Evaluation of Wheat/Non-Traditional Flour Composites

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Abstract

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We examine the nutritional effect of selected non-traditional grain samples added into wheat flour. In a form of flour, amaranth, quinoa, lupine, 5 hemp types, 2 teff types and 2 chia types were used for wheat flour substitution on a low and high level. Samples with amaranth and lupine flour showed the best improvement in terms of protein content (in the range between 21.1 and 26.0%). The highest total dietary fibre was found in lupine composites (7.1 and 9.8%). Hemp samples contained a significant amount of minerals in comparison with the control wheat sample (from 1.16% to 1.98%). According to the above-mentioned differences, flour composites containing single tested grains were distinguished by principal component analysis. All examined plant materials could be recommended for wheat flour fortification in terms of nutritional improvement. The addition of non-traditional flours partially changed both the volume and shape of laboratory prepared bread correspondingly to the type and added amount.

Keywords: dietary fibre; flour, minerals; proteins; resistant starch; baking test

The application of non-traditional components in cereal technology can often extend possibilities for production of new alternative cereal-based products. Amaranth, quinoa, lupine, hemp, teff, and chia milling products can be used for wheat flour fortification. The above-mentioned innovative components are known for a good chemical composition and can potentially improve the nutritional value of wheat cereal products.

Amaranth is a plant originally cultivated in South America. Main grain species include *A. hypochondriacus, A. cruentus*, and *A. caudatus*, which belong to the genus *Amaranthus* and family *Amaranthaceae.* High protein content (15%) with a significant amount of lysine was found in grain. The good nutritional value of amaranth is also characterised by a considerable amount of fibre, fat, and minerals (Escudero *et al.* 2004; Tömösközi *et al.* 2009; Kaur *et al.* 2010).

Quinoa is a pseudocereal initially grown in the Andean region in South America. *Chenopodium quinoa* Willd. belongs to the family *Chenopodiaceae* and genus *Chenopodium*. Quinoa seed contains a significant amount of protein (14–20%) with good digestibility and a considerable amount of lysine, methionine and cystine (Ruales & Nair 1992). High content of minerals and vitamins was reported in quinoa seeds (Jancurová *et al.* 2009).

Lupine is a plant grown in the Mediterranean area and in South America. *Lupineus albus* (white lupine) is common in Europe. Lupine belongs to the genus *Lupineus* and family *Leguminosae.* Its nutritional composition is interesting mainly due to high protein (30–40%) and dietary fibre (up to 50%) content. In comparison with other cereals, lupine protein contains a higher amount of lysine (Písaříková & Zralý 2010).

Hemp was traditionally produced especially for fibre and oil. It belongs to the genus *Cannabis* and family *Cannabaceae. Cannabis sativa* is the most widely grown species. Hempseed contains 20–25% of protein, 25–35% of oil, 10–15% of insoluble fibre and a rich array of minerals (Deferne & Pate 1996; Dimic *et al.* 2009). Hemp oil is beneficial for human nutrition due to its high portion of unsaturated fatty acids (Callaway 2004).

Teff is largely produced in Ethiopia. It is a cereal plant of the family *Poaceae*. Great benefit can be found in the mineral composition with a high amount of iron, calcium, and magnesium (Hager *et al.* 2012). In its seeds, moderate content of protein with great digestibility was found. However, low lysine in protein content was reported (Adebowale *et al.* 2011).

Chia is a plant categorised under the *Labiatae* family. It was natively planted in Mexico and Guatemala. From the nutrition point of view high protein content (16–26%) with no limiting factors in the amino acid composition is important (Ayerza & Coates 2011). Seed also contains a high amount of fat (30–33%) and dietary fibre (37–41%) (Ciftci *et al.* 2012).

Table 1 shows average values of the basic chemical composition of non-traditional grains, obtained from literature. According to this table, lupine is the best source of protein. Hemp has the lowest carbohydrate content and together with chia, it has the highest concentration of fat and fibre.

Non-traditional components can improve the chemical composition of wheat cereal products owing to their high protein, fibre, and fat contents and other elements positive to human health (vitamins, minerals, antioxidants) (Sanz-Penella *et al.* 2013). Furthermore, the combination of wheat and alternative flour can provide better overall essential amino acid balance, especially a higher lysine portion. However, this procedure can also affect technological properties of dough due to dilution of gluten, which is the main component responsible for the structure and volume of baked products (Dervas *et al.* 1999; Kohajdová *et al.* 2011). Besides that, the flavour and texture of composite cereal products can also be greatly influenced by unconventional plant materials. Therefore, the inclusion of an alternative ingredient could be significantly limited to maintain the product quality.

The scope of this study was to examine wheat flour composites in terms of chemical composition and possible nutritional benefits to human health. Samples of amaranth, quinoa, lupine, hemp, teff and chia flour were used for wheat flour fortification.

Table 1. Average value (in %) of basic components for tested non-traditional seeds

	Protein	Carbohydrates	Fat	Fibre
Amaranth[a]	17	66	6	21
Quinoa[b]	17	69	6	4
Lupine[c]	39	35	7	15
Hemp[d]	25	28	36	28
Teff[e]	12	63	2	3
Chia[f]	20	34	32	24

[a]Alvarez-Jubete *et al.* (2010); [b,c]Jancurová *et al.* (2009); [D]Callaway (2004); [e]Dimic *et al.* (2009); [f]Mohd *et al.* (2012)

Due to the presumed usage of all tested samples in the food industry, also bread quality was evaluated and compared with that of the wheat control.

MATERIAL AND METHODS

As prepared flour composites base, commercial wheat flour produced by the industrial mill Delta Praha in 2010 was used. It is characterised as a bright type (ash content 0.52%) with protein content 10.7%.

Samples of amaranth (A) and quinoa (Q) were originally produced in India and Ecuador, respectively. Both grain samples were bought in Country Life CZ shop (Czech Republic). For the flour form preparation, a Concept grinder, KM-5001 model (Elko Valenta, Choceň, Czech Republic), was used. Lupine (L) sample was grown in Austria and commercial fine flour was milled by the Natural Jihlava Company (Czech Republic). Five hemp (H) samples were used for this study, which differed in planting regime or seed treatment. Specimens H1, H2, and H3 were commercial fine flours milled from seeds produced in conventional regime (H1 and H2; E. Citterbartová's Company, Březí, Czech Republic) or in bio-planting (organic) regime (H3; Hanf & Natur, Marienheide, Germany). Hulled sample H4 and dehulled one H5 were produced by Hemp Production CZ (Czech Republic), and proper wholemeal flours were prepared using the grinder mentioned above. Flour samples T1 and T2 were obtained from white and brown botanical types of teff, respectively, and they represent a commercial product (Tobia Teff UK Ltd., London, UK). White and dark chia (CH) seeds were conventionally produced in Mexico and supplied by Aida Organic and Country Life CZ. Both samples were disintegrated to wholemeal flour CH1 and CH2, respectively. All samples of commercial flours were bought for the purpose of this research only.

Amaranth, quinoa, lupine, and hemp were mixed with wheat flour at ratios of 10 : 90 and 20 : 80. For teff flour testing, substitution levels of 20 and 30% were selected. Addition of 2.5 and 5.0% was used in the case of chia samples. Lower amounts of chia flour were used due to the limit set down by the European Union; recently, it was officially increased to 10% (Decision 2013/50/EU). For ANOVA statistics, the A, Q, and L samples were conjoined under non-traditional (NT) seed group. Hemp, teff and chia composite samples were included in hemp, teff, and chia groups, respectively.

Ash content was measured by combustion at 900°C according to the method defined by the Czech standard (ČSN 56 0512-8:1993). For determination of protein content, the Kjeldahl method (ČSN 56 0512-12:1995) was used, considering factor 6.25. Total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) were analysed using the enzymatic-gravimetric method with Megazyme assay kit (AOAC 985.29). Another Megazyme kit was used for measuring the amount of resistant starch (AOAC 2002.02).

Baking test was performed according to an internal method of the Institute of Chemical Technology in Prague. The procedure comprises dough preparation to a consistency of 600 units using a farinograph, fermenting for 50 min at 30°C and 90% RH and manual splitting to 70 g pieces and moulding by hand. Dough samples were allowed to leaven for 45 min at 30°C and 75% RH, and they were baked on a baking plate in a pre-steamed laboratory oven (14 min, 240°C). After 2 h of cooling in laboratory conditions, prepared bread was evaluated by specific bread volume and shape (height-to-diameter ratio) in triplicate. For the former feature, the rapeseed displacement method was used, and bread shape was measured using of a special rectangular device (Hrušková *et al.* 2006).

Nutritional enhancement caused by the addition of alternative plant raw materials was evaluated by analysis of variance (ANOVA) and correlation analysis using the Statistica 7.0 software (Hill & Lewicki 2007). Impacts of flour group (non-traditional samples containing A, Q, and L; hemp, teff, and chia ones) and flour addition level factors (FG and FL, respectively) with FG × FL interaction were quantified by F-test. Findings of the F-test were verified by the principal components method (PCA) – the first two principal components biplot of variables and cases (samples) was found as sufficient to describe differences in particular flour composites.

Table 2. Chemical composition (in %) of non-traditional and hemp seed composites

	Fortification	Ash	Protein	RS
Non-traditional seed				
M	–	0.52	10.7	0.4
M + A	10	0.71	21.1	0.4
	20	0.92	21.6	0.4
M + Q	10	0.74	19.9	0.2
	20	0.96	19.9	0.2
M + L	10	0.76	22.8	0.5
	20	1.00	26.0	0.5
M + T1	20	0.96	12.6	0.4
	30	1.15	12.6	0.5
M + T2	20	0.94	13.1	0.7
	30	1.11	13.1	0.6
M + CH1	2.5	0.59	11.0	0.6
	5.0	0.69	11.2	0.5
M + CH2	2.5	0.59	11.0	0.3
	5.0	0.69	11.2	0.4
SD		0.12	0.6	0.1
Hemp seed				
M	–	0.52	10.7	0.4
M + H1	10	1.24	15.2	0.3
	20	1.73	16.3	0.3
M + H2	10	1.28	15.1	0.2
	20	1.81	16.1	0.1
M + H3	10	1.34	15.6	0.4
	20	1.91	17.5	0.5
M + H4	10	1.30	15.4	0.4
	20	1.98	17.2	0.2
M + H5	10	1.16	14.8	0.3
	20	1.80	15.8	0.6
SD		0.12	0.6	0.1

M – wheat flour (control); A – amaranth flour; Q – quinoa flour; L – lupine flour; T – teff flour; CH – chia flour; H – hemp flour;. RS – resistant starch; SD – standard deviation

RESULTS AND DISCUSSION

The aim of this study was to examine the chemical composition of wheat flour composites with non-traditional grains. Results of the evaluation of ash, protein and resistant starch contents are summarised in Table 2. A high amount of ash was observed in all hemp samples. Amaranth and lupine flours were evaluated as the best source of protein. Figure 1 illustrates graphically the contents of all types of dietary fibre determined in the tested mixtures. Wheat flour with 20% of lupine had the highest content of TDF. Compared to fine wheat flour M, data demonstrate that all studied composite samples had better nutritional compositions.

Protein. Due to its nutritional significance and contribution to bread texture, an amount of protein was measured in all samples. Incorporation of non-traditional flours caused a different increase of protein content, reflecting the type and percentage of alternative component used. The lowest amount of protein was observed in teff and chia blends (results in the range between 11% and 13%) – it corresponds with findings of other studies (Saturni *et al.* 2010;

Ayerza & Coates 2011; Hager *et al.* 2012; Mohd Ali *et al.* 2012). The authors reported that the protein content was found to be equal or slightly higher in comparison with wheat. Amaranth largely improved the nutritional value of our composites in this regard (21.1 and 21.6%). However, other researchers found only a moderate protein amount in amaranth seed that could not cause such a significant improvement in the composite flour quality (Escudero *et al.* 1999; Sanz-Penella *et al.* 2013). Differences could be explained by variable environmental conditions during growth, and therefore different chemical composition among the analysed samples. The best results were obtained within the wheat-lupine blends (22.8% and 26.0%), which is in agreement with the lupine chemical composition published earlier (Dervas *et al.* 1999; Erbas *et al.* 2005).

The improvement of protein content for a higher fortification level was evident only in the case of lupine and hemp composite samples.

Minerals. Ash content matches with the quantity of minerals in a sample and it was found in higher concentration in composites containing hemp flour – the determined range was between 1.16 and 1.98%. High content in hempseed reported in literature confirms the beneficial effect of hemp on the nutritional composition (Callaway 2004). In an amount of minerals, a moderate improvement was determined for the rest of the examined samples; it corresponds with the chemical composition of non-traditional components published by Dervas *et al.* (1999), Ando *et al.* (2002), Kaur *et al.* (2010), Hager *et al.* (2012) or Mohd Ali *et al.* (2012). As presumed, the higher the fortification level, the higher the ash content that was determined independently of the botanical origin of added flour.

Resistant starch. Resistant starch (RS) has similar properties and biological functions like soluble dietary fibre. In fine wheat flour, 0.4% of RS was found. Only small differences were observed between the studied composite flours and the control sample. Bouzová (2011) measured RS content in amaranth and quinoa and these results correspond with small differences in the case of our composite flours. With regard to the small amount of RS detected, the addition of any non-traditional grain was not of high nutritional importance.

Dietary fibre. Dietary fibre is one of the most nutritionally important components in cereal products. Contrasted to wheat flour control, all studied composites showed a positive effect on the dietary fibre level (Figure 1). Despite the small addition to

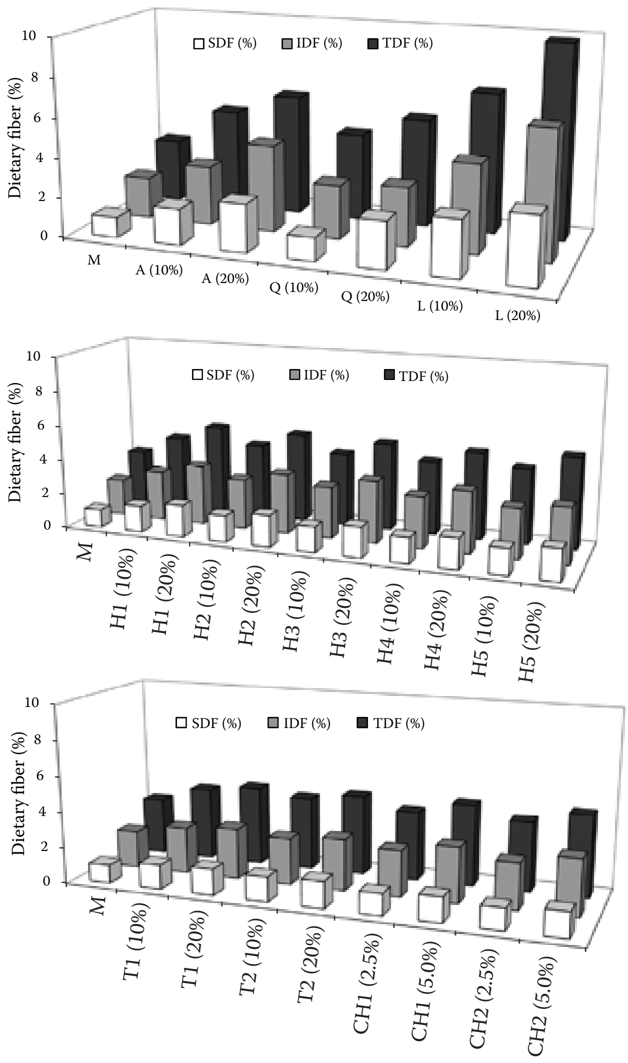

Figure 1. Dietary fibre content (A) amaranth, quinoa and lupine composites), (B) hemp composites, and (C) teff and chia composites

M – wheat flour (control); SDF, IDF, TDF – soluble, insoluble, and total dietary fibre

wheat flour, chia was revealed out as a good source of TDF (4.6% for 5% of both CH1 and CH2). Results correspond with the high dietary fibre content in chia seeds published by other researchers (Reyes-Caudillo *et al.* 2008; Segura-Campos *et al.* 2013). TDF in wheat-hemp blends ranged between 4.2 and 5.3%, and the highest improvement was found for 20% addition of H5 sample correspondingly to its hulled wholemeal character. Callaway (2004) reported a satisfying amount of TDF in hempseed, which is in agreement with observed increases related to the dietary fibre level in control M. A sufficient amount of dietary fibre was found in wheat-amaranth mixtures (5.1 and 6.2%); however, lupine was the best ingredient for wheat flour fortification (9.8% for 20% of L).

Table 3. Correlation analysis of chemical components (P = 99%)

	Ash	Protein	RS	IDF	SDF	TDF
Ash	1					
Protein	–	1				
RS	–	–	1			
IDF	–	0.754	–	1		
SDF	–	0.844	–	0.907	1	
TDF	–	0.815	–	0.968	0.957	1

RS – resistant starch; IDF, SDF, TDF – insoluble, soluble and total dietary fibre, respectively

Data presented by Erbas *et al.* (2005), Tömösközi *et al.* (2009), Alvarez-Jubete *et al.* (2010) or Písaříková and Zralý (2010) indicate that TDF content in both grains can vary greatly due to different environmental conditions (up to 20.6 and 50.4%, respectively). Besides the amount of TDF, the nutritional potential of dietary fibre could be examined on the basis of both IDF/SDF contents and their ratio (3 : 1 is recommended) (Reyes-Caudillo *et al.* 2008). All samples had a slightly lower SDF content in comparison with IDF and their ratios was in the range from 1.2 : 1 to 2.3 : 1. Only a small increase for all types of dietary fibre was caused by application of the higher fortification level.

Statistical analysis. Correlation analysis was used for the calculation of linear interrelation between quantities of various chemical components in the examined samples. Presented in Table 3, positive correlations were confirmed for all types of dietary fibre. A stronger positive relationship was found for protein and all three types of fibre, which corresponds with their location in the outer layer of seeds.

Analysis of variance (ANOVA) for two factors (four flour groups and two fortification levels) was calculated. A major statistical difference was found between H samples and all other flour groups (Table 4). According to ash content, only H and NT groups were completely distinguished. Diverse variance of protein content averages was found between NT and other groups (especially the CH one). However, no verifiable difference was observed for resistant starch data owing to the close range of determined values. The result of ANOVA for dietary fibre is presented in Figure 2, which illustrates only a partial variance for both analysed factors. Somewhat broader data scatter and thus better differentiation between flour groups could be seen for the SDF and high fortification level (variation a, ab, b).

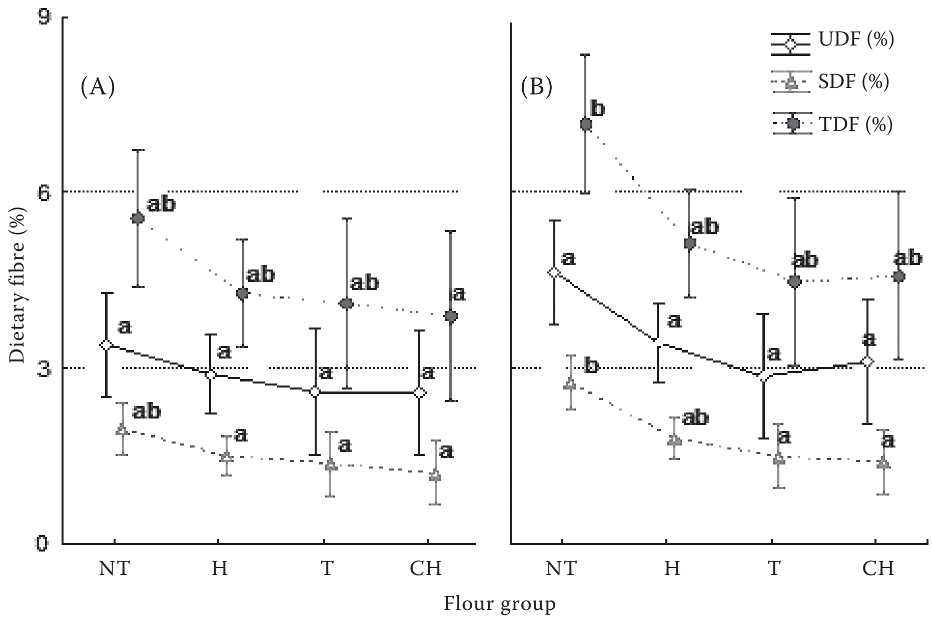

Figure 2. Analysis of variance of flour group and fortification level (A – low and B – hight) factors in relation to the dietary fibre content of composite flours

Flour groups: non-traditional – A, Q, L; hemp – H1, H2, H3, H4, H5; teff – T1, T2; chia – CH1, CH2

Fortification: low – 10% in NT and H groups, 20% in R group, 2.5% in CH group; high – 20, 30, and 5.0% in the above-mentioned groups

SDF, IDF, TDF – soluble, insoluble and total dietary fibre, respectively; RS – resistant starch

a–b – flour group averages for the particular dietary fibre components signed by the same letter are not significantly different (P = 95%)

Table 4. Analysis of variance of the influence of flour group (FG) and fortification level (FL) factors on the nutritional constituent content of tested flour composites (P = 95%)

Factor		Nutritional feature (%)		
FG	FL	ash	protein	RS
NT	low	0.74^{ab}	21.3^{c}	0.37^{a}
	high	0.96^{c}	22.5^{c}	0.38^{a}
H	low	1.26^{d}	15.2^{ab}	0.32^{a}
	high	1.85^{e}	16.6^{b}	0.34^{a}
T	low	0.95^{bc}	12.8^{ab}	0.57^{a}
	high	1.13^{cd}	12.8^{ab}	0.56^{a}
CH	low	0.59^{a}	11.0^{a}	0.43^{a}
	high	0.69^{a}	11.2^{a}	0.48^{a}

Flour group: non-traditional – A, Q, L; hemp – H1, H2, H3, H4, H5; teff – T1, T2; chia – CH1, CH2
Fortification level: low – 10% in NT and H groups, 20% in T group, 2.5% in CH group; high – 20%, 30% and 5.0% within the above-mentioned groups
SDF, IDF, TDF – soluble, insoluble and total dietary fibre, respectively; RS – resistant starch
$^{a–e}$flour group averages in the particular columns signed by the same letter are not significantly different (P = 95%)

F-test was used to compare influences of flour group and fortification level and their interaction on the nutritional composition of studied composites. According to Table 5, the chemical composition was mainly influenced by the type of non-traditional flour used. In the case of ash content only, significant interaction of both factors was observed.

Further statistical assessment was based on principal component analysis (PCA). Figure 3 shows that data variance was sufficiently explained by the first two principal components (61% by PC1 and 21% by PC2). Protein and dietary fibre contents were associated with PC1, while ash and resistant starch variance was explained by PC2. Within the plot, composites containing chia or teff flour as well as amaranth or lupine ones were distinguished from wheat-hemp blends mainly on the basis of resistant starch content and dietary fibre content, respectively. The group of hemp composite samples was characterised by the highest level of ash and protein contents. That flour group positioning confirms the F-test conclusion that a stronger effect is related to the flour type used.

Table 5. Comparison of the influence of flour group (FG) and fortification level (FL) factors and their interaction on the nutritional composition of composite flours (F-test)

Factor	F-value for nutritional feature					
	ash	protein	IDF	SDF	TDF	RS
FG	$13^{\dagger}$	$171^{\dagger\dagger\dagger}$	8	$9^{\dagger}$	$15^{\dagger}$	$101^{\dagger\dagger}$
FL	7	12	15	7	24	0
FG × FL	$21^{\dagger\dagger\dagger}$	0	0	1	0	0

SDF, IDF, TDF – soluble, insoluble and total dietary fibre, respectively; RS – resistant starch
F-values provable at $^{\dagger}P$ = 95%, $^{\dagger\dagger}P$ = 99%, and $^{\dagger\dagger\dagger}P$ = 99.9%

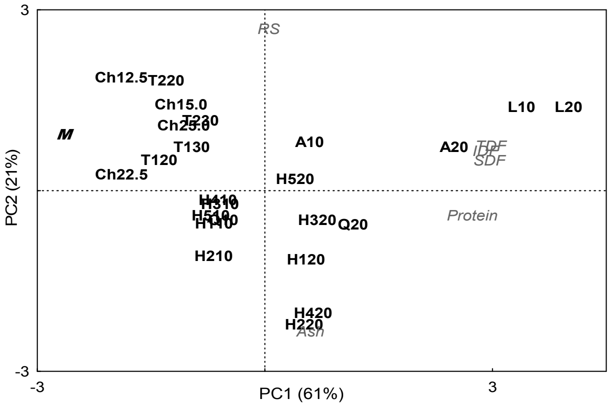

Figure 3. (A) PC1 × PC2 biplot for flour composites and nutritional constituents (for abbreviations see Table 2)

Baking test results. The quality of laboratory prepared bread was evaluated in terms of bread volume and shape, because both parameters are preferentially perceived by consumers. Control wheat bread volume reached 313 ml/100 g, and its vaulting was standard (height-to-diameter ratio 0.63). Within the NT group, the addition of A, Q as well as L flour lessened the bread volume. The negative effect of all three non-traditional flours corresponded with their amount in recipe, and the strongest impact was observed for wheat-lupine bread (decrease about 40%; Figure 4).

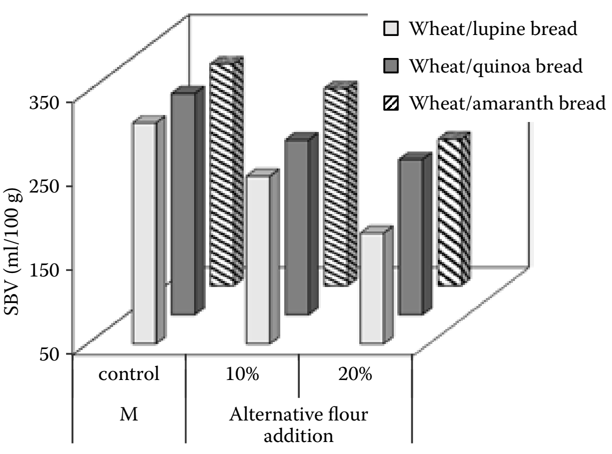

Figure 4. Comparison of amaranth, quinoa, and lupine flour effects on specific bread volume (SBV); M – wheat flour

Table 6. Baking test results of wheat and selected composite bread

	Fortification (%)	Specific bread volume (ml/100 g)	Bread shape (–)
M	-	313	0.63
M + T1	20	257	0.43
	30	135	0.23
M + T2	20	277	0.45
	30	186	0.37
M + CH1	2.5	396	0.60
	5.0	388	0.76
M + CH2	2.5	391	0.60
	5.0	392	0.61
M + H1	10	295	0.47
	20	252	0.55
M + H3	10	285	0.51
	20	246	0.45
SD		5	0.03

M – wheat flour (control); T – teff flour; CH – chia flour; H – hemp flour. SD – standard deviation

Wheat flour replacement by teff one also affected bread volumes, but both tested types caused approximately the same diminishing rate. Compared to the control M, the volume of bread containing 30% teff fell up to a half. Correspondingly to that, the bread shape was flatter in dependence on the added amount (Table 6). In the case of hemp fortification, chosen H1 and H3 samples decreased bread volumes comparably to teff flour. According to bakery product sizes, the above-mentioned hemp flour types could not be distinguished; at 20% substitution, specific bread volume decreased about one-tenth compared to the less fortified one. The shape of wheat-hemp bread samples was not influenced either by hemp type or by added level (Table 6). Consumer bread quality improvement was determined for both chia types tested – the bread volume increased approximately about 25%, and baking test results were similar for both fortification rates. The shape of wheat/chia bread was at least comparable to the control one (Table 6).

Although the nutritional benefit of tested non-traditional grains could not be doubted, their inclusion in the bread recipe was not reflected in bread consumer quality features such as bread volume and shape.

CONCLUSION

This study showed that non-traditional grains are good ingredients for wheat flour fortification. All examined samples of composite flour had better nutritional composition than the wheat control in terms of protein, minerals and dietary fibre. Amaranth and lupine flours were evaluated as the best source of protein. Despite the small addition to wheat flour, wheat-chia blends contained a high amount of dietary fibre. Wheat flour fortified with all hemp types had exceptional minerals content. The amount of alternative grain used for fortification could be limited with respect to standard bakery technology. According to the type and added amount of non-traditional flour, the consumer quality of laboratory prepared bread was changed. Although the nutritional benefit of tested non-traditional grains could not be doubted, their inclusion in the bread recipe caused a worsening of both bread volume and vaulting.

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