INVESTIGATION OF TOTAL PROTEIN CONTENT AND AMINO ACID COMPOSITION OF WHOLE GRAIN FLOUR BLEND FOR PASTA PRODUCTION

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Abstract
The purpose of the current research was to investigate the total protein content and amino acid composition of flour blend made from several types of whole grain flour for pasta production. Conventional rye, hull-less barley, triticale and wheat grain was used in the experiments. For the flour blend obtaining white wheat flour type 550 was used. Using standard methods the following quality parameters were analysed: protein content in grain and flour samples by using InfratecTM model 1241 Grain Analyzer, in flour blend – by AACC 46–20, amino acid content by LVS ISO 13903:2005. In the present research it was determined that it is possible to increase the total protein content in wheat flour type 550 by 11% if adding whole wheat or whole triticale flour, and by 7% if adding whole grain flour of hull-less barley. Higher total amino acid content was obtained for whole wheat flour sample; lower – for whole rye flour sample. Significantly lower total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour. No significant differences (p=0.779) were found in the analysed essential amino acid content made of different flour blend – the content of essential amino acids in the analysed flour blend samples was very similar.
Key words: grain, protein, amino acids, pasta, blend.

Introduction

Whole grain consists of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk. The principal anatomical components - the starchy endosperm, germ and bran - are present in the same relative proportions as they exist in the intact kernel (Healtgrain forum…, 2013). Currently, no common internationally accepted definition exists. Therefore, exact definitions of whole grain and whole grain foods processed in various ways, and knowledge about the components providing health effects in food as consumed, are crucial issues for whole grain research and recommendations (Andersson et al., 2014). It has been shown in epidemiological and certain intervention studies that whole grain cereals, containing a multitude of bioactive components, may protect against obesity, diabetes, cardiovascular diseases (CVD), hypertension and certain cancers (Fardet, 2010; Okarter and Liu, 2010; Lillioja et al., 2013). It was suggested that aleurone in bran, which is rich in minerals (magnesium and zinc), protein and certain bioactive components such as ferulic acid, is certainly more than just indigestible dietary fibre and was thus concluded to be a critical grain constituent for health effects (Lillioja et al., 2013).

Whole wheat flour (WWF), one of the most common and important whole grain, retains wheat bran and germ, and acts as a rich source of dietary fibre, vitamins, minerals and antioxidants. From epidemiological evidence and clinical trials, many studies have shown that the long-term intake of whole grain can provide tangible benefits to sufferers of chronic diseases such as diabetes mellitus, obesity, and cancer leading to a greater focus on whole grain foods. Corresponding dietary recommendations have also recommended consumers to eat more whole grain. Meanwhile, many specialty foods have been produced: whole wheat bread, whole wheat pasta, whole wheat noodles and some local traditional snacks (Wang et al., 2014). Wheat (*Triticum spp.*) is the main cereal crop used for human consumption in many areas worldwide. Traditionally, pasta is manufactured solely from durum wheat (*Triticum durum* Desf.), which results in a product considered to be of superior quality, to pasta made from cheaper common wheat (*Triticum aestivum* L.) or a blend of the two species. Nevertheless, price differentials between both wheat species could provide some manufacturers with the incentive to benefit economically from undeclared addition of common wheat. The manufacture of pasta from mixtures of durum and common wheat without adequate labelling is usually considered as adulteration. Durum wheat pasta for export outside the European Union may contain a maximum of 3% common wheat from unavoidable adventitious contamination during agricultural processing. Pasta is consumed in large quantities throughout the world. Scientific research has been undertaken to understand the parameters influencing pasta processing and the final product quality. It is firm and resilient with no surface stickiness and little if any cooking losses (Sissons et al., 2005; Troccoli et al., 2000). The quality of dietary protein is also important in determining changes in protein metabolism. The requirement for dietary protein consists of two components:

1. Nutritionally essential amino acids (isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val)) under all conditions, and for conditionally essential amino acids (arginine (Arg),

cysteine (Cys), glutamine (Gln), glycine (Gly), histidine (His), proline (Pro), taurine, and tyrosine (Tyr)) under specific physiological and pathological conditions.

2. Nonspecific nitrogen for the synthesis of the nutritionally dispensable amino acids (aspartic acid, asparagine, glutamic acid, alanine, and serine) and other physiologically important nitrogen-containing compounds such as nucleic acids, creatine, and porphyrins (Mauro, 2007).

Gluten is the main structure forming protein in the flour and responsible for the elastic characteristics of the dough (Gallaghe et al., 2004). These properties derive largely from the gluten proteins, which form a continuous viscoelastic network within the dough. Gluten contains hundreds of protein components, which are present either as monomers or, linked by interchain disulphide bonds, as oligo- and polymers (Wrigley et al., 1998). They are unique in terms of their amino acid composition, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups. Traditionally, gluten proteins have been divided into gliadins and glutenins, according to their polymerisation properties: gliadins are monomeric proteins that form only intra-molecular disulphide bonds, if present, whereas glutenins are polymeric proteins whose subunits are held together by inter-molecular disulphide bonds, although intra-chain bonds are also present. Among these storage proteins, glutenins (polymeric proteins) have been shown to be extremely important in determining rheological properties (Jia et al., 1996). Gluten proteins are susceptible to heat treatment and their behaviours subjected to relatively high temperatures have been studied by a number. It was shown that molecular size of the glutenin aggregates increases and, hence, their extractability decreases (Weegels et al., 1994b). At the macromolecular level, pasta is essentially a large protein network formed by irreversible protein–protein crosslinks through thermal dehydration, which encapsulates starch granules (Drawe, 2001). Bran from whole grain flour can interfere with water migration during this step, increasing water retention within the pasta (Villeneuve et al., 2007). Bran and germ particles also disrupt the continuity of the protein network, resulting in weaker, less firm pasta (Manthey et al., 2002). The structure of cooked pasta is generally described as a compact matrix, with the starch granules trapped in the network formed by the gluten proteins (Cunin et al., 1995). The resulting structure is responsible for the peculiar sensorial and nutritional properties of pasta, and its formation relates to the characteristics of the starting material and to various steps of the technological process (Scanlon et al., 2005). Rye (*Secale cereale*) could be exploited more efficiently in new types of cereal products due to its positive health effects. Nowadays, its use is limited mainly as a result of the problems arising from its flavour; not all European consumers are familiar with the somewhat foreign, rye-like flavour, perceived as bitter and intense. However, rye consumption in Europe might increase if ingredients were produced with the specific rye-like flavour modified to a slightly milder one, without significantly decreasing the contents of fibre and bioactive compounds in the rye (Heiniö et al., 2003). Triticale (*Triticosecale wittmack*) is a type of small grain created by genetically combining wheat and rye. Triticale grain, flours, and prepared products are available through both health food and commercial outlets on a limited basis. The data indicate that while the nutritional quality of triticale is considered superior to wheat, the higher ash content, lower milling yields of flour, and inferior loaf volume and texture distract from commercial baking use of triticale. In comparison with bread wheat, triticale has low gluten content, efficient gluten viscoelasticity and, therefore, inferior bread-making quality (Doxastakis et al., 2002). Hull-less barley (*Hordeum vulgare L.*) has been intensively investigated in respect to its food, feed and industrial applications. The advantage of hull-less barley compared to hulled barley in food uses is that pearling is not needed, so that the outer part of the endosperm, the aleurone, which contains proteins with essential amino acids and vitamins, is retained, as well as other bioactive compounds. Another advantage of hull-less barley is that there is no hull hampering the milling process. It can be milled using conventional equipment available for wheat milling, with extraction yields of 73%. Hull-less barley flour has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies (Andersson et al., 2004). Conventional farming has played an important role in improving food and fibre productivity to meet human demands but has been largely dependent on intensive inputs of synthetic fertilizers, pesticides, and herbicides. Certain conventional farming practices and associated chemical inputs have raised many environmental and public health concerns. Prominent among these are the reduction in biodiversity, environmental contamination, and soil erosion. Public concerns over environmental health and food quality and safety have led to an increasing interest in alternative farming practices with both lower inputs of synthetic chemicals and greater dependence on natural biological processes (Tu et al., 2006).

In the scientific literature practically cannot be found data about whole grain flour made form hull-less barley, rye, triticale and wheat application in pasta production, as well as protein content and amino acid composition of possible whole flour blend for pasta making. Therefore, the purpose of the current research was developed as follows – to investigate the total protein content and amino acid composition in dry blends made from several types of whole grain flour for pasta production.

Materials and Methods

The study was carried out at the Agronomy Research Laboratory of Latvia University of Agriculture (LUA), at the scientific laboratories of Faculty of Food Technology at Latvia University of Agriculture and at the laboratory of the JSC "Jelgavas dzirnavas" (Latvia), at the laboratories of Biotechnology and Veterinary Medicine Scientific Institute "Sigra" (Latvia).

Conventional rye (′Kaupo′) harvested from State Priekuli Plant Breeding Institute (Latvia) in 2012, hull-less barley (line ′PR 5099′) and triticale (line ′9405-23′), as well as wheat (′Zentos′) grain form LUA research station „Peterlauki" (Latvia) were used in the experiments. To obtain the flour blend, white wheat flour type 550 (Latvia) was used.

Before experiments grain was grounded in laboratory mill Hawos (Hawos Kornmuhle GmbH, Germany) obtaining fine whole grain flour.

At the previous experiments the following optimal wheat flour type 550 and whole grain proportion was obtained: 70% type 550 wheat flour and 30% whole wheat flour (W/W); 80% type 550 wheat flour and 20% whole rye flour (W/R); 70% type 550 wheat flour and 30% whole triticale flour (W/T); 80% type 550 wheat flour and 20% whole hull-less barley flour (W/H).

For measurement of protein content in analysed grain samples the InfratecTM model 1241 Grain Analyzer from Foss Tecator AB has been used for the spectroscopic investigation of the grain samples according to the express method ISO 12099. The instrument had an extended wavelength range of 570 – 1100 nm (Petterssona et al., 2003).

The total protein content of flour blend was determined under the standard method AACC 46–20 by means of a Kjeldal method.

The total and essential amino acid content in flour and flour blend was measured by HPLC Waters Alliance 2695/3100MS/2998FD (Waters Corporation, USA) using LVS ISO 13903:2005 standard method.

Microsoft Excel software was used for the research purpose to calculate the mean arithmetic values and standard deviations of the mathematical data used in the research. SPSS 20.0 software was used to determine the significance of research results, which were analysed using the two-factor ANOVA analyses to explore the impact of factors and their interaction, and the significance effect (p-value).

Results and Discussion

Protein is the second most common constituent of cereal grain, following starch. Depending on cereal species, variety, and agronomic conditions, the protein content in cereals can range from 5 to 20%. The type and amount of protein in cereal grain are important in terms of nutritional values as well as impacts on functional properties of food or feed containing the protein (Shewry, 2007).

Significant differences ($p=0.004$) in total protein content were found in the analysed whole grain flour samples. Lower protein content was obtained in rye flour 108 ± 3 g kg^{-1}, higher in wheat as 146 ± 1 g kg^{-1}, respectively (Figure 1). However, it is necessary to mention that there were not found any significant differences ($p=0.774$) between wheat and triticale whole grain flour protein content – results were very similar, as 146 ± 1g kg^{-1} and 143 ± 2 g kg^{-1}, respectively. The differences in total protein in the analysed flour samples could be explained mainly with grain individuality.

For the comparison, wheat flour type 550 protein content is 105 ± 2 g kg^{-1}, which is very similar to rye whole grain flour protein content and significantly

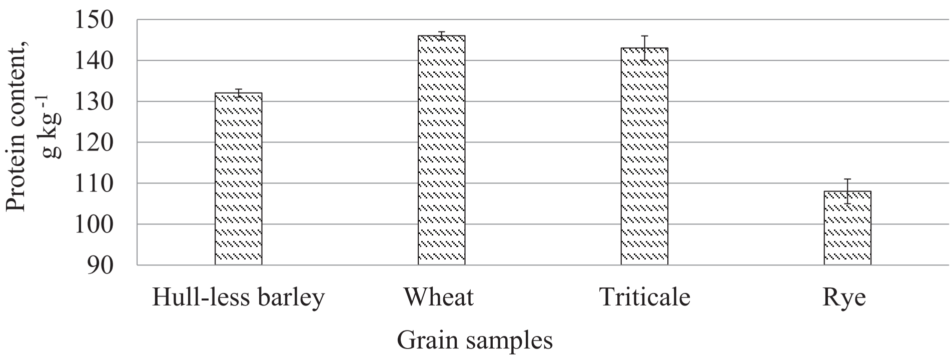

Figure 1. Total protein content in grain.

lower (p=0.001) comparing with whole grain hull-less barley, wheat and triticale flour.

Higher protein content in whole grain flour mainly could be explained with all grain (including cover) grinding. Because of their different production systems and levels of fertiliser use, it is difficult to obtain comparative values for the protein contents of different cereals. However, consideration of values reported indicates that relatively small differences exist within and between species and that these are amplified by environmental factors. Thus, ranges of 58 – 77 g kg^{-1} of protein on a dry weight basis have been quoted for rice; 80 – 150 g kg^{-1} for barley and 90 – 110 g kg^{-1} for maize. However, these ranges almost certainly reflect the impact of environment as well as genotype. For example, if grain nitrogen varied from 1.27 to 2.01% (about 72 – 115 g kg^{-1} protein) in a single variety of barley in which N fertilisation (Shewry, 2007).

Within the present experiment it was ascertained that it is possible to increase the total protein content in wheat flour type 550 by adding several types of whole grain flour in several proportions (Figure 2). As the results of our research demonstrate, it is possible to increase the total protein content by 11% in wheat flour type 550 by adding whole wheat or whole triticale flour. As a result, the flour blend for pasta production could be with higher nutritive value. No significant differences (p=0.417) were found in protein content between the analysed flour blends by adding rye flour (Figure 2). However, it is possible to increase the protein content in flour blend by 7% (which is not significant - p=0.068) if adding the hull-less barley whole grain flour. Therefore, it is possible to foresee higher protein content in pasta made from wheat flour type 550 and whole grain wheat flour, as well as from wheat flour type 550 and whole grain triticale flour.

Cereals and cereal products contain variable amounts of free amino acids depending largely on the species, cultivar and growing conditions. Free amino acids in raw materials of heat-treated foods take part in the Maillard reaction, which is important for cereal food quality (Mustafa et al., 2007).

During the present experiment significant differences (p=0.004) in the total amino acid content were obtained for analysed whole grain flour samples in dry matter (Table 1). Higher total amino acid content

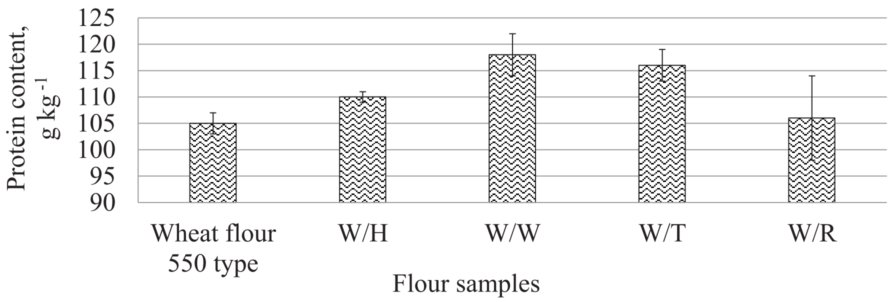

Figure 2. Total protein content of flour blend.

W/H – 80% type 550 wheat flour and 20% whole hull-less barley flour; W/W – 70% type 550 wheat flour and 30% whole wheat flour; W/T – 70% type 550 wheat flour and 30% whole triticale flour; W/R – 80% type 550 wheat flour and 20% whole rye flour

Table 1

Amino acid composition of analysed flour samples

Samples	Total amino acid content, g kg^{-1}	Essential amino acids total content g kg^{-1}
Whole rye grain	13.525±0.004	5.101±0.001
Whole hull-less barley grain	14.764±0.001	5.354±0.011
Whole triticale grain	15.115±0.012	5.361±0.007
Whole wheat grain	21.148±0.001	9.617±0.004
Wheat flour type 550	16.170±0.008	6.458±0.001
70% type 550 wheat and 30% whole wheat (W/W)	17.626±0.018	7.406±0.012
70% type 550 wheat and 30% whole triticale (W/T)	15.817±0.008	6.129±0.008
80% type 550 wheat and 20% whole rye (W/R)	15.641±0.004	6.186±0.004
80% type 550 wheat and 20% whole hull-less barley (W/H)	15.889±0.048	6.237±0.008

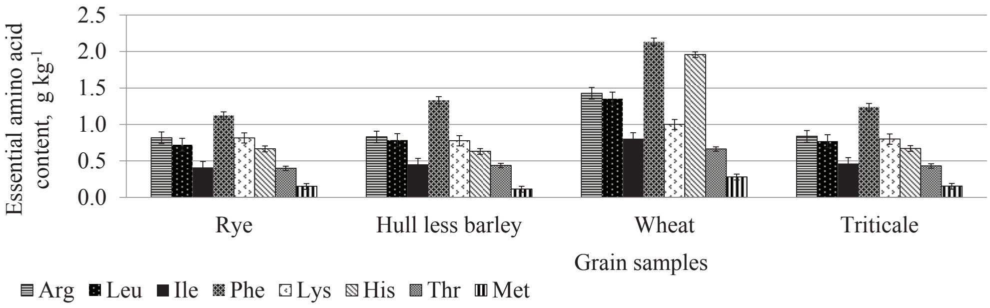

Figure 3. Essential amino acid content in whole grain flour samples.

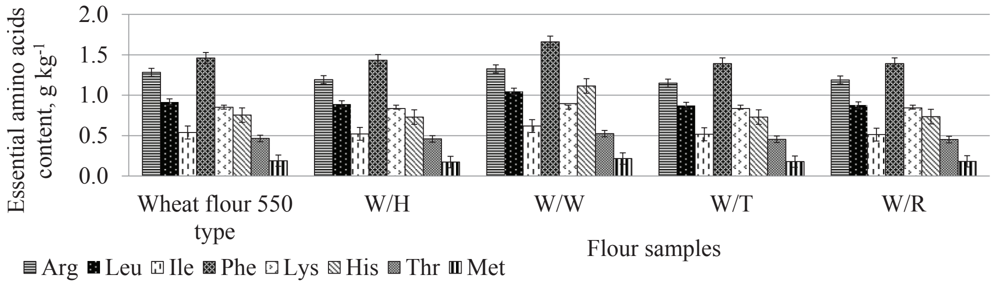

Figure 4. Essential amino acid content in flour blends.

W/H – 80% type 550 wheat flour and 20% whole hull-less barley flour; W/W – 70% type 550 wheat flour and 30% whole wheat flour; W/T – 70% type 550 wheat flour and 30% whole triticale flour; W/R – 80% type 550 wheat flour and 20% whole rye flour

was obtained for whole wheat flour sample, lower – for whole rye flour sample. Differences could mainly be explained with grain individuality. Mathematical data analyses show, that there are no found significant differences ($p=0.068$) in the total amino acid content between: whole rye and whole hull-less barley flour ($p>0.05$), whole hull-less barley and whole triticale flour ($p>0.05$). However, significantly lower ($p<0.05$) total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour (Table 1). Consideration of values reported indicates that relatively small differences exist within and between varieties and that these are amplified by environmental factors (Shewry, 2007).

In the present research it was found that wheat flour type 550 has a significantly higher ($p=0.05$) total amino acid content comparing with made flour blends (Table 1), with whole triticale, and rye and hull-less barley flour. The obtained results could mainly be explained with lower total amino acid content in same analysed whole grain flour samples. However, it is necessary to indicate that by mixing wheat flour type 550 with whole grain wheat flour, it is possible to increase the total content of amino acids by 8%, comparing with wheat flour type 550 amino acid results. It could be explained with the fact of all grain milling for whole grain flour production, and in the scientific literature (Mustafa et al., 2007) it is mentioned that an essential part of amino acid concentrates in the aleurone layer of the grain. Very similar results were obtained during essential amino acid total content analyses (Table 1).

It is necessary to mention that amino acids are not stored in the body like fats or carbohydrates; there are no specialized cells in the body to maintain a reservoir. Of course, amino acids are ubiquitous, being present in structural proteins, enzymes, transport proteins, etc. Some of these proteins (notably serum albumin) can be degraded under conditions of fasting or starvation, to release free amino acids. Dietary intake of amino acids is typically not balanced to exactly match the body's demands for various amino acids. Amino acids taken via the diet must be chemically modified and rearranged to provide adequate levels of all the amino acids needed. There is a large number of pathways in the body for balancing the pool of amino acids, both for synthesis and for degradation. The number of enzymes involved creates a great potential for genetic diseases. Furthermore, disruption (by mutation of just one enzyme) in the metabolism of only one amino acid can have profound consequences for growth and development; some of the genetic diseases are fatal. Adult humans are unable to synthesize all twenty amino acids needed for protein synthesis; those which cannot be synthesized and which must then be acquired via the diet are referred to as essential.

The ten which the body can synthesize on its own are nonessential (Essential and nonessential…, 2014). Therefore, it was very necessary to analyse essential amino acid composition in whole grain flour samples and in flour blend.

It was found that phenylalanine presence in the analysed whole grain flour samples is more pronounced, comparing to others amino acids (Figure 3). However, in smaller amount methionine was obtained in all analysed flour samples. In the scientific literature it is mentioned that lysine is the limiting amino acid for all cereals. Whole wheat flour, as aleurone and embryo tissues of grain, do contain higher contents of essential amino acids (Shewry, 2007). Such results differ from those obtained during the present research: the amount of lysine in all analysed whole flour samples was higher than histidine, or threonine, or methionine (Figure 3).

Lysine is one of the most limiting amino acids in plants consumed by humans and livestock. Recent genetic, molecular, and biochemical evidence suggests that lysine synthesis and catabolism are regulated by complex mechanisms (Ferreira et al., 2005). However, within experiments no significant differences ($p=0.447$) were found in lysine content between analysed whole grain samples.

In our study we have found significant differences ($p=0.067$) composed of phenylalanine. Lower phenylalanine content was obtained in the flour blend with whole triticale grain flour as 1.39 ± 0.07 g kg^{-1}, however, higher analysed amino acids content was found in flour blend with whole wheat grain flour as 1.66 ± 0.06 g kg^{-1}, respectively. The found differences could be explained with the fact that aleurone and embryo tissues of grain contain higher contents of essential amino acids (Shewry, 2007). No significant difference ($p=0.799$) was found among the other essential amino acids in analysed whole grain flour samples (Figure 4).

Conclusions

1. Lower protein content was obtained in whole grain rye flour 108 ± 3 g kg^{-1}, higher in wheat – 146 ± 1 g kg^{-1}, respectively. There are not found significant differences ($p=0.774$) between wheat and triticale whole grain flour protein content – the obtained results were very similar, as 146 ± 1 g kg^{-1} and 143 ± 2 g kg^{-1}, respectively.
2. It is possible to increase the total protein content in wheat flour type 550 by adding several types of whole grain flour. It is possible to increase the total protein content in wheat flour type 550 by 11% if adding whole wheat or whole triticale flour and by 7% if adding hull-less barley whole grain flour.
3. Higher total amino acid content was obtained for whole wheat flour sample (21.148 g kg^{-1}); lower – for whole rye flour sample (13.525 g kg^{-1}).
4. No significant differences were found in the total amino acid content between whole rye and whole hull-less barley flour ($p>0.05$), whole hull-less barley and whole triticale flour ($p>0.05$). However, significantly lower ($p<0.05$) total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour.
5. In this study significant differences ($p=0.067$) were found composed of phenylalanine. Lower phenylalanine content was obtained in the flour blend with whole triticale grain flour as 1.39 ± 0.07 g kg^{-1}, however, higher analysed amino acid content was found in flour blend with whole wheat grain flour - 1.66 ± 0.06 g kg^{-1}, respectively.

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