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Reducing of acrylamide formation in wheat biscuits supplemented with flaxseed and lupine

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ABSTRACT

The use of pseudo-cereals for wheat products making is to fortify the deficiency of nutritional value in wheat flour. However rich in proteins plant additives could increase acrylamide content in baked products. The present study was focused on acrylamide reduction in wheat flour biscuits supplemented with lupine and defatted flaxseed flour treated by solid state (SSF) and submerged (SMF) fermentations by *Lactobacillus sakei*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* strains. After fermentation the decrease in asparagine was on average of 67.6 and 80.6%, and reducing sugar contents were reduced by 18 and 79.4% in flaxseed and lupine, respectively. The most effective acrylamide reduction in biscuits (78 and 85%, respectively) was reached using *P. acidilactici* for flaxseed (SMF) and lupine (SSF). It was found a significant effects of lupine or flaxseed addition ($F(1.24) = 4032$, $P < 0.001$), fermentation method ($F(1.24) = 22{,}371$, $P < 0.001$), type of microorganisms applied for the fermentation ($F(2.24) = 5907$, $P < 0.001$) and interaction of these factors ($F(7.24) = 40{,}001$, $P < 0.001$) on acrylamide concentration in wheat biscuits. Using *L. sakei* for SSF of flaxseed and SMF of lupine acrylamide was reduced on average by 83.4%. Fermented lupine and flaxseed could have potential application for production of safe and high nutritional value biscuits.

1. Introduction

Use of refined ingredients makes biscuits lack of grain components that are supposed to be protective of health (Fardet, 2010). The major constituents of biscuits are typically wheat flour, sucrose and fat, making this products a rather energy dense cereal food (Sozer, Cicerelli, Heiniö, & Poutanen, 2014).

Flaxseeds were recognized as one of the richest dietary sources of lignans, and the amount of enterolignans formed from flaxseed (10,433.2 nmol/g) is of another magnitude than from cereal products (Bartkiene, Juodeikiene, & Basinskiene, 2012). Flaxseeds may be associated with decreased risk of breast cancer, and demonstrates antiproliferative effects in breast tissue of women at risk of breast cancer and may protect against primary breast cancer (Flower et al., 2014). Therefore, the most valuable flaxseed products for thermal treated food preparation from a nutrition point of view are dried and defatted flaxseed, because during the baking process 50–60% of fatty acids are transferred to *trans*-isomers (Bartkiene et al., 2009).

Human intervention studies have demonstrated that sweet lupine exert several physiological benefits (Fechner, Kiehntopf, & Jahreis, 2014). The mean protein content of lupin is significantly higher compared to pea and similar to soya bean (Bahr, Fechner, Hasenkopf, Mittermaier, & Jahreis, 2014). Among other amino acids, in lupine seeds is high amount of asparagine (Kaczmarek, Kasprowicz-Potocka, Hejdysz, Mikuła, & Rutkowski, 2014).

Free amino acids, mainly asparagine, and reducing sugars are important precursors to acrylamide in foods and that processing conditions, such as temperature, water activity and matrix, influence its formation and degradation (Keramat, LeBail, Prost, & Jafari,

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2011). Estimation of acrylamide occurrence in food commodities is of great concern in many countries. The amount of acrylamide is extremely high in biscuits (Krishnakumar & Visvanathan, 2014). Therefore, biscuits are a popular cereal food category, consumed as breakfast items and as snacks.

Reduction of acrylamide content in cereal products can be achieved by fermentation with selected lactic acid bacteria (LAB) (Bartkiene et al., 2013a,b). Also, it is known that lactic acid fermentation could improve digestibility of lupine proteins (Bartkiene, Krungleviciute, Juodeikiene, Vidmantiene, & Maknickiene, 2014). But biscuit dough is a complex system containing lower amount of water (Sudha, Chetana, & Reddy, 2014), and in this case solid state fermentation (SSF) will be more adaptable. Nowadays, a lot of experiments are published about the biscuits functional value increasing (Himeda et al., 2014; Jayalaxmi, Vijayalakshmi, Durgannavar, & Chandru, 2015; Onabanjo & Dickson, 2014; Pasqualonea et al., 2015; Kaura, Sandhub, Arorab, & Sharmac, 2015). The research was focused on an increase of antioxidant activity, analysis of dietary fibre compounds and sensory properties of the biscuits. In our opinion, the influence of new ingredients included in biscuits formula should be analysed more complex, and one of safety parameters, such as acrylamide formation, should be evaluated. The formation of undesirable compounds during manufacturing process is unavoidable, and in the case to reduce them the different technological solutions could be used.

The present study was undertaken to develop the functional bakery goods, enriched with flaxseed and lupine nutritional components. The solid state (SSF) and submerged (SMF) fermentations by bacteriocins producing strains were applied for treatment of flaxseed and lupine seeds in case to reduce acrylamide contents in the end product.

2. Materials and methods

2.1. Materials

Wheat flour (type 550D, falling number 350 s, gluten 270 g/kg, ash 6.8 g/kg) obtained from JSC Kauno grudai (Kaunas, Lithuania), margarine (80% fat) (Vilnius margarine factory, Lithuania), sugar, eggs, salt, baking powder (composition: sodium pyrophosphate, sodium bicarbonate, wheat flour; by weight 40/40/20, respectively) purchased from the local supermarket were used for biscuits preparation. Partially defatted flaxseed (*Linum usitatissimum* L.) flours (BioFlax, Poland) contained protein 40 g/100 g, fat 9 g/100 g, carbohydrate 3 g/100 g, cellulose content 36 g/100 g). The blue lupine seeds (*Lupinus angustifolius* L.) No. 1734 with low alkaloid content (<0.1%) were obtained from the Lithuanian Institute of Agriculture (Voke, Lithuania) in 2014. The *Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-9, *Lactobacillus sakei* KTU05-6, previously isolated from spontaneous rye sourdough (Digaitiene, Hansen, Juodeikiene, Eidukonyte, & Josephsen, 2012) were cultured at 30 °C temperature for 48 h in MRS medium (CM0359, Oxoid, Hampshire, United Kingdom) prior to be used.

2.2. Fermentation of plant material

Flaxseed or lupine flour (200 g) were mixed with water and 2% (w/v) of pure culture of *P. acidilactici*, *P. pentosaceus*, *L. sakei*. Water content was calculated with a reference to moisture content of the raw materials and required humidity of the end product for solid state fermentation (SSF) (moisture content 45%) and for submerged fermentation (SMF) (moisture content 65%). Fermentation was carried out for 48 h at optimal temperatures for lactic acid bacteria (LAB) cultivation: 32 °C (*P. acidilactici*), 35 °C (*P. pentosaceus*) and 30 °C (*L. sakei*). Six different sourdoughs from each plant were made using different LAB and different fermentation technologies (SSF and SMF). The pH values of sourdoughs were measured and recorded with a pH electrode (PP-15, Sartorius, Gottingen, Germany). Total titratable acidity (TTA) was determined on a 10 g sample (sourdough) homogenized with 90 mL of distilled water and expressed as millilitres of 0.1 mol/L NaOH to achieve a pH of 8.2.

2.3. Microbiological analysis of fermented plant material

Ten grams of sample were homogenized with 90 mL of saline (sodium chloride 9 g/L). Serial dilutions of 10^{-4}–10^{-8} with saline were used for sample dilution. Sterile MRS agar (CM0361, Oxoid) of 5 mm of thickness was used for the bacteria growth (Man, Rogosa, Sharpe) in Petri plates. The plates were separately seeded with the sample suspension using sowing in surface and were incubated under anaerobic conditions at 30 °C for 72 h. The number of bacteria colonies was calculated and expressed as a log of colony forming units per gram of sample (cfu/g).

2.4. Determination of α-amylase and protease activity in fermented plant samples

The α-amylase levels excreted by single LAB were determined by starch-iodine method described by Nguyen, Rezessy-Szabó, Claeyssens, Stals, and Hoschke (2002). The mode of action of the LAB protease was determined by Sigma–Aldrich non-specific protease assay.

2.5. Determination of reducing saccharides

Reducing saccharides in flaxseed, lupine and wheat flour were determined using a Waters HPLC system (Waters, Milford, Massachusetts, USA) with a photodiode array detector and a Waters Micromass ZQ-2000 mass detector (Bartkiene et al., 2013b).

2.6. Determination of asparagine by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

Asparagine was determined in fermented and non fermented flaxseed and lupine samples, and in wheat flour by Qtrap 5500 tandem quadrupole mass spectrometer (AB Sciex, Framingham, Massachusetts, USA) equipped with an electrospray ionization (ESI) source coupled with an Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters) (Bartkiene et al., 2015).

2.7. Biscuit formula and preparation

2.7.1. Main formula for biscuit preparation

Wheat flour 300 g, margarine 100 g, saccharose 80 g, eggs 80 g, salt 1 g, baking powder 1.5 g. Test samples were prepared by addition of flaxseed and lupine fermented with each of LAB using different processing (SSF and SMF) to the main biscuit dough at levels of 25 g, 50 g and 75 g. With each bacteria were prepared six biscuit dough samples with flaxseed and six with lupine. Control samples were prepared with 25 g, 50 g and 75 g of non fermented plants. Sugar and fat were creamed in a mixer (Guangzhou R & M Machinery, Guangdong, China). Eggs were added to this cream and mixed for 0.5 min to obtain a homogeneous cream. Finally, flour was added and mixed for 1 min to obtain a homogeneous dough. Biscuits were formed manually by rolling with rolling machine “Roll S5B” (Vicenza, Italy), the thickness of the dough was 2.5 mm, and stamping was carried out by hands. Biscuits were baked in a deck oven (MIWE, Michael Wenz, Germany) at 220 °C for 10 min.

The control sample was prepared without plant additives.

2.8. Determination of acrylamide by LC-MS/MS

For acrylamide analysis, all biscuits made using the one formula were milled and obtained powder was used for acrylamide evaluation as described by Bartkiene et al. (2013a). All measurements were made in triplicate.

2.9. Colour measurement

The colour characteristics of the biscuits were evaluated using CIEL*a*b* system (CromaMeter CR-400, Conica Minolta, Japan). L* is a measure of lightness from completely opaque (0) to completely white (100). a* is a measure of redness (or −a* of greenness) and b* is a measure of yellowness (or −b* of blueness) (Mc Guire, 1992).

2.10. Statistical analysis

All analytical experiments were carried out in triplicate. In order to evaluate an influence of three different factors (fermentation method, non-traditional cereals additive and application of several microorganisms) and their interaction on acrylamide content in the final product, data were subjected to analysis of the three-way analysis of variance (ANOVA) and the Tukey HSD test as post-hoc test (statistical program R 3.2.1, R Core Team, 2015). The results were referred to statistically significant at $P < 0.05$.

3. Results and discussion

3.1. The effectiveness of lactobacilli fermentation on defatted flaxseed and lupine

The cell number of LAB during flaxseed fermentation was ranging from the 7.90 log cfu/g to the 9.45 log cfu/g (in SMF and SSF with *L. sakei* samples, respectively) (Table 1), while *P. acidilactici* and *P. pentosaceus* cell numbers in SMF samples were higher by 9.4% and 13.3%, and in SSF samples were lower by 5.5% and 12.6%, respectively. After 48 h of fermentation with *L. sakei*, *P. acidilactici* and *P. pentosaceus* the lowest pH (4.16; 4.27 and 4.43, respectively) and highest TTA (9.4; 15.4 and 16.0, respectively) were found in SMF flaxseed samples (Table 1). Flaxseed treatment by SSF gave pH higher by 32.7; 15.8 and 9.6%, and TTA lower by 46.8; 64.9 and 62.5%, respectively, compared to SMF (Table 1).

The optimum pH of sourdough LAB is typically between 5.0 and 6.0 (Messens, Neysens, Vansieleghem, Vanderhoeven, & De Vuyst, 2002). Because the pH but not the organic acid concentration limits the growth of LAB in sourdough, the selection of cereal substrates with a high buffering capacity, allows the production of sourdough with a high concentration of organic acids and a corresponding high TTA (Gobbetti & Gänzle, 2013). It was not found a significant relation between cell number of LAB and pH and TTA ($R^2 = 0.3076$ and $R^2 = 0.2234$, respectively) in flaxseed.

In all cases higher viable cells counts of LAB were calculated in SSF lupine samples (ranging from 8.9 to 9.2 log cfu/g), in compare with SMF (ranging from 6.5 to 8.7 log cfu/g). After 48 h of fermentation pH of SSF and SMF lupine was ranging from 4.03 to 4.81 (in SMF with *P. pentosaceus* lupine and in SSF with *P. acidilactici* lupine, respectively), being lower than that after 24 h of fermentation (Table 1). In all analysed SMF samples pH was found lower, than in SSF (7.3%, 13.0% and 15.8%, in samples fermented with *P. pentosaceus*, *L. sakei* and *P. acidilactici*, respectively).

Rapid LAB growth and acidification are the basic aims of fermentation (Corbo, Bevilacqua, Campaniello, Speranza, & Sinigaglia, 2014). Upon prolonged fermentation, higher metabolite concentrations are produced (Ravyts & De Vuyst, 2011). The competitiveness of LAB are not only influenced by the technological parameters but are also depended on the cereal substrate, i.e., the unique sugar composition of certain flours (Moroni, Arendt, & Dal Bello, 2010). In opposite to the pH results, we found that TTA of fermented lupine was higher in SSF samples in compare with SMF samples, and all in experiment used LAB shown good possibilities to grow in lupine substrate (Table 1).

3.2. Protease and α-amylase activities of lactic acid bacteria in SMF and SSF treated plants

Proteolytic enzymes are directly involved in flavour and texture development and are indirectly involved in the maximalization of microbial cell growth by provision of essential amino acids (Matthews et al., 2004) and acrylamide reduction (Bartkiene et al., 2013a). Proteolytic enzymes activities of sourdough LAB determine the accumulation of (bioactive) peptides, amino acids, and amino acid metabolites in dough (Gänzle, 2014). Higher protease activity was found in SMF flaxseed, in compare with SSF (83.53% higher in with *P. acidilactici* fermented samples; 81.89% higher in with *P. pentosaceus* fermented samples; 48.79% higher in with *L. sakei* fermented samples, than in SSF, respectively) (Fig. 1). It was found a significant correlation between pH and protease activity in fermented flaxseed ($R^2 = 0.6792$; $P = 0.0437$).

Relatively great difference in the excreted protease level in regard to the single LAB used was noticed in lupine (Fig. 1). The *L. sakei* showed the highest protease activity (90.2 AU/g), followed by the *P. acidilactici* (84.0 AU/g), followed by the *P. pentosaceus* (75.6 AU/g). The lower protease activities were found in SSF conditions. Therefore, significant correlation between fermented

Table 1
Lactic acid bacteria (LAB) counts, pH, total titratable acidity (TTA) of the fermented plants.

Parameters	*L. sakei*		*P. acidilactici*		*P. pentosaceus*	
	SMF	SSF	SMF	SSF	SMF	SSF
Flaxseed						
LAB, cfu/g	7.90 ± 0.09a	9.45 ± 0.03bc	8.72 ± 0.05b	8.93 ± 0.04b	9.11 ± 0.08b	8.26 ± 0.04a
pH after 24 h	4.34 ± 0.02a	6.40 ± 0.01d	4.52 ± 0.02a	5.21 ± 0.03c	4.61 ± 0.01ab	5.20 ± 0.01c
pH after 48 h	4.16 ± 0.01a	6.18 ± 0.01d	4.27 ± 0.01a	5.07 ± 0.01c	4.43 ± 0.02ab	4.90 ± 0.01c
TTA after 48 h	9.4 ± 0.08d	5.0 ± 0.05a	15.4 ± 0.04e	5.4 ± 0.05b	16.0 ± 0.07e	6.0 ± 0.04c
Lupine						
LAB, cfu/g	7.30 ± 0.03b	9.20 ± 0.05cd	6.50 ± 0.04a	8.95 ± 0.02c	8.70 ± 0.03c	8.90 ± 0.04c
pH after 24 h	4.96 ± 0.03b	6.11 ± 0.03d	4.33 ± 0.03a	5.53 ± 0.03c	4.15 ± 0.03a	5.49 ± 0.03c
pH after 48 h	4.08 ± 0.03a	4.69 ± 0.03c	4.05 ± 0.03a	4.81 ± 0.03cd	4.03 ± 0.03a	4.35 ± 0.03b
TTA after 48 h	16.4 ± 0.04a	19.6 ± 0.04d	18.6 ± 0.04c	19.8 ± 0.04d	17.4 ± 0.04b	23.0 ± 0.04e

Data are presented as mean value ± standard deviation from triplicate experiments. Mean values listed in rows with different letters are significantly different ($P < 0.05$). SMF – submerged fermentation; SSF – solid state fermentation; TTA – total titratable acidity.

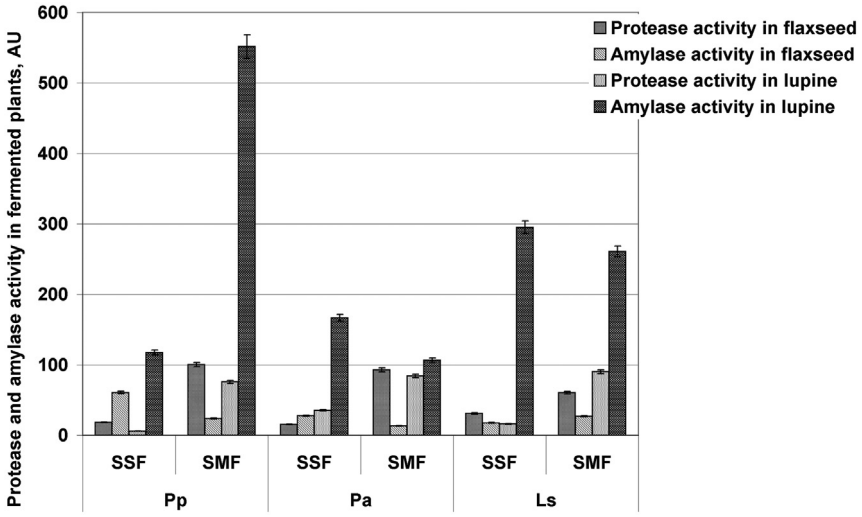

Fig. 1. Protease and amylase activities in flaxseed and lupine samples fermented by different LAB: Ls – *L. sakei*; Pa – *P. acidilactici*; Pp – *P. pentosaceus*. SMF – submerged fermentation; SSF – solid state fermentation.

lupine samples pH and protease activity was not found.

Cereal fermentations generally differs from most other fermentations because the final product is often not consumed but further processed to bread, crackers etc (De Valdez, Gerez, Torino, & Rollán, 2010). The aim of using a starter culture is to ferment sugars, improve nutritional attributes, decrease pH, which reduces the Maillard reactions initiated by heat (Bloom et al., 2009). The complete genome sequencing and annotations revealed the existence of amylase genes in almost all LAB (Petrova, Petrov, & Stoyancheva, 2013). LAB could be a source of potential amylolytic enzymes (Pranoto, Anggrahini, & Efendi, 2013). Despite the fact that a limiting factor for acrylamide formation is free asparagine (Loaec et al., 2014), the acrylamide content could be reduced by using LAB with lower amylolytic activity, thus lowering the reducing sugar content (Bartkiene et al., 2013a).

The highest amylase activity was found in SSF with *P. pentosaceus* defatted flaxseed (60.57 AU/g). In other fermented samples amylase activity was found from 2.2 to 4.6 times lower (in SSF and SMF with *P. acidilactici* defated flaxseed, respectively) (Fig. 1). There was no correlation between fermented samples (defatted flaxseed and lupine) pH and amylase activity. The amylase activities excreted in lupine sourdoughs prepared with *P. acidilactici*, *P. pentosaceus* and *L. sakei* after 48 h of fermentation were found to be ranging from 106.5 AU/g to 552.0 AU/g (in SSF with *P. acidilactici* and in SMF with *P. pentosaceus* lupine, respectively) (Fig. 1).

3.3. The effect of fermentation on asparagine and reducing saccharides content in plants

In pursuit of an improvement, a side from the revision of food processing which could limit the formation of acrylamide, the food industry has adopted another strategy, namely a better selection of raw materials which have low contents of the two acrylamide precursors, asparagine and reducing saccharides (FDE, 2014). Asparagine and reducing saccharides contents in wheat flour were of 140.8 mg/kg and 38.0 g/kg, respectively. Fermentation decreased asparagine and reducing saccharides content in flaxseed, in compare with non fermented samples (from 67.63 and to 17.93%, respectively) (Table 2). Fermentation with selected LAB reduced asparagine content in lupine samples from 78% to 83% (in SMF with *P. pentosaceus* lupine, and in SMF with *P. acidilactici* lupine, respectively). After 48 h fermentation of lupine sourdough reducing saccharides content in lupine samples was reduced from 40% to 64.9% (in SMF with *L. sakei* lupine, and in SMF with *P. acidilactici* lupine, respectively) (Table 2). As maximum L-asparaginase activity obtained with sucrose as carbon source (Anamika, Neeraja, Swarnalatha, Venkateshwar, & Venkateswar, 2013) it could be the reason, that in biscuits dough with high sucrose asparagine content was reduced by enzymes excreated by LAB.

3.4. The effect of fermented flaxseed and lupine addition on acrylamide content in biscuits

In all cases, non fermented flaxseed increased acrylamide content in biscuits, and the highest acrylamide content was found in biscuits with a higher amount of flaxseed (103.2 μg/kg) (Table 3). The addition of non fermented flaxseed (25 g, 50 g, 75 g) increased acrylamide content in biscuits by 70.9; 74.2 and 78.3%, respectively compared to control samples. The most effective decrease in acrylamide were reached in biscuits with 25 g of flaxseed treated by SSF with *L. sakei* (6 times), by SMF with *P. acidilactici* (6.4 times), and by SMF with *P. pentosaceus* (8.4 times).

Acrylamide content in biscuits samples increased by increasing the amount of non fermented lupine in biscuits formula (increase of 71, 74 and 78% in biscuits with 25 g; 50 g and 75 g non fermented lupine, respectively) (Table 3). The opposite tendencies were established in biscuits with fermented lupine. In most cases in biscuits with fermented lupine additives was found lower amount of acrylamide, in compare with control biscuits samples, and in compare with biscuits samples without lupine. The lowest amount of acrylamide was found in biscuits with 50 g of SSF with *L. sakei* and *P. pentosaceus* lupine additives (16.80 μg/kg and 12.61 μg/kg, respectively), and in biscuits with 75 g of SSF with *L. sakei* and *P. pentosaceus* lupine additives (10.01 μg/kg and 14.10 μg/kg, respectively).

According to European Food Safety Authority report the mean acrylamide content in biscuits was found 86 μg/kg (EFSA, 2012). As safety evaluation, it was estimated the tolerable daily intake (TDI) of acrylamide for neurotoxicity to be 40 μg/kg per day and for cancer to be 2.6 μg/kg per day (Tardiff, Gargas, Kirman, Carson, & Sweeney, 2010). Mitigation strategies propose modifying the product formulations or processing conditions. The food industry has investigated pathways of acrylamide formation. As a result

Table 2
Asparagine (mg/kg) and reducing sugar (g/kg) contents in non fermented and fermented flaxseed and lupine.

Parameters	Non fermented	*L. sakei*		*P. acidilactici*		*P. pentosaceus*	
		SMF	SSF	SMF	SSF	SMF	SSF
Flaxseed							
Asparagine	15.21 ± 0.05e	5.0 ± 0.03c	4.96 ± 0.02c	4.98 ± 0.06c	4.23 ± 0.04a	5.62 ± 0.05d	4.71 ± 0.02b
Reducing sugars	8.03 ± 0.05d	7.41 ± 0.03c	7.20 ± 0.02c	6.13 ± 0.01a	6.07 ± 0.04a	6.42 ± 0.07ab	6.28 ± 0.04a
Lupine							
Asparagine	28.20 ± 0.03d	5.61 ± 0.03b	5.32 ± 0.02b	4.83 ± 0.04a	5.01 ± 0.02a	6.13 ± 0.04c	5.84 ± 0.03bc
Reducing sugars	52.03 ± 0.25e	31.22 ± 0.16d	27.26 ± 0.23c	16.53 ± 0.21a	20.02 ± 0.14b	26.48 ± 0.17c	25.28 ± 0.34c

Data are presented as means ± standard deviation of triplicate determinations. Mean values listed in rows with different letters are significantly different ($P < 0.05$). SMF – submerged fermentation; SSF – solid state fermentation.

Table 3
Acrylamide contents (μg/kg dw) in biscuits supplemented with non fermented and fermented by different LAB flaxseed and lupine.

Biscuit samples	Flaxseed		Lupine	
Control	26.6 ± 0.17			
Non fermented				
25	91.3 ± 0.15k		93.5 ± 0.11m	
50	103.2 ± 0.20m		112.3 ± 0.28n	
75	122.7 ± 0.36n		123.7 ± 0.39p	
Biscuit samples	Flaxseed		Lupine	
	SSF	SMF	SSF	SMF
Control	26.6 ± 0.17			
Fermented by *P. pentosaceus*				
25	81.0 ± 0.03h	10.9 ± 0.18a	10.0 ± 0.14a	16.8 ± 0.12c
50	82.9 ± 0.09h	17.8 ± 0.09b	14.2 ± 0.08b	21.2 ± 0.16d
75	91.2 ± 0.06k	54.4 ± 0.21g	26.3 ± 0.10d	54.4 ± 0.28f
Fermented by *P. acidilactici*				
25	18.0 ± 0.18b	<10	18.8 ± 0.22c	11.0 ± 0.19a
50	37.9 ± 0.10e	22.6 ± 0.04c	48.0 ± 0.31e	17.7 ± 0.32c
75	40.7 ± 0.11f	33.1 ± 0.03d	77.8 ± 0.47h	65.0 ± 0.40g
Fermented by *L. sakei*				
25	<10	22.3 ± 0.09c	74.7 ± 0.18h	12.6 ± 0.09b
50	13.9 ± 0.15a	43.4 ± 0.25f	79.3 ± 0.23hk	21.7 ± 0.25d
75	29.8 ± 0.24d	50.2 ± 0.10g	84.1 ± 0.17k	21.9 ± 0.10d

Data are presented as means ± standard deviation of triplicate experiments. Mean values listed in columns within groups with different letters are significantly different ($P < 0.05$). Control – wheat flour biscuits without plant additives. 25, 50, 75 – amounts of additive (g) in biscuits. SMF – submerged fermentation; SSF – solid state fermentation; dw – dry weight.

voluntary measures were developed, such as the so-called 'toolbox' approach, which provides guidance to help producers and processors to identify ways to lower acrylamide in their respective products (CIAA, 2006,2009). During the joint workshop organised by the European Commission and the CIAA it was concluded that there had been only limited success in reducing acrylamide formation in cereals and cereal products in relation to recipe formulation and processing conditions (Konings, Ashby, Hamlet, & Thompson, 2007). However, there were some promising leads for the future, for example, the use of the enzyme asparaginase, which is now listed as a separate tool in the acrylamide toolbox (CIAA, 2009). Claus, Mongili, Weisz, Schieber, and Carle (2008) demonstrated that superficial application of cysteine to the dough of wheat bread and bread rolls prior to baking showed acrylamide lowering potential. Furthermore, addition of cysteine to the dough led to remarkably lower acrylamide levels in the bread. Among these, using asparaginase, eliminating reducing sugars, replacing ammonium bicarbonate with alternative leavening agents, addition of amino acids or protein hydrolizates, addition of calcium, and lowering baking temperature are the most prominent applications for bakery products (Acar, Pollio, Monaco, Fogliano, & Gökmen, 2012). The Maillard reaction requires a reducing saccharides such as glucose, fructose or maltose, although sucrose can participate if it is first hydrolysed through enzymic, thermal or acid-catalysed reaction (De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2009).

According to Claus, Schieber, and Carle (2008) review on cereal products, the most promising field for acrylamide reduction is the addition of low molecular weight additives such as polyphenols, which so far have not been applied in cereal products. Such additives ideally combine acrylamide reduction with little or no changes in product technology or, most importantly, sensory quality. In our experiment used plant additives could be interpreted as a biological raw material consisting a complex of micro and macro elements. Therefore, asparagine reduction in plant additives by using fermentation there was not a crucial factor in the reduction of acrylamide in biscuits.

Results of the ANOVA test indicated that there is a significant effect of lupine or flaxseed addition ($F(1.24) = 4032$, $P < 0.001$), fermentation method ($F(1.24) = 22{,}371$, $P < 0.001$), type of microorganisms applied for the fermentation ($F(2.24) = 5907$, $P < 0.001$) and interaction of these factors ($F(7.24) = 40{,}001$, $P < 0.001$) on acrylamide concentration in wheat biscuits. Since the interaction between factors has been determined as statistically significant no further statistical analysis of main factors were performed.

Tukey's HSD test was performed after ANOVA test with purpose to determine which groups in the results set have notable differences. Results of this test clearly indicates significant ($P < 0.001$ in all tests) differences between all three-way interaction groups.

3.5. Colour characteristics of the biscuits

For bakery products, colour is one of the most important attributes and critical acceptance by the consumers. In this study, the effect of plant additives on biscuits colour was investigated (Table 4). All biscuits samples with flaxseed additives were found to have lightness (L*) lower from 17% to 30% compared with biscuits without flaxseed. The values of a* of all the biscuits samples have a similar range. Higher value of b* was found in samples with flaxseed additives compared with biscuits without flaxseed (from 17 to 46%, of biscuits with 25 g with *P. acidilactici* SSF flaxseed and of biscuits with 75 g with *P. pentosaceus* SMF flaxseed).

The flaxseed and lupine additives in all cases reduced the brightness of biscuits: the addition of non fermented flaxseed and lupine additives influence the lightness (L^*) lower by 22.7% (*Pediococci*) and by 29.7% (*L. sakei*) (Table 4), and by 48.9% (*Pediococci*) and by 63.1% (*L. sakei*) (Table 5), respectively. SSF as well as SMF fermentations of flaxseed slightly increased (by 5.4–16.8% *Pediococci* and by 5.3–6.9% *L. sakei*) the brightness of biscuits (Table 4), but in case of fermented lupine the increase in brightness was higher (by 24.1–40.7% *Pediococci* and by 56.4–61.3% *L. sakei*) (Table 5) compared to non fermented samples.

The values of b^* was found higher by 20.5–32.4% and by 43.5–62.7% in biscuits with non fermented flaxseed (Table 4) and

Table 4
Colour characteristics of the biscuits supplemented with non fermented and fermented by different LAB flaxseed.

Biscuit samples	L^*		a^*		b^*	
Control	87.9 ± 0.21e		2.01 ± 0.14e		21.23 ± 0.10a	
With non fermented flaxseed						
25	62.13 ± 0.11a		0.32 ± 0.14a		26.77 ± 0.13b	
50	61.41 ± 0.24a		4.77 ± 0.17h		29.31 ± 0.21c	
75	68.10 ± 0.17b		4.82 ± 0.19h		31.42 ± 0.23d	
Biscuit samples	L^*		a^*		b^*	
	SSF	SMF	SSF	SMF	SSF	SMF
Control	87.9 ± 0.21e		2.01 ± 0.14e		21.23 ± 0.10a	
With fermented by *P. pentosaceus* flaxseed						
25	73.23 ± 0.22d	65.32 ± 0.22b	0.70 ± 0.11b	1.84 ± 0.12d	28.35 ± 0.25c	38.64 ± 0.10g
50	71.46 ± 0.31c	69.69 ± 0.31c	0.90 ± 0.01b	3.60 ± 0.09g	32.73 ± 0.13e	27.61 ± 0.23b
75	71.03 ± 0.17c	70.26 ± 0.16c	1.26 ± 0.09c	4.68 ± 0.15h	26.78 ± 0.10b	39.92 ± 0.21g
With fermented by *P. acidilactici* flaxseed						
25	69.61 ± 0.11c	66.10 ± 0.34b	3.75 ± 0.13g	1.27 ± 0.16c	25.62 ± 0.31b	31.95 ± 0.20d
50	69.39 ± 0.32c	72.11 ± 0.14c	3.14 ± 0.22f	5.20 ± 0.10j	30.56 ± 0.20d	28.15 ± 0.32c
75	72.88 ± 0.10d	73.96 ± 0.16d	1.87 ± 0.14d	3.02 ± 0.18f	26.93 ± 0.14b	29.69 ± 0.24c
With fermented by *L. sakei* flaxseed						
25	69.16 ± 0.10c	65.23 ± 0.12b	0.80 ± 0.17b	4.71 ± 0.20h	28.70 ± 0.23c	35.21 ± 0.17f
50	71.46 ± 0.21c	69.34 ± 0.33c	3.71 ± 0.11g	3.06 ± 0.12f	27.38 ± 0.16b	28.24 ± 0.22c
75	73.17 ± 0.41d	72.90 ± 0.10c	1.85 ± 0.10d	1.26 ± 0.09c	29.96 ± 0.10d	29.00 ± 0.19c

Data are presented as means ± standard deviation of triplicate experiments. Mean values listed in columns with different letters are significantly different ($P < 0.05$). Controls – wheat flour biscuits without plant additives. 25, 50, 75 – amounts of additive (g) in biscuits. SMF – submerged fermentation; SSF – solid state fermentation; dw – dry weight.

Table 5
Colour characteristics of the biscuits supplemented with non fermented and fermented by different LAB lupine.

Biscuit samples	L^*		a^*		b^*	
Control	87.9 ± 0.21k		2.01 ± 0.14a		21.23 ± 0.10a	
With non fermented lupine						
25	49.30 ± 0.14c		8.36 ± 0.12h		37.59 ± 0.15f	
50	40.56 ± 0.12b		10.36 ± 0.09k		49.36 ± 0.21g	
75	32.47 ± 0.31a		11.90 ± 0.17k		56.98 ± 0.19h	
Biscuit samples	L^*		a^*		b^*	
	SSF	SMF	SSF	SMF	SSF	SMF
Control	87.9 ± 0.21k		2.01 ± 0.14a		21.23 ± 0.10a	
With fermented by *P. pentosaceus* lupine						
25	59.21 ± 0.24d	70.82 ± 0.15f	8.47 ± 0.11k	3.04 ± 0.09c	30.03 ± 0.13c	30.43 ± 0.19c
50	59.56 ± 0.20d	70.87 ± 0.14f	7.55 ± 0.19h	3.04 ± 0.04c	30.17 ± 0.24c	30.35 ± 0.18c
75	65.88 ± 0.11e	75.79 ± 0.13g	5.49 ± 0.23f	2.68 ± 0.07b	27.51 ± 0.21b	31.79 ± 0.24d
With fermented by *P. acidilactici* lupine						
25	65.89 ± 0.24e	70.18 ± 0.17f	5.28 ± 0.17f	5.80 ± 0.07g	27.81 ± 0.38b	33.08 ± 0.30d
50	64.59 ± 0.11e	68.31 ± 0.26f	5.39 ± 0.07f	5.84 ± 0.10g	30.65 ± 0.43c	30.10 ± 0.13c
75	68.27 ± 0.10f	73.85 ± 0.31g	4.57 ± 0.15e	2.86 ± 0.12b	30.71 ± 0.56c	29.60 ± 0.42c
With fermented by *L. sakei* lupine						
25	74.52 ± 0.48g	75.36 ± 0.18g	3.53 ± 0.14d	4.69 ± 0.23e	30.06 ± 0.21c	32.41 ± 0.48d
50	76.68 ± 0.19g	77.34 ± 0.23g	3.19 ± 0.10c	3.75 ± 0.23d	31.73 ± 0.27d	33.64 ± 0.45d
75	83.88 ± 0.31h	79.48 ± 0.27h	2.99 ± 0.23bc	2.65 ± 0.10b	34.54 ± 0.19e	34.49 ± 0.36e

Data are presented as means ± standard deviation of triplicate experiments. Mean values listed in columns with different letters are significantly different ($P < 0.05$). Controls – wheat flour biscuits without plant additives. 25, 50, 75 – amounts of additive (g) in biscuits. SMF – submerged fermentation; SSF – solid state fermentation; dw – dry weight.

lupine (Table 5) compared to control. SSF and SMF of flaxseed with *P. pentosaceus* increased the b^* values of biscuits by 35.1 and 45.1%, respectively compared to control (Table 4). SSF fermentation influenced the yellowness of biscuits prepared with lupine at a lower level: the increase in b^* value was of 22.8% (SSF with both *Pediococci*) and 38.5% (SSF by *L. sakei*), as well as SMF fermentation of lupine increased b^* values by 30.4% (pediococci) and by 36.9% (*L. sakei*) compared to control (Table 5). Lupine flour has been described as having a pale yellow colour (Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2014), and this could have influence on higher values of b^* in biscuits with non fermented lupine additives. Carotenoids, which are characterized by giving yellow colour for lupine flour, could be reduced by lupine wholemeal fermentation, and this could have influence on lower values of b^* in biscuits with fermented lupine additives. Also, lupine additives significantly ($P < 0.05$) increased the redness (a^*) of biscuits (Tables 4 and 5) providing the acceptable light brown colour. Changes in the browning of bakery products are mainly caused by the Maillard reaction and caramelisation (Kawai, Matsusaki, Hando, & Hagura, 2013).

4. Conclusions

All in experiment tested LAB showed good growth, reducing pH and increasing TTA in flaxseed and lupine substrates. Fermentation decreased asparagine and reducing saccharides contents in

fermented plants and reduced acrylamide content in biscuits. Each of factors lupine or flaxseed addition ($F(1.24) = 4032$; $P < 0.001$), fermentation method ($F(1.24) = 22{,}371$; $P < 0.001$), type of microorganisms applied for the fermentation ($F(2.24) = 5907$; $P < 0.001$), had the significant effect on acrylamide concentration in wheat biscuits, and the most effective ($F(7.24) = 40{,}001$; $P < 0.001$) was the interaction of these factors. The use of *P. acidilactici* for SMF of flaxseed and SSF of lupine influenced the most effective acrylamide reduction (by 78 and 85%, respectively) in biscuits. Using *L. sakei* for SSF of flaxseed and SMF of lupine acrylamide was reduced on average by 83.4%. Our study indicates that fermented flaxseed and lupine additives could have a great potential in the development of higher nutritional value bakery products with low acrylamide content.

References

Acar, O. C., Pollio, M., Monaco, D. I., Fogliano, R., & Gökmen, V. (2012). Effect of calciumon acrylamide level and sensory properties of cookies. *Food and Bioprocess Technology, 5*, 519–526.

Anamika, M., Neeraja, Y., Swarnalatha, J., Venkateshwar, S., & Venkateswar, L. R. (2013). Optimisation of nutrients for L-asparaginase production using Aspergillus terreus. *World Journal of Pharmacy and Pharmaceutical Sciences, 2*, 5582–5590.

Bahr, M., Fechner, A., Hasenkopf, K., Mittermaier, S., & Jahreis, G. (2014). Chemical composition of dehulled seeds of selected lupin cultivars in comparison to pea and soya bean. *LWT – Food Science and Technology, 59*, 587–590.

Bartkiene, E., Jakobsone, I., Juodeikiene, G., Vidmantiene, D., Pugajeva, I., & Bartkevics, V. (2013a). Study on the reduction of acrylamide in mixed rye bread by fermentation with bacteriocin-like inhibitory substances producing lactic acid bacteria in combination with *Aspergillus niger* glucoamylase. *Food Control, 30*, 35–40.

Bartkiene, E., Jakobsone, I., Juodeikiene, G., Vidmantiene, D., Pugajeva, I., & Bartkevics, V. (2013b). Effect of lactic acid fermentation of lupine wholemeal on acrylamide content and quality characteristics of wheat-lupine bread. *International Journal of Food Sciences and Nutrition, 64*, 890–896.

Bartkiene, E., Jakobsone, I., Pugajeva, I., Bartkevics, V., Vidmantiene, D., & Juodeikiene, G. (2015). Influence of the addition of *Helianthus tuberosus* L. fermented with different lactobacilli on acrylamide content in biscuits. *International Journal of Food Science & Technology, 50*, 431–439.

Bartkiene, E., Juodeikiene, G., & Basinskiene, L. (2012). In vitro fermentative production of plant lignans from cereal products in relationship with constituents of non-starch polysaccharides. *Food Technology and Biotechnology, 50*, 237–245.

Bartkiene, E., Juodeikiene, G., Vidmantiene, D., Zaborskiene, G., Basinskiene, L., & Kunkulberga, D. (2009). The influence of flaxseed on wheat bread quality. *Žemdirbystė – Agriculture., 96*, 181–196.

Bartkiene, E., Krungleviciute, V., Juodeikiene, G., Vidmantiene, D., & Maknickiene, Z. (2014). Solid state fermentation with lactic acid bacteria to improve the nutritional quality of lupin and soya bean. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.6827.

Bloom, M., Baardseth, P., Sundt, T. W., & Slinde, E. (2009). Lactic acid fermentation reduces acrylamide formed during production of fried potato products. *Aspects of Applied Biology, 97*, 67–73.

CIAA (Confederation of the European Food and Drink Industries). (2006). *The CIAA acrylamide toolbox. Confederation of the European Food and Drink Industries*. Brussels: CIAA.

CIAA (Confederation of the European Food and Drink Industries). (2009). *Rev 12. The CIAA acrylamide toolbox. Confederation of the European Food and Drink Industries*. Brussels: CIAA.

Claus, A., Mongili, M., Weisz, G., Schieber, A., & Carle, R. (2008). Impact of formulation and technological factors on the acrylamide content of wheat bread and bread rolls. *Journal of Cereal Science, 47*, 546–554.

Claus, A., Schieber, A., & Carle, R. (2008). Acrylamide in cereal products: a review. *Journal of Cereal Science, 47*, 118–133.

Corbo, M. R., Bevilacqua, A., Campaniello, D., Speranza, B., & Sinigaglia, M. (2014). Selection of promising lactic acid bacteria as starter cultures for sourdough: using a step-by-step approach through quantitative analyses and statistics. *Journal of the Science of Food and Agriculture, 94*, 1772–1780.

De Valdez, G. F., Gerez, C. L., Torino, M. I., & Rollán, G. (2010). New trends in cerealbased products using lactic acid bacteria. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (p. 393). Iowa, USA: John Wiley and Sons. http://dx.doi.org/10.1002/9780813820866.ch15.

De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2009). Role of precursors on the kinetics of acrylamide formation and elimination under low moisture conditions using a multiresponse approach e part I: effect of the type of sugar. *Food Chemistry, 114*, 116–126.

Digaitiene, A., Hansen, A. S., Juodeikiene, G., Eidukonyte, D., & Josephsen, J. (2012). Lactic acid bacteria isolated from rye sourdouhs produce bacteriocin-like inhibitory substances active against *Bacillus subtilis* and fungy. *Journal of Applied Microbiology, 112*, 732–742.

EFSA (European Food Safety Authority). (2012). Update on acrylamide levels in food from monitoring years 2007–2010. *EFSA Journal, 10*, 2938.

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of wholegrain cereals: what is beyond fibre? *Nutrition Research Reviews, 23*, 65–134.

FDE (Food Drink Europe). (2014). *Acrylamide toolbox 2013*. Available from http://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2013.pdf.

Fechner, A., Kiehntopf, M., & Jahreis, G. (2014). The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hypercholesterolemic adults. *Journal of Nutrition, 144*, 599–607.

Flower, G., Fritz, H., Balneaves, L. G., Verma, S., Skidmore, B., Fernandes, R., et al. (2014). Flax and breast cancer: a systematic review. *Integrative Cancer Therapies, 13*, 181–192.

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology, 37*, 2–10.

Gobbetti, M., & Gänzle, M. (2013). *Handbook on sourdough biotechnology*. New York Heidelberg Dordrecht London: Springer. http://dx.doi.org/10.1007/978-1-4614-5425-0. ISBN: 978-1-4614-5425-0 (eBook).

Himeda, M., Yanou, N. N., Fombang, E., Facho, B., Kitissou, P., Mbofung, C. M. F., et al. (2014). Chemical composition, functional and sensory characteristics of wheat-taro composite flours and biscuits. *Journal of Food Science and Technology, 51*(9), 1893–1901.

Jayalaxmi, B., Vijayalakshmi, D., Durgannavar, N. A., & Chandru, R. (2015). Mango peel: a potential source of natural bioactive phyto-nutrients in functional food. *Asian Journal of Dairy and Food Research, 34*(1), 75–77.

Kaczmarek, S. A., Kasprowicz-Potocka, M., Hejdysz, M., Mikuła, R., & Rutkowski, A. (2014). The nutritional value of narrow-leafed lupin *(Lupinus angustifolius)* for broilers. *Journal of Animal and Feed Sciences, 23*, 160–166.

Kaura, M., Sandhub, K. S., Arorab, A. P., & Sharmac, A. (2015). Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: physicochemical and sensory properties. *LWT – Food Science and Technology, 62*(1), 628–632.

Kawai, K., Matsusaki, K., Hando, K., & Hagura, Y. (2013). Temperature-dependent quality characteristics of pre-dehydrated cookies: structure, browning, texture, in vitro starch digestibility, and the effect on blood glucose levels in mice. *Food Chemistry, 141*, 223–228.

Keramat, J., LeBail, A., Prost, C., & Jafari, M. (2011). Acrylamide in baking products: a review article. *Food Bioprocess Technology, 4*, 530–543.

Konings, E. J. M., Ashby, P., Hamlet, C. G., & Thompson, G. A. K. (2007). Acrylamide in cereal and cereal products: a review on progress in level reduction. *Food Additives & Contaminants, 24*, 47–60.

Krishnakumar, T., & Visvanathan, R. (2014). Acrylamide in food products: a review. *Journal Food Process Technology, 5*, 7. http://dx.doi.org/10.4172/2157-7110.1000344.

Loaec, G., Niquet-Léridon, C., Henry, N., Jacolot, P., Volpoet, G., Goudemand, E., et al. (2014). Effects of variety, agronomic factors, and drying on the amount of free asparagine and crude protein in chicory. Correlation with the acrylamide formation during roasting. *Food Research International, 63*, 299–305.

Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P., & Jiranek, V. (2004). Lactic acid bacteria as a potential source of enzymes for use in Vinification. *Applied and Environmental Microbiology, 70*, 5715–5731.

Mc Guire, R. G. (1992). Reporting of objective color measurements. *Horticultural Science, 27*, 1254–1255.

Messens, W., Neysens, P., Vansieleghem, W., Vanderhoeven, J., & De Vuyst, L. (2002). Modeling growth and bacteriocin production by Lactobacillus amylovorus DCE 471 in response to temperature and pH values used for sourdough fermentation. *Applied Environental Microbiology, 68*, 1431–1435.

Moroni, A. V., Arendt, E. K., & Dal Bello, F. (2010). Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. *Food Microbiology, 28*, 497–502.

Nguyen, Q. D., Rezessy-Szabó, J. M., Claeyssens, M., Stals, I., & Hoschke, A. (2002). Purification and characterisation of amylolytic enzymes from thermophilic fungus *Thermomyces lanuginosus* strain ATCC 34626. *Enzyme and Microbial Technology, 31*, 345–352.

Onabanjo, O. O., & Dickson, A. I. (2014). Nutritional, functional and sensory properties of biscuit produced from wheat-sweet potato composite. *Journal of Food Technology Research, 1*(3), 111–121.

Pasqualonea, A., Biancoa, A. M., Paradisoa, V. M., Summoa, C., Gambacortaa, G., Caponioa, F., et al. (2015). Production and characterization of functional biscuits obtained from purple wheat. *Food Chemistry, 180*, 64–70.

Petrova, P., Petrov, K., & Stoyancheva, G. (2013). Starch-modifying enzymes of lactic acid bacteria – structures, properties, and applications. *Starch/Starke, 65*, 34–47.

Pranoto, Y., Anggrahini, S., & Efendi, Z. (2013). Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience, 2*, 46–52.

R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Ravyts, F., & De Vuyst, L. (2011). Prevalence and impact of single-strain starter cultures of lactic acid bacteria on metabolite formation in sourdough. *Food Microbiology, 28*, 1129–1139.

Sozer, N., Cicerelli, L., Heiniö, R. L., & Poutanen, K. (2014). Effect of wheat bran addition on in vitro starch digestibility, physicomechanical and sensory

properties of biscuits. *Journal of Cereal Science, 60*, 105–113.
Sudha, M. L., Chetana, R., & Reddy, S. Y. (2014). Effect of microencapsulated fat powders on rheological characteristics of biscuit dough and quality of biscuits. *Journal of Food Science and Technology, 1*(12), 3984–3990.
Tardiff, G. T., Gargas, M. L., Kirman, C. R., Carson, M. L., & Sweeney, L. M. (2010). Estimation of safe dietary intake levels of acrylamide for humans. *Food and Chemical Toxicology, 48*, 658–667.
Villarino, C. B., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., & Johnson, S. (2014). The effects of bread-making process factors on Australian sweet lupin-wheat bread quality characteristics. *International Journal of Food Science and Technology, 49*(1), 2373–2381.