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THE INFLUENCE OF PLANT-BASED PROTEIN INGREDIENTS ON THE QUALITY OF HIGH-PROTEIN BREAD

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Abstract

The aim of this work was to analyse the influence of plant-based protein ingredients added to bread on the overall quality of the end product. The analysis of the recent literature revealed a great interest in using plant-based protein ingredients versus animal-based ingredients. The main previous improvements consisted in the production of the bread in which about 15% wheat flour was replaced with various protein isolates from wheat, corn, potato, carob, peas, soya, lupine and beans. The impact on the properties of the dough (gluten aggregation, gluing behaviour, rheology) as well as on the quality of the bread (volume, crumb structure, crumb hardness) was investigated. The protein-rich ingredients affected gluten aggregation, gluing and determined the weakening of the gluten network in dough containing potato and pea proteins. Also, the literature indicated a high importance of the consumers' preference regarding the inclusion of functional foods with added protein in their daily diets, so that they reach the levels of intake necessary to achieve the health effects at present.

Key words*: gluten, high-protein bread, pea, plant-based proteins, soya.*

INTRODUCTION

One of the most consumed products in the world, which plays an important role in the human diet is bread (Henchion et. al., 2017). Bread is a simple food that is obtained usually from wheat flour, yeast and water. But this simple recipe can be modified or improved by using different types of flours and ingredients in order to increase its nutritional value, to obtain products for special diets and to answer to the consumers' preferences. Bread is a source of complex calories and carbohydrates (Gomez et. al., 2008), but the proteins from bread contain low levels of essential amino acids, such as lysine and threonine. In addition, the use of refined white flour reduces the nutritional density and fiber content of white bread compared to whole grain bread. It is known that the amino acid composition of legumes is complementary to that of cereals (Boye et al., 2010; Anderson et al., 2009), and they are also rich in bioactive compounds, such as fibers and phytochemicals, that is why legumes flour is often added to wheat flour in bread making process.

The nutritional properties of wheat bread can be improved by including a percentage of alternative flours from other cereals or legumes. Therefore, due to the fact that bread is a food in which other ingredients can be easily incorporated, a wide range of plant rich bakery products can be produced. The nutritional value of a food product is given by the protein digestibility value, so the quality of the proteins is very important for the characterization of the nutritional properties of a product (Bonnand-Ducasse et al., 2010; Belc et al., 2020).

Protein is a dietary essential macronutrient needed for a healthy structure and function of human's body. The quality of the proteins can be quantified according to the quantity and the profile of the essential amino acids, as well as based on the real value of the digestibility of the essential amino acids.

Protein density can be quantified based on the amount of total calories consumed to meet the daily requirement of all essential amino acids (Robert et al., 2018; Gherghina et al., 2015). The use of plant proteins provides additional nutrients and can improve the nutrient supply to consumers (Mao and Miao, 2015).

Terms of "high protein" are found in various forms, but according to Regulation (EC) no. 1924/2006, a product is considered "rich in protein" if 20% of its calories are supplied by protein. The plant ingredients sources of proteins can be classified, based on the protein content, as: flour (protein less than 65% d.m.), concentrates (protein more than 65% d.m.) and isolates (protein higher than 90% d.m.) (Boye et al., 2010). The protein content of the isolates depends on the processing of raw ingredients and this does not always reach a protein level of 90% d.m (Arntfield and Maskus, 2011).
The general objective of this paper is to identify and analyse low cost and efficient sources of plant-proteins, in order to develop new, modern, protein enriched foods.

Plant based proteins ingredients

Based on the scientific literature search, partial substitution of wheat flour with protein-rich ingredients from numerous sources was applied for bread production: legumes (Villarino et al., 2015; Turfani et al., 2017; Marchais et al., 2011), cereals (Bugusu et al., 2002), pseudo-cereals (Sanz-Penella et al., 2013) and dairy proteins (Kenny et al., 2000). The previous studied proteins sources were: wheat (gluten - *Glue*), corn (zein - *Zea mays*), potato *(Solanum tuberosum)*, carob/tomato (*Ceratonia siliqua*), peas (*Pisum sativum*), lupine (*Lupinus angustifolius*) and beans (*Vicia faba*) and they were tested regarding the impact on high-protein breads (Day, 2011; Crepon et al., 2010). Pea proteins have gained popularity in food and pharmaceutical systems due to the relatively lower content of anti-nutrients compared to soy protein (Nirali et al., 2019).
In the study conducted by Andrea et al., 2019, five commercially available high protein ingredients were used: potato protein isolate, pea protein isolate, redwood meal, gluten vital and corn protein (zein), protein isolate from blue lupine and a fine fraction high in protein from beans. In this study, 15% of wheat flour was replaced with different protein flours of different concentrations.
Therefore, the red bean meal and bean meal had the lowest levels of protein concentration (55.04% d.m.) respectively (61.25% d.m.); pea flour and gluten vital had a protein content of 80.19% d.m. and 83.11% d.m., respectively, and corn and potato flour had a protein content of over 90-91.79% d.m.
In another study the protein sources used were: flour (21.34% protein); redcurrant flour (43.17% protein); pea protein isolate (80.74% protein) and soybean meal (38.14% protein). This time the researchers chose to adjust the amount of protein in each formulation to obtain the same percentage of protein (1.5%) (Miñarro et al., 2012).
In the other studies, wheat flour was replaced approx. up to 30%, depending on the type of the used protein ingredients (flour, concentrate or protein isolate). Studies showed that the replacement of 30% from the wheat flour with bean flour with a protein content of 35.7 ± 1.2% d.m. increased the protein content from 11.6 to 16.5% d.m. In other researches it was found that the simple addition of bean flour resulted in an increase of approx. 15-30% of free peptides and amino acids, during fermentation (Rossana et al., 2017). Following the study in which 15% of the wheat flour was replaced by protein-rich ingredients, the protein content of floury mixture increased 3.5 times in comparison with wheat flour which had a protein content of 14.09% d.m. (Andrea et al., 2019).
Other plant protein sources, like quinoa and amaranth, were recently taken into consideration. Quinoa has a protein content of 12-20%, although this can be modified depending on its species. Its protein amount is at least equal to the amount of milk protein, containing also essential amino acids (Abugoch, 2009; Repo-Carrasco-Valencia & Serna, 2011; Yıldız et al., 2014).
Quinoa was compared to other cereals in terms of protein content as the quinoa flour had a protein content of 14.12%, rice flour 6.81%, barley flour 9.91%, wheat flour 13.68%, corn flour 9.42%, rye flour 10.34 and sorghum flour 10.62% (Aybuke and Nevin, 2019). In his study, Gostin (2019) tested the addition of quinoa flour to white wheat and wholemeal wheat flour the samples being obtained from (1) white wheat flour (WF, control, protein 13.2%), (2) wholemeal wheat flour substituted with 33 g/100 g (33%) white wheat flour (protein 13.7%), (3) wholemeal wheat flour (protein 14.0%), (4) wholemeal wheat flour substituted with 10 g/100 g (10%) quinoa flour (protein

13.0%), (5) white wheat flour substituted with 10 g/100 g quinoa flour (protein 13.2%), and (6) quinoa flour (protein 12.9%). Compared with the control bread (wheat flour), all breads with the addition of quinoa flour were perceived to be twice as salty and six times as bitter. Therefore, bread with quinoa flour added had very low acceptability.

According to previous studies quinoa flour can be used up to 10-13% in bread with wheat flour or corn flour for gluten-free products (Aybuke and Nevin, 2019).

Rheological analysis

Proteins have a strong influence on the rheological properties of the dough (Rafa et al., 2013; Choi and Han, 2001). Proteins, in addition to their nutritional properties, have also functional properties that play an important role in the formulation and processing of foods. The functional properties of these proteins are: the solubility capacity, the water and fat binding capacity and the foaming capacity (Boye et al., 2010; Korus et al., 2009).

In a previous study in which flour mixtures were analyzed, it was found that in the samples with raw redcurrant flour and lentil flour the dough's behavior was not different from the control but the redcurrant flour (10%) made the dough sticky (Turfani et al., 2017; Ahmed et al., 2013). During the formation of the dough with the addition of potato it was found a lack of elasticity and a higher viscosity, this could be influenced by the fact that it was applied at the same time mixing for all the recipes, and in the case of the potato exceeded the tolerance of gluten mix (Andrea H. et al., 2019). The amount of water added to the dough formation differs depending on the protein added. The amount of water varied between 523 g per sample in the case of albumin and 739 g in the case of soy protein, and the intermediate variants were lupine (568 g), collagen (617 g) and peas (639 g) (Rafa et al, 2013; Choi and Han, 2001).

When adding lupine flour (35% protein content) in a proportion of 5 and 10% respectively, it was found that the water incorporation time in the flour was higher, therefore the dough formation time increased with approximately 1.5 minutes compared to the control sample (only with flour), but not depending on the use lupin quantitative.

It was also found that samples with the addition of lupine flour have a higher dough stability, a stronger dough than the wheat flour control sample (Paraskevopoulou et al., 2010; Witczak et al., 2012).

Several tests were done to prepare, three different types of bread using a 30% level of wheat flour substitution with raw peas, sprinkled peas or fried peas. After the analysis of dough formation with the Mixolab equipment, it was found that pea flour-wheat flour mixtures had a lower water absorption than the control (wheat flour) and the dough formation time was similar with the control. But differences were found in the stability of the dough and its resistance to mechanical mixing. The resistance was reduced in all the three cases. (Millar et al., 2019).

Regarding the rheological analyses for four samples of chickpea flour, redcurrant flour, pea protein isolate and soybean meal, revealed no significant differences were found. The analyses were performed at Brabender Farinograph where 15% of wheat flour was replaced with protein flour of different percentages, respectively flour (control flour) 14.09%, gluten 83.11%, zein 91.79, potato 55.04, peas 80.19, lupine 94.51, beans faba 61.25% (Angioloni & Collar, 2012).

It was noticed that the absorption of water was higher in tomato, gluten and peas 69.8%, 70.2%, respectively 71.7%, even in potato and lupine, 65.2%, respectively 66.2%, compared to wheat flour 63.0%, when using goddess and beans was made only with the exception of the goddess (60.8%), the beans (62.2%) (Andrea H. et al., 2019).

In another study in which lupine flour and soybeans replaced 5-10% of white wheat flour, the dough stability and tolerance index increased (Kaack et al., 2002).

Loss of moisture when baking

An important parameter that influences the bread shelf-life is the loss of moisture during baking and this leads to the formation of a dry crust and therefore determines the early aging of the product. Moisture loss can be influenced by the water binding ability of the dough ingredients. Addition of lupine protein isolate has been reported to delay bread staling, and the addition of dietary fiber to bread has been

reported to delay drying (Kaack et al., 2006; Kiosseoglou et al., 2014).
As a result of the moisture determination analyses, it was found that the sample of Australian sweet lupine bread had lower moisture loss during baking (Villarino et al., 2015; Alvarez et al., 2010).

Texture
In the ordinary production of bread, wheat is used because of its properties that provide the desired texture through the formation of gluten network (Popa et al., 2014).
The effects of the Australian sweet lupine variety on the physical characteristics of the bread were evaluated by Villarino et al. (2015); Alvarez et al. (2010). Adding Australian sweet lupine flour to wheat bread resulted in a reduced bread volume and a strong texture, due to disruption of the gluten matrix by non-elastic lupine proteins and high-water absorption of Australian sweet lupine dietary fiber. The influence of the lipid and protein components of Australian sweet lupine flour on the texture properties of bread was observed, but the instrumental textural properties of Australian sweet lupine wheat bread did not differ significantly from those of wheat only bread (Villarino et al., 2015). Any difference in the size of Australian sweet lupine flour particles may in turn affect the volume of bread. The particle size reduction of refined wheat flour substitutes (bran or whole wheat) either increased or decreased the volume of bread. After increasing the percentage of protein by replacing wheat flour with bean flour, it was observed that the bread hardness increased with about 30-50% in bread with added bean flour (Andrea et al., 2019). Partial replacement of wheat flour with pea protein isolate (Marchais et al., 2011; Hogan et al., 2012) and lupine flour (Villarino et al., 2015) has been reported previously to decrease specific volume and increase hardness. Following the studies done by Bugusu et al. (2002) and Turfani et al. (2017) it was found that the volume of bread increased in samples with the addition of carob flour and zein (from corn).
Regarding hardness, one of the important characteristic of bread, the study compares the control sample (wheat flour; 11,81N) with plant protein added as follow; 11.81 N corn/corn (15.10 N), peas (16.68 N), potato (19.02 N), lupine (20.11 N) and faba beans (20.11 N) (Andrea H. et al., 2019).The addition of lupine flour (35% protein content) in a of 5 or 10% resulted in increasing the values of the hardness of the bread compared to the control bread with wheat flour. Bread hardness also increased significantly after 24 and 48 h subsequent tests, but compared to the wheat flour control sample, lupine flour samples had a softer texture, probably due to the high-water content (Paraskevopoulou et al., 2010; Sabanis et al., 2006).

Color
The addition of different ingredients rich in protein often determine changes in the color of the final baked products.
The bakery samples with beans were the darkest ($L^* = 57.07$), and the most light sample was the one with the zein (protein concentration 91.79% d.m.) ($L^* = 72.80$), being relatively close to the one with the potato protein isolate ($L^* = 70.58$), the control sample having a value of $L^* = 71.84$.
The color of the crust ($L*$) was measured by a Colorimeter CR-400 (Konica Minolta, Japan) using the CIE $L*a*b*$ color space (Andrea et al., 2019). The values of the samples with redcurrant, lupine, vital gluten and peas had contained values between 62.33 (carob) and 66.83 (peas).
A color difference was observed between the samples with the added pea flour (in the form of raw, sprouted and fried) and the control sample (only wheat flour). The samples with added pea protein in different forms had a lower value of color parameter L^*, which results in a darker color. This may be influenced by the increase in Maillard browning reactions as a result of increased protein content (Millar et al., 2019).
Samples of bread enriched with: chickpea flour (21.34% protein); carob germ flour (43.17% protein); pea protein isolate (80.74% protein); and soy flour (38.14% protein) were subjected to color analysis and is noticed the darker color was in the sample with carob germ flour ($L^* = 73.52$) and the lightest color was at the sample with the addition of pea protein isolate ($L^* = 77.40$) (Miñarro et al., 2012).

Consumer acceptability
Acceptability regarding the appearance of a gluten-free bread with added pea protein was

slightly lower than that of the control (corn starch, potato starch, pectin, guar gum, yeast, sugar, salt, oil and water), but the samples with the addition of lupine and soy protein had a very low acceptability. The possible causes for which the latter had a low acceptability, was the specific volume and their compact structure. Bread with added protein from peas had a high degree of acceptability also in terms of color and odor evaluation, this having a particularly pleasant odor compared to the control sample (Rafa et al., 2013; Choi and Han, 2001).
When replacing wheat flour with 5-10% lupine flour and soybean flour, the parameters remained as good as in the case of wheat flour, but when increasing the substitution with more than 10% there were reported changes in the rheology of the dough, especially in the volume, weight and texture of the bread, therefore the acceptability was lower (Doxastakis et al., 2002).
In another study wheat flour (control sample with approximately 9.9% protein) was analyzed with the addition of skimmed soybean meal (approximately 48.9% protein), in the following mixtures: wheat flour with added 3% soybean meal (mixture containing about 11.8% protein), wheat flour with added 7% soybean meal (mixture containing about 14.0% protein) and another sample of wheat flour with added 7% soybean meal and 3% sugar (mixture having approximately 14.2% protein). The protein content of the flour mixture with 3%, respectively 7% soybean meal increased by 21.4%, respectively 29.1% without wheat flour. Breads were prepared from these mixtures which were subsequently subjected to sensory analysis. The samples were tested by a number of 145 panellists and the sensory characteristics sought were: breaking resistance, appearance, aroma and taste, crust texture and general properties of acceptability. From the appearance point of view, it was observed that the color of the crust has changed from white-yellow to yellow-brown with the increase of the added soybean meal percentage added. The breaking resistance increased slightly with the increase in the percentage of soybean meal added to wheat flour. The aroma and taste were more acceptable in the samples with small amount of added soy flour, being similar with the ones of control sample (only wheat flour).
The acceptability was higher in samples with the addition of 3% soybean meal, compared to samples with a higher percentage of soybean meal (Mashayekh et al., 2008).
In another study, made by Miñarro et al., (2012) the sensory analysis was carried out with the help of consumers, who tested four bread samples. The control sample was with corn starch and the other ones enriched with different proteins: chickpea flour, carob germ flour, pea protein isolate and soy flour. Bread with the addition of carob germ flour had the highest hardness values compering with the control in the after five days of storage. The sample with the addition of chickpea flour had the most volume while the bread with the addition of carob germ flour recorded the lowest volume. In terms of flavor and taste, they did not show significant differences, these being accepted by consumers. The highest score for the overall appearance was obtained by the bread with the addition of soy flour.
Leguminous flour affected the dough rheology and bread quality by altering key features such as its specific volume, structure and texture. After several experiments, Turfani et al. (2017) found that a 5% flour replacement with legume substitutes does not influence the dough formation, but if the percentage increases for example to 10%, then the volume of the bread is negatively affected. The reduced volume of bread is related to the vegetable fiber content and the legume proteins (Sivam et al., 2010).

CONCLUSIONS

Usually, the products are improved by adding proteins of animal origin but nowadays, the interest is focused on using mostly proteins from plant sources as they can bring special nutritional value due to the fiber content, they have a lower processing cost and do not have such a negative influence on the environment. Bread dough with added lupine is harder than bread dough with wheat flour. In the samples with wheat flour and pea addition, a lower water absorption was registered during the formation of the dough, which led to the differences in the stability of the dough and the increase of resistance to mechanical mixing. The acceptability of the products is influenced by the shelf life, which is related to the loss of moisture during baking which leads in the first phase to the formation of a drier cave. The addition of Australian sweet lupine flour to wheat bread has reduced bread volume and the hardness increased. Partial replacement with bean flour, pea protein isolate and lupine flour resulted to hard hardness of bread. Pea protein isolate and lupine flour have a reducing effect on the specific volume of bread, carob flour and zein (corn) having a positive effect. Acceptability was directly influenced by the volume and texture of the bread, therefore all samples with a lower volume compared to the control sample had a lower acceptability. It is a continuous challenge to find technological procedures to facilitate the addition of plant proteins into foods in order to answer to the consumers' willingness to include functional foods with added protein in their daily diets.

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