.020
5	30 ± 1.8^{c}	31 ± 2.3^{c}	33 ± 1.7^{c}	33 ± 3.2^{b}	0.132	1.6 ± 0.06^{b}	1.6 ± 0.36	1.5 ± 0.34	1.7 ± 0.11^{ab}	0.634
p	< 0.001	< 0.001	< 0.001	0.004		< 0.001	0.794	0.142	< 0.001	

^{a,b,c,d}Values within a column with different small superscript letters are significantly different ($p < 0.05$). ^{A,B,C}Values across rows with different capital superscript letters are significantly different ($p < 0.05$). p: p-value. (n = 6).

Table 5
Estimated glycemic index (eGI) of sourdough tef breads of different storage ages in days.

ST	Sourdough proportion%					Sourdough proportion%				p
	0	10	20	30		0	10	20	30	
	eGI_G				p	eGI_{Gr}				
Fresh	75 ± 9^{b} (45)	83 ± 7^{b} (44)	85 ± 3^{c} (44)	89 ± 1^{c} (44)	0.062	72 ± 2.4^{cA}	82 ± 11^{cAB}	77 ± 0.88^{cAB}	86 ± 1.7^{bB}	0.026
1	72 ± 5^{b} (46)	70 ± 6^{a} (45)	74 ± 3^{b} (46)	74 ± 9^{b} (45)	0.784	58 ± 8.5^{b}	67 ± 10^{b}	62 ± 4.5^{b}	54 ± 11.5^{a}	0.283
2	66 ± 2^{abA} (48)	69 ± 3^{aAB} (47)	74 ± 3^{bB} (48)	73 ± 5^{bB} (45)	0.025	51 ± 2.2^{bA}	55 ± 4.3^{bAB}	62 ± 5.3^{bB}	60 ± 4.01^{aAB}	0.034
5	57 ± 3^{aA} (53)	67 ± 2^{aB} (48)	63 ± 3^{aAB} (51)	62 ± 7^{aAB} (45)	0.009	39 ± 3.8^{aA}	50 ± 2.3^{aBC}	45 ± 3.9^{aAB}	52 ± 1.0^{aC}	0.001
p	0.002	0.002	< 0.001	< 0.001		< 0.001	0.001	< 0.001	< 0.001	

eGIG = (0.549 × HI) + 39:71, eGIGr = (0.862 × HI) + 8.198. [a,b,c]Values within a column with different small superscript letters are significantly different ($p < 0.05$). [A,B]Values across rows with different capital superscript letters are significantly different ($p < 0.05$). p: p-value. ST, storage time in days. Values in brackets () are the dry matter contents of the breads. (n = 6).

However, this effect could not be speculated in this study as digestion was *in vitro*. The LAB starter used in this study could produce such acids in that the breads may result in a different trend of GI in *in vivo* digestion.

The highest and lowest eGI were recorded for fresh and 5 days old breads in all the breads, regardless of the amount of sourdough (Table 5). High eGI in the range of 94–137 and 79–99, respectively of fresh *injera* (fermented flat bread) and thick porridge were reported (Shumoy & Raes, 2017a). The reason why tef *injera* and porridge exhibited much higher eGI than the tef breads could be attributed to the difference in processing, ingredients but mainly the moisture contents of the products. The moisture content of the fresh *injera* and porridge, ranging from 71 to 73% and 59–66%, respectively (Shumoy & Raes, 2017a), was very high compared to that of 47–56% in tef bread. At high water content (water/starch > 1.5) and temperature of 50–80 °C, starches undergo a complete gelatinization leading to higher GI (Wang & Copeland, 2013).

Formulation of gluten free breads involves high water levels imposing disadvantages of higher GI and shorter shelf-life. In fact, it was reported that higher levels of water during bread processing lowered the RS in bread (Dewettinck et al., 2008) which could lead to high GI. Although the breads in this study contained higher water levels, they showed lower GI compared to GI (83–96) of corn and potato sourdough breads with lower water levels (26–46%). This indicates that there is fundamental difference in the native starches that makes tef a potential cereal for low GI food products.

When fresh white wheat bread is used as a reference to calculate the hydrolysis index (HI), food products can be classified, as low GI (GI < 60), medium GI (GI = 60–85) and high GI (GI > 85) (Shumoy & Raes, 2017a). Based on this classification, fresh tef breads showed a medium eGI except for breads that contained 30% sourdough. Interestingly, after one day of room temperature storage, the eGI of all the breads fell into the lower medium category of GI. As aging of the breads increased up to 5 days, the eGI even goes down to the low category. This study reports for the first time on the *in vitro* eGI of conventionally prepared tef breads as eaten. So far, Wolter et al. (2013), had reported an eGI of 74 for conventional tef bread, however the breads were frozen (at least not mentioned if it was done on the fresh breads).

This study showed that the same bread prepared from a particular cereal could have significantly different GI depending on its freshness level. Breads in the contemporary bakery and/or supermarkets can be found at different freshness level. Thus, when reporting GI of food products, it is worthy to indicate the duration and the temperature at which the samples were stored. Results of this study could have importance to help consumers in choosing the type of breads based on their personalized requirements. Fresh breads could have the best quality in terms of organoleptic properties. Nonetheless from a nutritional and/or health point of view, particularly GI, breads of 1 or 2 days old could be important to control blood glucose level. To that end, breads of 5 days old could be consumed if their safety is not compromised.

Although, GI is considered as the best indicator of blood glucose release of starchy food products, digestibility based starch fractions could also be a good indicator if complemented with the GI results (Haydersah et al., 2012). To assess this, Pearson's correlation analysis was conducted on data generated: The RDS content of all the breads was strongly correlated to their corresponding eGI ($r = 0.79$, $p < 0.001$), while the SDS and RS were negatively correlated ($r = -0.67$, $p < 0.001$) and ($r = -0.52$, $p < 0.001$), respectively. Therefore, depending on the values of the Pearson's correlation coefficient (r), the effect of the contents of starch fractions of tef bread on the resulting GI is dependent on RDS > SDS > RS in a decreasing order. The eGI of the breads also showed a strong negative correlation with the age of the breads ($r = -0.76$, $p < 0.001$) while it exhibited a weak positive correlation with the added sourdough proportion ($r = 0.32$, $p = 0.05$). The other result was the correlation of the aging duration of the breads with their starch fractions. The RDS, SDS and RS contents strongly correlated with the duration of bread storages ($r = -0.79$, $p < 0.001$), ($r = 0.50$, $p < 0.001$) and ($r = 0.72$, $p = 0.05$), respectively. Unlike RDS and SDS which did not show any correlation with the added sourdough, the RS showed meaningful positive correlation with sourdough ($r = 0.48$, $p < 0.001$). Sourdough bread of sorghum and quinoa showed higher RS than their non-sourdough counterparts (Wolter et al., 2014).

Moreover, use of HPMC, sugar and salt as ingredients could impact eGI. Inclusion of 5% HPMC decreased the rise of postprandial blood glucose level in rats (Brockman, Chen, & Gallaher, 2012). However, the HPMC used in this study contributed to the softness which in turn can increase the rate of starch digestibility resulting in high GI (Fardet et al., 2006). Salt accelerates rate of starch digestion and glucose absorption thereby it may have increased the GI of the breads in this study (Thorburn, Brand, & Truswell, 1986). During fermentation, sugar is used as the source of energy for the yeast in that its effect on GI depends on how much of the original sugar is found in the bread.

3.6. Tef protein content and in vitro digestibility

Brown and white tef flours (Table 1) showed similar protein content in the range of 9–11. The *in vitro* protein digestibility (IVPD) of the brown and white tef flours and their sourdough and non-sourdough breads are given in Fig. 3. The IVPD of the tef flour and bread ranged from 69 to 72% but did not show any significant difference ($p < 0.05$). Previously flour and *injera*, a traditional fermented flat pancake prepared from different tef varieties, exhibited protein content and IVPD, respectively in the range of 8.5–9.4 g/100 g dm and 71–75 (Shumoy et al., 2018). Tef has similar protein content compared to other cereals (Shumoy & Raes, 2017b), however, it could be a good source of protein as its IVPD was higher than other *gluten-free* cereals, such as finger millet (48%) (Antony & Chandra, 1998), sorghum and maize (59–67%) (Duodu et al., 2002).

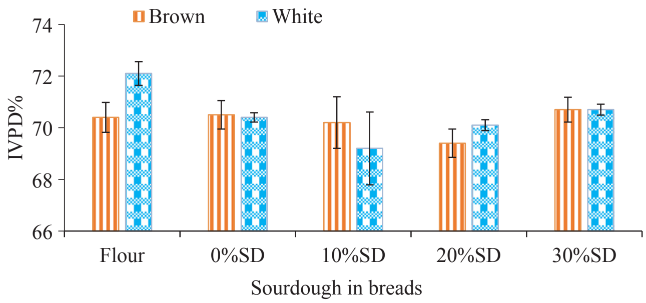

Fig. 3. *In vitro* protein digestibility tef flour and bread.

4. Conclusions

Incorporation of sourdough slightly increased the RS, without however, significantly affecting the RDS and SDS contents. Addition of sourdough could increase the eGI of fresh tef breads. Fresh tef breads resulted in medium eGI, however, after 1 or 2 days of storage, they fell into a low and lower medium category of eGI. Consumption of breads after 1 or 2 days storage could be a good option to attain a lower GI. The effect of sourdough addition on shelf life and organoleptic properties, as well as staling rate of tef bread is worthy of study. Nutritionally, tef could be a potential source of protein.

5. Funding

This work was supported by Erasmus Mundus Action 2 partnership program of the European Union within the CARIBU project [grant number: strand 1/Lot L18].

References

AACC (1999). Determination of Falling Number. Retrieved from *Approved method of the American association of cerial chemists* (pp. 1–4). (9th ed.). St.Paul, MN: American Association of Cereial Chemists, Inc.
Akinola, S. (2017). Lactic acid bacteria and yeast diversities in spontaneously fermented millet sourdoughs. *Journal of Microbiology, Biotechnology and Food Sciences, 6*, 1030–1035.
Antony, U., & Chandra, T. S. (1998). Antinutrient reduction and enhancement in protein, starch, and mineral availability in fermented flour of finger millet (Eleusine coracana). *Journal of Agricultural and Food Chemistry, 46*(97), 2578–2582.
AOAC. (1995). AOAC Association of Official Analytical Chemists- Method 46-11.02. Crude protein- improved Kjeldahl method. (16th ed.). Arlington, Virginia, USA.
Arendt, A. E. K., Ryan, L. A. M., & Bello, F. D. (2007). Impact of sourdough on the texture of bread. *Food Microbiology, 24*, 165–174.
Brockman, D. A., Chen, X., & Gallaher, D. D. (2012). Hydroxypropyl methylcellulose, a viscous soluble fiber, reduces insulin resistance and decreases fatty liver in Zucker Diabetic Fatty rats. *Nutrition and Metabolism, 9*, 1–12.
Bultosa, G. (2007). Physicochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc.) Trotter] grain varieties. *Journal of Applied Sciences Research, 3*(12), 2042–2050.
Cappa, C., Lucisano, M., Raineri, A., Fongaro, L., Foschino, R., & Mariotti, M. (2016). Gluten-free bread: Influence of sourdough and compressed yeast on proofing and baking properties. Retrieved from *Foods, 5*, 69–80. http://www.mdpi.com/2304-8158/5/4/69.
De La Hera, E., Rosell, C. M., & Gomez, M. (2014). Effect of water content and flour particle size on gluten-free bread quality and digestibility. *Food Chemistry, 151*, 526–531.
Debonne, E., Leyn, I. De, Verwaeren, J., Moens, S., Devlieghere, F., Eeckhout, M., & Bockstaele, F. Van (2018). The in fl uence of natural oils of blackcurrant, black cumin seed, thyme and wheat germ on dough and bread technological and microbiological quality. *LWT – Food Science and Technology, 93*, 212–219.
Dewettinck, K., Van Bockstaele, F., Kuhne, B., Van de Walle, D., Courtens, T. M., & Gellynck, X. (2008). Nutritional value of bread: Influence of processing, food interaction and consumer perception. *Journal of Cereal Science, 48*(2), 243–257.
Duodu, K. G., Nunes, A., Delgadillo, I., Parker, M. L., Mills, E. N. C., Belton, P. S., & Taylor, J. R. N. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science, 35*(2), 161–174.
Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurment of nutrittionally important starch fractions. *European Journal of Cinical Nutrition, 46*, 33–50.
Fardet, A., Leenhardt, F., Lioger, D., Scalbert, A., & Remesy, C. (2006). Parameters controlling the glycaemic response to breads. *Nutrition Research Reviews, 19*, 18–25.
Goni, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437.
Granfeldt, Y., Bjorck, I., Drews, A., & Tovar, J. (1992). An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. *European Journal of Clinical Nutrition, 46*(2), 649–660.
Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice, 103*(2), 137–149.
Hager, A. S., & Arendt, E. K. (2013). Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat. *Food Hydrocolloids, 32*(1), 195–203.
Hager, A. S., Wolter, A., Czerny, M., Bez, J., Zannini, E., Arendt, E. K., & Czerny, M. (2012). Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. *European Food Research and Technology, 235*(2), 333–344.
Hager, A. S., Wolter, A., Jacob, F., Zannini, E., & Arendt, E. K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *Journal of Cereal Science, 56*(2), 239–247.
Haydersah, J., Chevallier, I., Rochette, I., Mouquet-Rivier, C., Picq, C., Marianne-Pépin, T., ... Guyot, J. P. (2012). Fermentation by amylolytic lactic acid bacteria and consequences for starch digestibility of plantain, breadfruit, and sweet potato flours. *Journal of Food Science, 77*(8), 466–472.
Hellemans, T., Abera, G., Leyn, I. De, Meeren, P. Van, Der, Bockstaele, Van, F., ... De, B. (2017). Composition, Granular Structure, and Pasting Properties of Native Starch Extracted from Plectranthus edulis (Oromo dinich) Tubers. *Journal of Food Science, 82*, 2794–2804.
Hsu, H. W., Satterlee, D. L., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42*, 1269–1273.
Kweon, M. (2010). Falling number in wheat. Retrieved September 6, 2017, from https://www.ars.usda.gov/.
Lappi, J., Selinheimo, E., Schwab, U., Katina, K., Lehtinen, P., Mykkänen, H., ... Poutanen, K. (2010). Sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. *Journal of Cereal Science, 51*(1), 152–158.
Liljeberg, H., & Bjorck, I. (1996). Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: Studies in humans and rats using test products with added organic acids or an organic salt. *American Journal of Clinical Nutrition, 64*, 886–893.
Marti, A., Marengo, M., Bonomi, F., Casiraghi, M. C., Franzetti, L., Pagani, M. A., & Iametti, S. (2017). Molecular features of fermented teff flour relate to its suitability for the production of enriched gluten-free bread. *LWT – Food Science and Technology, 78*(2017), 296–302.
Patel, H., Chandra, S., Alexander, S., Soble, J., & Williams, K. A. (2017). Plant-based nutrition: An essential component of cardiovascular disease prevention and management. *Current Cardiology Reports, 104*, 1–10.
Rachel, H., Lu, S., Chang, Y. H., & Chiang, W. (2015). Effects of cooking, retrogradation and drying on starch digestibility in instant rice making. *Journal of Cereal Science, 65*(2015), 154–161.
Rinaldi, M., Paciulli, M., Caligiani, A., Scazzina, F., & Chiavaro, E. (2017). Sourdough fermentation and chestnut flour in gluten-free bread: A shelf-life evaluation. *Food Chemistry, 224*(2017), 144–152.
Sakhare, S. D., Inamdar, A. A., & Soumya, C. (2004). Effect of flour particle size on microstructural, rheological and physico-sensory characteristics of bread and south Indian parotta. *Journal of Food Science and Technology, 51*, 4108–4113.
Segura, M. E. M., & Rosell, C. M. (2011). Chemical composition and starch digestibility of different gluten-free breads. *Plant Foods for Human Nutrition, 66*(3), 224–230.
Shumoy, H., Pattyn, S., & Raes, K. (2018). Tef protein: Solubility characterization, in-vitro digestibility and its suitability as a gluten free ingredient. *LWT – Food Science and Technology, 89*, 697–703.
Shumoy, H., & Raes, K. (2017a). In vitro starch hydrolysis and estimated glycemic index of tef porridge and injera. *Food Chemistry, 229*, 381–387.
Shumoy, H., & Raes, K. (2017b). Tef : The rising ancient cereal : What do we know about its nutritional and health benefits? *Plant Foods for Human Nutrition, 72*, 335–344.
Thorburn, A. W., Brand, J. C., & Truswell, A. S. (1986). Salt and the glycaemic response. *British Medical Journal, 292*, 1697–1699.
Wang, S., & Copeland, L. (2013). Molecular disassembly of starch granules during gelatinization and its effect on starch digestibility: A review. *Food & Function, 4*(11), 1564.
Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety, 14*(5), 568–585.
Wolter, A., Hager, A.-S. S., Zannini, E., & Arendt, E. K. (2014). Influence of sourdough on in vitro starch digestibility and predicted glycemic indices of gluten-free breads. *Food and Function, 5*, 564–572.
Wolter, A., Hager, A. S., Zannini, E., & Arendt, E. K. (2013). In vitro starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread. *Journal of Cereal Science, 58*(3), 431–436.
Wolter, A., Hager, A. S., Zannini, E., Galle, S., Gänzle, M. G., Waters, D. M., & Arendt, E. K. (2014). Evaluation of exopolysaccharide producing Weissella cibaria MG1 strain for the production of sourdough from various flours. *Food Microbiology, 37*, 44–50.