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AMINO ACIDS COMPOSITION OF WHEAT-GERMINATED LEGUMES COMPOSITE FLOURS

Florin URSACHI, Denisa ATUDOREI, Mădălina UNGUREANU-IUGA, Georgiana Gabriela CODINĂ

"Stefan cel Mare" University of Suceava, Faculty of Food Engineering, 720229, Suceava, Romania

Corresponding author email: codina@fia.usv.ro

Abstract

The aim of this study was to analyze the amino acids content of different legumes types (beans, lentil, soybean, chickpea and lupine) in a raw and germinated form. Also the effect of different levels (0%, 2.5%, 5%, 7.5%, 10%, 15%, 20%, 25%) of legumes addition in a germinated and raw form in refined wheat flour has been discussed. According to our data for the wheat- bean mix, lentil-wheat flour, soybean-wheat flour mix, lupine-wheat flour mix the highest amount of essential amino acid were recorded for the histidine whereas for the mix between chickpea-wheat flour the highest amount of the essential amino acid were recorded for valine. Regarding the amount of non-essential amino acids content the highest levels were obatined for glutamic acid for all the mixes between germinated legumes and wheat flour and the lowest one for glycine.

Key words*: amino acids content, germinated legumes flour, legumes flour, wheat flour.*

INTRODUCTION

Wheat flour is the main raw material for bakery products. However, the proteins from their content are deficient in essential amino acids, such as lysine, tryptophan, threonine, etc. (Laze et al., 2019). Different composite flours can be used to correct this deficit by partial substitution of wheat flour with other grain products such as legumes ones. Their use in wheat flour addition is recommended after some processing techniques in order to minimize their antinutrients contents and unpleasant flavor. Such a technique is the germination one which besides reducing their antinutrients compounds it also improves their nutritional content (Atudorei & Codină, 2020). Various studies have shown that germination increases the availability of nutrients, such as amino acids, minerals, vitamins, etc. (Ohanenye et al., 2020; El-Suhaibani et al., 2020) and at the same time reduces antinutritional compounds in seeds such as protein inhibitors, hemagglutinins, antivitamins, phytates (Singh & Sharma, 2017; Sokrab, Mohamed-Ahmed & Babiker, 2012). The international literature has pointed out that the germination process increases the amount of phenolic compounds, chemical compounds with antioxidant action (Atudorei & Codină, 2020). At the same time, the germination process activates the hydrolytic enzymes in the grains, which promotes the digestion of compounds such as starch and proteins (Han et al., 2016). It seems that germination process activate grains endo-enzymes, such as proteases and amylases, which hydrolises macromolecular substances such as proteins and carbohydrates and improve the digestibility of nutritional compounds from grains. However, the germination process does not significantly affect the content of macromolecular compounds from grains, although the amylose content appears to slightly decrease with the increase of the total sugar amount (Atudorei & Codină, 2020). From the protein point of view, the nutritional value of legumes is improved by increasing their content in essential amino acids available such as lysine, arginine, tyrosine, tryptophan, methionine due to the action of proteolytic enzymes which was synthesized during germination process on proteins (Boye et al., 2010). More, some inhibitors like trypsin, chymotrypsin which may have adverse effects on protein digestibility may be eliminated during germination process. This fact is desirable since protein in these forms has a higher digestibility.

Adding them in a germinated form in wheat flour can lead to a significant increase in the essential amino acid content of wheat flour. This represents the main raw material for bread

making. Bread is the main source of vegetable protein in the human body, which covers about 1/5-1/3 of the total protein requirement and about 2/3 of the body's vegetable protein requirement (Segal, 2002). Due to the inadequate balance of essential amino acids in bread, the biological value of bread proteins is relatively low. This deficiency can be corrected by adding germinated legumes in wheat flour which improves its nitrogen balance and, as a result, increases the assimilation coefficient of amino acids in bread. In our study was used as legumes in a germinated form for addition in a refined wheat flour lentil, lupine, bean, soybean and chickpea. According to the literature, these type of legumes present a higher amount of protein (17-40% dw) compared to the wheat flour (3-7% dw) (Atudorei & Codină, 2020). More, the supplementation of wheat flour with this type of germinated legumes will complement the deficiencies in these amino acids from the cereal-based products (Patrascu et al., 2019). From the amino acid content point of view, lupine is higher in arginine and leucine, whereas the amount of methionine is lower compared to other legumes (Martínez-Villaluenga et al., 2006). Chickpeas has a higher amount of arginine, leucine and lysine and beans of lysine, leucine, phenylalanine, tyrosine. Soy and lentils have a high amount of leucine and lysine (Boye et al., 2010). The aim of this paper was to present an analysis of the evolution of amino acids content in legumes: lentil (LN), lupine (LpN), bean (BN), soybean (SN) and chickpea (CN) at the initial time and after 4 days of germination: germinated lentil (LG), germinated lupine (LpG), germinated bean (BG), germinated soybean (SG), germinated chickpea (CG) and the impact of its addition in a refined wheat flour at different levels in order to improve its quality from the amino acids content point of view.

MATERIALS AND METHODS

Materials

The legumes used in this study (harvest 2019) were the following: bean (*Phaseolus vulgaris*), soybean (*Glycine max* L.), lentil (*Lens culinaris* Merr.), chickpea (*Cicer aretinium* L.) and lupine (*Lupinus albus*). Refined wheat flour (WF) of 650 type (2019 harvest) was provided by S.C. Dizing S.R.L. company (Brusturi, Neamț, Romania). The wheat flour (WF) and legumes in a native and germinated form physical-chemical characteristics have been reported in a previously study (Atudorei et al., 2021).

Legumes germination process

The germination process was made after a method previously described (Atudorei et al., 2021). Shortly, before germination, legumes grains were soaked at a temperature of 20°C for 6 hours in the case of lentil and soybean and for 12 hours in the case of beans, lupine and chickpeas. The germination took place in dark conditions for 4 days on filter paper at 25°C and 80% humidity. In order to reduce their humidity the samples were lyophilized at -50°C, for 72 h, at 4.2 Pa pressure. To obtain wheat-germinated legumes composite flours the lyophilized germinated legumes were grinded and mixed in various amounts in wheat flour.

Amino acid quantification

The amino acids determination was done by using the EZ:Faast kit (Phenomenex, Germany) and consisted of a solid phase extraction step and a derivatization and liquid/liquid extraction step. The solid phase extraction was performed via a sorbent packed tip that bended amino acids while allowing interfering compounds to flow through. Amino acids on sorbent were then extruded into the sample vial and quickly derivatized with reagent at room temperature in aqueous solution. Derivatized amino acids concomitantly migrate to the organic layer for additional separation from interfering compounds. Organic layer was then removed, evaporated, and suspended again in dissolution solvent and analyzed on a Shimadzu GC/MS system (GC MS-QP 2010 Plus, Shimadzu, Kyoto, Japan) with a Zebron ZB-AAA GC column. The essential amino acids obtained from the raw materials in a native and germinated form were: histidine (His), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val). The non-essential amino acids content obtained were: glutamine (Gln), glycine (Gly), proline (Pro), tyrosine (Tyr), alanine (Ala), asparagine (Asp), aspartic acid (Asx), glutamic acid (Glu), serine (Ser).

RESULTS AND DISCUSSIONS

The essential amino acid content of the raw materials used are shown in Figure 1.

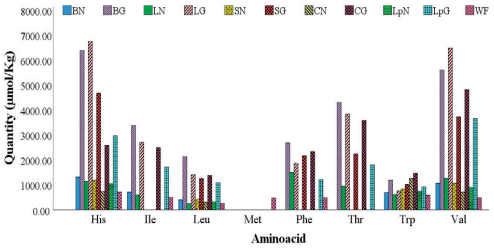

Figure 1. Raw materials essential amino acids contents

According to the data obtained the wheat flour presented lower levels of essential amino acids compared to the legumes flour. Also the legumes flour in a germinated form (after 4 days of germination) presented in generall higher levels of essential amino acids compared to the non germinated legumes. This data are in agreement with those reported by other authors which also noticed an increase of the essential amino acid content through the germination process (Atudorei & Codină, 2020). For germinated legumes flour the highest levels for essential amino acids were recorded for histidine, followed by valine and threonine while the lowest level were recorded for tryptophan. The highest amounts for essential amino acids from germinated legumes were recorded for germinated lentil followed by germinated bean. Also non-essential amino acids content were determined for wheat flour, legumes and germinated legumes (after 4 days) the data obtained being presented in Figure 2.

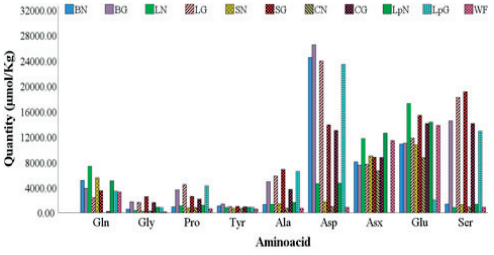

Figure 2. Raw materials non-essential amino acids contents

Generally, among the non-essential amino acids the highest amounts were recorded for the asparagine followed by serine and glutamic acid. The highest amounts for asparagine were recorded for germinated bean and the highest amount for serine for germinated soybean. More, some essential amino acids were not detected in raw legumes but appeared after germination. For example phenylalanine and tryptophan were not present in native bean, soybean, chickpea and lupine but were detected in their germinated form. Similar data has also been reported by Kuo et al. (2003).

Regarding the wheat flour it presented high amounts for essential amino acids such as histidine, isoleucine, methionine, fenilalanine, tryptofan, valine. From the non-essential amino acids the highest amount was obtained for serine and aspartic acid these data being in agreement with those reported by Mustafa et al. (2007). In wheat flour the highest amount obtained was those for non-essential amino acids glutamic acid of which value were higher than those obtained for legumes and germinated legumes flours.

The esential and non-essential amino-acids content of the composite flour for the wheat-bean mixes are shown in Figures 3 and 4.

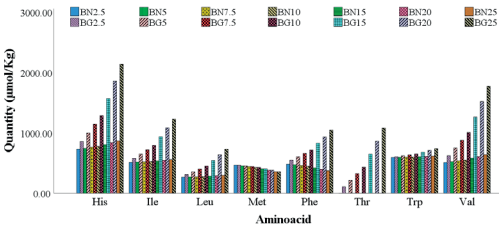

Figure 3. Wheat-bean composite flours essential amino acids contents

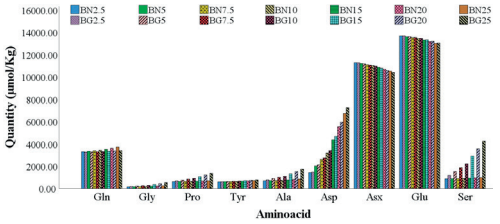

Figure 4. Wheat-bean composite flours essential amino acids contents

As it may be seen the highest amount of essential amino acids were obtained for histidine, fallowed by valine, isoleucine, threonine, phenylalanine, leucine of which value increased with the increase level of germinated bean flour addition in wheat flour.

For non-essential amino acids content the highest value was recorded for glutamic acid followed by aspartic acid, asparagine and glutamine. High levels of glutamic acid for bean have also been reported by Kuo et al. (2003). Although the wheat flour presented a higher level of glutamic acid compared to the bean flour in a raw and germinated form, the amount of this amino acid in the final product decreased by wheat flour substitution with bean flours.
The amino acids content for wheat-lentil mixes are shown in Figures 5 and 6.

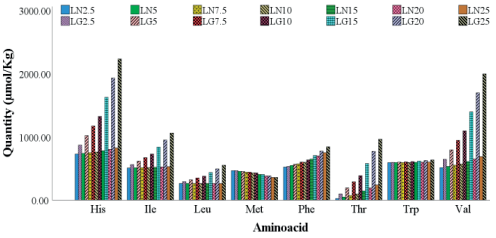

Figure 5. Wheat-lentil composite flours essential amino acids contents

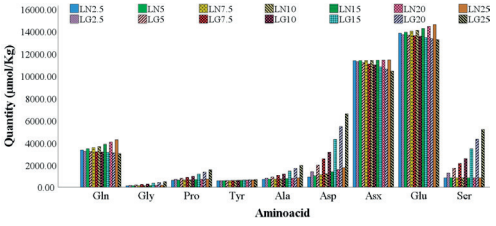

Figure 6. Wheat-lentil composite flours non-essential amino acids contents

According to the data obtained the highest levels for amino acids were obtained for histidine, valine, isoleucine, threonine and phenylalanine. This data are in agreement with those reported by Kahraman (2016) which also found high values for these amino acids type in different lentil varieties. By wheat flour substitution with lentil in a raw or germinated form the value of these amino acids increased. For non-essential amino acids content the highest levels were obtained for glutamic acid, aspartic acid, glutamine, and asparagine. However, the values for aspartic acid and glutamic acid decreased with the increase level of lentil flour addition in wheat flour due to the fact that wheat flour contain high levels of these amino acids compared to the lentil one. More, the mixes with germinated lentil addition in wheat flour presents low levels of these amino acids compared to the mixes with raw lentil flour addition in wheat flour. This is explainable since raw lentil contains high levels of aspartic acid and glutamic acid compared to the germinated one. These data are in agreement with those reported by Kuo et al. (2003) which also obtained a decrease of these amino acids during the germination period of lentil.
The mixes between wheat and soybean flours present the essential and non-essential amino acids content as it may be seen in Figures 7 and 8.

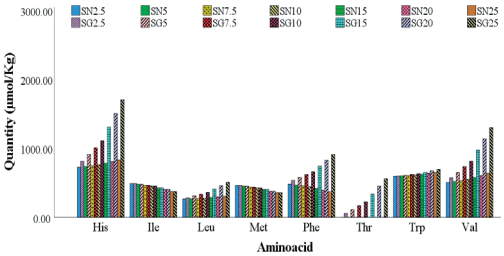

Figure 7. Wheat-soybean composite flours essential amino acids contents

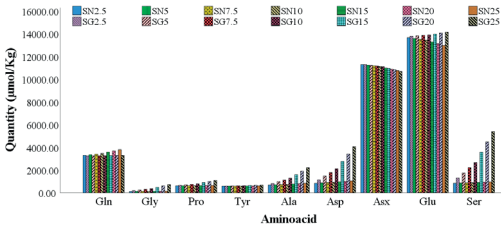

Figure 8. Wheat-soybean composite flours non-essential amino acids contents

For wheat-soybean composite mixes the highest values for essential amino acids were obtained for histidine, valine, phenylalanine, and tryptophan. These values are higher for wheat-germinated soybean mixes than for the wheat-raw soybean mixes. These are due to the fact that during germination these amino acids content increased in soybean (Bueno et al., 2020). For the non-essential amino acids the highest values were obtained for glutamic acid for which these values increased with the increase level of germinated soybean and decreased when raw soybean were added in wheat flour. Similar data has also been reported by Martínez-Villaluenga et al. (2006) which also obtained an increase of this amino acid for germinated soybean. Others high values of non-essential amino acids were obtained for aspartic acid which decreased with

the increase level of soybean addition, serine and asparagine when high levels of germinated soybean were added in wheat flour.
The data for amino acids content for the wheat-chickpea composite flours are shown in Figures 9 and 10.

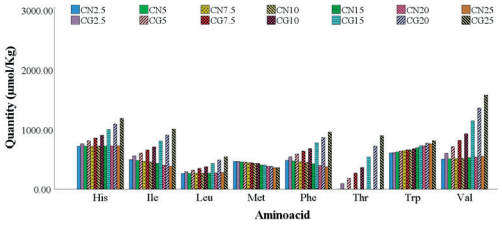

Figure 9. Wheat-chickpea composite flours essential amino acids contents

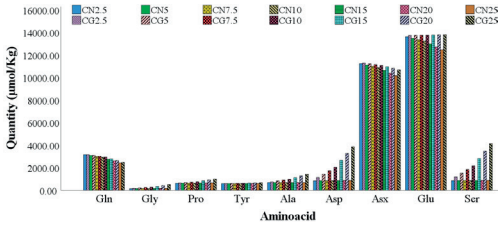

Figure 10. Wheat-chickpea composite flours non-essential amino acids contents

As it may be seen from the data obtained the highest values were obtained for essential amino acids valine, histidine, isoleucine for the mix samples with germinated chickpea addition in wheat flour. More, threonine has only been detected when germinated chickpea was incorporated in wheat flour. The increase of these amino acids values after chickpea germination has also been reported by others (Fernandez & Berry, 1988; Atudorei & Codină, 2020). From the non-essential amino acids point of view the highest levels were recorded for glutamic acid, aspartic acid, asparagine, serine for the mixes between wheat flour and germinated chickpea flour. High levels of glutamic acid and aspartic acid has also been reported for chickpea by Ghribi et al. (2015).
The essential and non-essential amino acid content for the mixes between wheat flour and germinated lupine flour are shown in Figures 11 and 12.
According to the data obtained the highest levels of essential amino acids were recorded for histidine and tryptophan for the mixes between wheat flour and raw lupine flour.

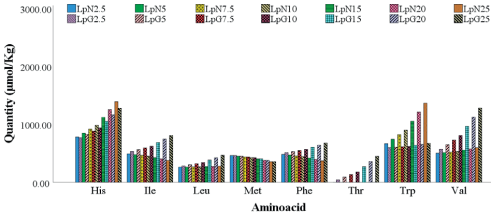

Figure 11. Wheat-lupine composite flours essential amino acids contents

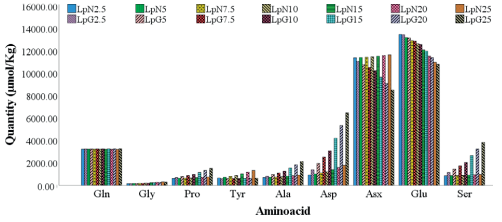

Figure 12. Wheat-lupine composite flours non-essential amino acids contents

Also high levels were recorded for valine, isoleucine and phenylalanine but only for the mixes when germinated lupine flour were incorporated in wheat flour. More, threonine was detected only for germinated lupine flour addition in wheat flour. This fact was explainable since it is well known that lupine is poor in threonine (Ahmed, 2014). The highest levels of non-essential amino acids content were obtained for glutamic acid and aspartic acid for the mixes between wheat flour and raw lupine and for asparagine, serine, glutamine for the mixes between wheat flour and germinated lupine flour. These facts are due to the lupine flour composition which is higher in some amino acids in raw form and lower one in germinated form. Similar data has also been reported by Villacrés et al. (2015) which obtained a decreased of glutamic acid and aspartic acid and an increase of serine for germinated lupine compared to the raw lupine one.

CONCLUSIONS

Compared to the legumes flours, the refined wheat flour presented lower amounts of essential amino-acids like histidine, leucine, tryptofan, valine. Generally, through germination the amino acid content of the legumes increased and even more, some essential amino acid which were not present in

legumes flours were detected in germinated ones. Generally, by wheat flour substitution with germinated legumes flours the amino acids content increased. These values increased with the increase level of germinated legume type flour addition in refined wheat flour. The highest amounts of essential amino acids were recorded for the histidine followed by valine for the mixes formed from germinated bean-wheat flour, germinated lentil-wheat flour, germinated soybean-wheat flour and germinated lupine-wheat flour whereas for the mix between germinated chickpea-wheat flour the highest amounts were recorded for valine followed by histidine. Except methionine, all the essential amino acids of the wheat flour were improved when germinated legumes flours were added in wheat flour.

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