Effect of yellow pea flour addition on wheat flour dough and bread quality

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Abstract
The aim of this study was to analyze the effect of yellow pea flour addition (5%, 10%, 15%, 20%) in wheat flour 650 type on dough microstructure by epifluorescence light microscopy (EFLM), including the analysis of dough rheological properties by using the Mixolab device and analysis of bread quality from the physical, crumb cell, textural and sensory characteristics points of view. To EFLM it seems that with the increase level of YPF substitution the protein content from the dough system was in an increased amount that surrounded more and more the starch granules. To Mixolab it was noticed an improvement of the shelf life of the finished bakery products due to the fact that the maximum consistency during stage five (C5) and the difference of the points C5 and C4 decreased.

Regarding the bread quality characteristics with the increased level of YPF substitution, it was obvious that the loaf volume, porosity, elasticity decreased, whereas the crumb structure seemed less compact and the maximum hardness increased. However, from the sensory point of view the bread with a substitution level up to 10% YPF addition was well received.

Keywords: bread quality, Epifluorescence Light Microscopy Mixolab, yellow pea flour, wheat flour

1. Introduction
Worldwide, the food and bakery products market has a dynamic character and contributes to increased competition due to technical and scientific progress, as well as consumer demand. Internationally, many varieties of breads are produced and the number increases constantly in order to satisfy consumers. Bread with added pea flour is one of these new products in consumer demand due to the nutritional qualities of peas. Pea (Pisum sativum L.) is one of the most important vegetables grown worldwide on approx. 6.59 million hectares, with an annual production of 9.83 million tons (A.K. PARIHAR & al. [1]). Pea is one of the main crops traded globally and represents roughly 35–40 % of the total trade (Q. SUN & al. [2]), with 80% of pea production coming from Russia and China (V. DILIS & A. TRICHOPOULOU [3]). Moreover, pea is gaining interest: both the yellow and green cotyledon selections have been considered for use in food products, in vegetable form and as flour. Dry pea flour is regarded as an essential dietary protein source, due to its application in meat products, constituent of milk substitutes, as texturized protein for snack foods, used in producing cereal products such as bakery products, cakes, breads, cookies or pastas, but also for its industrial application as a thickening agent in a number of food industries (B. BREŽNÁ & al. [4], S.A. WANI & P. KUMAR [5], L.H. McKEE & T.A. LATNER [6]). Research has proved for example that noodles containing 20% yellow pea flour exhibited satisfactory sensorial attributes, good texture, protein content, a reduction in glucose release and an improved protein digestibility (C.R. SHREENITHEE & P. PRABHASANKAR [7], S. 12888
BHARATH KUMAR & P. PRABHASANKAR [8]). Pea products have been demonstrated to increase protein content of spaghetti products by about 23%, the textural quality was similar to durum spaghetti (M.L. SUDHA & K. LEELAVATHI [9]).

Various studies have been carried out examining the utility of pea flour. Pea flour was used successfully as a prebiotic in the manufacture of fermented milk (F. ZARE & al.[10], D. GRANATO & al. [11], F. ZARE & al. [12]) to obtain meat products with improved protein content (K. KAACK & L. PEDERSEN [13]), baking cakes with reduced fat and sugar content (K. KAACK & L. PEDERSEN [14]), and used as the source of starch and fibers in various food products. It seems that yellow pea flour held the ability to succeed as emulsifiers and foaming agents (R.E. ALUKO & al. [15]). Some studies were made by using yellow pea flour and purple potato flour blends to yield antioxidant rich and natural pigmented extruded snacks by means of extrusion cooking technology (B. NAYAK & al. [16]). Others determined that yellow pea flour can also be used to obtain innovative low-glycemic foods (C.P.F. MARINANGELI & al. [17], C.P. MARINANGELI & P.J. JONES [18]). Consequently, the practicality of yellow pea flour can further be evaluated to advance our understanding of its effect in convenience foods (B. BREZNÁ & al. [4]).

From the nutritional point of view pea is a suitable source of macronutrients, proteins polysaccharides (starch, dietary fibers) and micronutrients, minerals, vitamins, and other healthy bioactive elements (A. ANGIOLONI & C. COLLAR [20]). The major antioxidants found in peas are vitamin C, carotenoids and various phenolic compounds (M. DUEÑAS & al. [21]). It is known that peas are an excellent source of digestible protein, and that protein content depends on different factors, from genetics to those related to growing conditions (W.J. DAHL & al. [22]). The protein is a globulin type protein which can vary between 21.2% and 32.9%, meaning that peas are a good source of essential amino acids for humans: trypsin, lysine, arginine, valine and methionine. Pea protein is appealing to the food industry due to its nutritional properties, availability, functionality, a relatively low raw material cost, minimal allergic reactions, and non-GMO (genetically modified organism) status (A.K. PARIHAR & al. [1], A.K. STONE & al. [23]).

Carbohydrates are the most important constituent of peas, representing up to 56–74% of the dry matter. Peas’ starch content ranges between 36.9%–49.0% of the seed dry matter. They are also a reliable source of both soluble and insoluble fibers (L.H. McKEE & T.A. LATNER [24]). This quality of peas is important in preventing diverticulitis, constipation and bowel cancer. It was determined that the addition of pea hull fibers in food caused a substantial intensification (7.5–24 %) of bowel movement regularity (W.J. DAHL & al. [22]). Moreover, it has been determined that whole peas and fractioned pea’s flour can reduce insulin levels by 13.5% and 9.8 %, respectively (C.P. MARINANGELI & P.J. JONES [19]). In another example, bread containing 17% pea hull fiber was found to cause substantial decline in glycemic response. Soluble dietary fiber found in peas could similarly be suitable in reducing and steadying blood cholesterol by diminishing the re-absorption of bile specific acids (C.A. PATTERSON & al. [25], M.S. LUNDE & al. [26]). Peas are also known as a consistent source of omega-3 fats via alpha-linolenic acid (ALA), thus being functional in the prevention and controlling of cardiovascular health. Another functional component of peas is quercetin, a flavonoid which is found as a glycoside with an attached sugar molecule. Antioxidants flavonoids help prevent cancer by inhibiting cancer cell proliferation, stimulating detoxification enzymes and promoting cell differentiation (C.A. PATTERSON & al. [25]).

Some studies were made on the effects of a hypo-energetic diet rich with pea fiber. They established that bread enhanced with pea fiber resulted in a longer period of satiety compared to regular bread (M.S. LUNDE & al. [26]). Pea starch (46–49% of seed dry weight) has been considered as potential food ingredient. When pea starch is added to food products Romanian Biotechnological Letters, Vol. 22, No. 5, 2017 12889
such as biscuits, pasta, bread, nutritional bars and cereal, it can boost their fibers content without impacting taste. As a functional ingredient, it is used in the recipe of bakery products and changes bread’s sensorial quality when added up to 10–20% flour replacement. Including pea flour into bread manufacturing recipe delivered a substantial increase in the amount of total dietary fiber in the end-product, from 3.94 (control sample) to 6.53 g/100 g, by adding 30% pea flour. Nonetheless, substituting regular flour with up to 20% pea flour permitted conservation of extensional, mechanical and viscometric parameters with insignificant obstruction of dough machinability (S.M. TOSH & S. YADA [27], S. SIMSEK & al. [28]). By incorporating field pea hulls in flour the water hydration capacity improved, yet several differences were prominent in gassing power and Mixograph characteristics compared to sample controls. Although water absorption increased, the specific volume of bread decreased as the proportion of field pea fibers increased in the recipe. Also, bread crust colour became lighter and more yellowish as the percentage of pea fibers increased. Shape/shred and grain/texture were evaluated as exceptional in breads supplemented with 10% field pea hulls, compared to a good evaluation of breads containing 15% pea fibers. Bread supplemented with 15% pea hull fibers, exhibited a total dietary fibers of 15% compared to only 3% in control samples (J.M. SANZ-PENELLA & al. [29]).

This paper analyses the effect of yellow pea flour addition (5%, 10%, 15%, 20%) in wheat flour 650 type on dough microstructure, dough rheological properties and bread quality from the physical, crumb cell, textural and sensory characteristics points of view. To our knowledge such a complete study on the effect of yellow pea flour on its behaviour on technological process and bread quality has never been done.

2. Materials and Methods

2.1. Materials

Commercial wheat flour 650 type (harvest 2014) was milled on an experimental Buhler mill from S.C. Mopan S.A. Suceava. Flour quality tests according to Romanian, or international standard methods, indicated the following values: 14.5 g/100 g moisture (ICC 110/1), 0.65 g/100g ash (ICC 104/1), 12.6 g/100g protein (ICC 105/2), wet gluten 34 g/100g (ICC106/1), gluten deformation index 8 mm (SR 90:2007), and falling number 380 s (ICC 107/1). The yellow pea flour (YPF) presented according to international standard methods the following values: 11.8 g/100g moisture content (ICC 110/1), 2.44 g/100g protein (ICC 105/2) content and 1.3 g/100g fat content (ICC 110/1). The analyses were made in duplicate and the values obtained are the average expression. Pea flour replaced the wheat flour at the levels of 5, 10, 15 and 20%.

2.2. Methods

Dough physical characteristics from yellow pea flour-wheat flour blends were analyzed by using the Mixolab device (Chopin, Tripette et Renaud, Paris, France) according to the standard method (ICC 173). For this purpose the Chopin + protocol was used at a rotational speed of 80 rpm.

Dough microstructure was analyzed by using the epifluorescence light microscopy (EFLM) with Motic AE 31 (Motic, Òptic Industrial Group, Xiamen, PR China). Dough samples were prepared from the wheat flour of 650 type and yellow pea flour addition of 0, 5, 10, 15, 20%. After 5 minutes of mixing, the protein and starch granules were analyzed by using fluorochromes rhodamine B which stains the proteins in red and fluoresceine (FITC) which stains the starch granules in green. To quantify the image obtained by EFLM it was used the RGB mode from the image processing program ImageJ (v. 1.45, National Institutes of Health, Bethesda, MD) according to G.G. CODINĂ & S. MIRONEASA [30].
The bread formula contained flour (100 g), dried yeast *Saccharomyces cerevisiae* (2 g), sodium chloride (2 g), yellow pea flour (0, 5, 10, 15 and 20%) and deionized water according to the water absorption of the yellow pea flour-wheat flour blends (56.3, 57.2, 57.7, 58.3 and 58.5%). The ingredients were mixed in a laboratory mixer for 15 min and then modeled and fermented at 30°C for 60 minutes at 85% relative humidity and baked in an oven (Caboto PF8004D, Italy) for 30 min at 180 ± 5°C. After 2 h after baking the bread quality was evaluated for its physical, textural, crumb structure and its sensorial characteristics.

The bread physical properties such as loaf volume, porosity, elasticity were measured according to the SR 91:2007, the bread crumb structure was analyzed with an MoticSMZ–140 stereo microscope with the 20x objective (a resolution of 2048 x 1536 pixels) and the bread textural properties (hardness, cohesiveness, elasticity, gumminess and chewiness) with a Mark-10- ESM301 texture analyzer. The values obtained are the average expression of the analyses that were made in duplicate.

The bread sensory characteristics were determined by using a panel of thirteen semi-trained judges with a preference method of a 9 hedonic scale (1- dislike extremely, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like nor dislike, 6 - like slightly, 7 - like moderately, 8 - like very much, 9 - like extremely).

### 3. Results and Discussion

#### 3.1. The effect of yellow pea flour on the EFLM analysis of dough

The dough microstructure is shown in Figure 1. It can be noticed that, as the substitution level of YPF in wheat flour occurred, the protein amount from the dough system increased while the starch granules decreased.

![Figure 1. Microstructure taken by EFLM of wheat dough with yellow pea flour (YPF) at various levels](image)

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This modification in dough microstructure may be explained due to the fact that YPF contain a high amount of protein. It is known that the two fluorochromes have different affinity in wheat flour dough. Rhodamine B is labeling the protein in red and fluoresceine is labeling the starch granules in green. Therefore, a high amount of protein in the yellow pea flour- wheat flour dough would lead to an increased protein amount and decreased starch granules due to the protein content of YPF. In all samples, the proteins seemed glued together enveloped by the starch granules. With the increased level of YPF substitution, it seemed that the protein content from the dough system was in an increased amount that surrounded more and more the starch granules.

3.2. The effect of yellow pea flour on the dough physical characteristics obtained with a Mixolab

The results obtained are shown in Table 1. In terms of technological advantage it can be noticed a statistically significant but relatively low increase of the water absorption capacity with the increase substitution of yellow pea flour level, the sample with 5% YPF addition presenting a significant higher CH value \(t=56.9, p<0.001\) as compared to the control sample.

<table>
<thead>
<tr>
<th>Mixolab parameters and falling number values</th>
<th>The levels of replacement with yellow pea flour (%) of wheat flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN (s)</td>
<td>0%</td>
</tr>
<tr>
<td>CH (%)</td>
<td>56.3±0.02</td>
</tr>
<tr>
<td>ST (min)</td>
<td>8.02±0.84</td>
</tr>
<tr>
<td>DT (min)</td>
<td>1.42±1.52</td>
</tr>
<tr>
<td>C1 (N-m)</td>
<td>1.10±0.01</td>
</tr>
<tr>
<td>C2 (N-m)</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>C1-2 (N-m)</td>
<td>0.70±0.02</td>
</tr>
<tr>
<td>C3 (N-m)</td>
<td>1.83±0.16</td>
</tr>
<tr>
<td>T_C3 (°C)</td>
<td>73.7±0.10</td>
</tr>
<tr>
<td>C3-2 (N-m)</td>
<td>1.43±0.15</td>
</tr>
<tr>
<td>C4 (N-m)</td>
<td>1.84±0.40</td>
</tr>
<tr>
<td>T_C4 (°C)</td>
<td>85.40±0.77</td>
</tr>
<tr>
<td>C5 (N-m)</td>
<td>2.71±0.58</td>
</tr>
<tr>
<td>T_C5 (°C)</td>
<td>61.4±0.19</td>
</tr>
<tr>
<td>C5-4 (N-m)</td>
<td>0.87±0.19</td>
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</table>

The falling number value presented some changes as compared to the control sample which significant increases \((t=37.4, p<0.001)\) up to 34.7% for the yellow pea flour level addition of 15% in wheat flour. In the stage 1 of the Mixolab curve dough development time increased with the increase substitution level of YPF while dough stability decreased proportional with the increase level of YPF addition with almost 50% compared to the control.
sample. An increase in water absorption, dough development time and a decrease of dough stability by incorporation of pea flour in wheat flour were also reported by Z. KOHAJDOVÁ & al. [31] which attributed this increase to the ability of proteins to absorb water, limiting the water availability for the gluten network development. Also this differentiated behavior of YPF-wheat flour dough could be due to the reduction of the gluten content from the dough system, the main responsible for the deteriorations of the dough rheological properties. It is known that the yellow pea flour contains a high amount of protein (the one used in our experiments having an amount of 25.3%). Even so the maximum consistency during stage 2 (C2) and the difference between the points C1 and C2 corresponding to the protein weakening stage was insignificant ($p > 0.1$), dough consistency and proteolytic enzymatic activity from dough system being close for all the four samples with YPF addition compared to the control sample. In the gelatinization phase corresponding to the second heating stage the dough consistency increased up to 15% YPF substitution level and then decreased. In this stage the temperature of the dough with YPF addition at starch gelatinization increased indicating a delay of starch gelatinization. As regards the dough consistency during stage four, we obtained a decrease in value, the lowest value being obtained for the 20% YPF substitution level. Regarding the dough temperature ($T_{C4}$) in this stage it was noticed a higher value of this parameter compared to the control sample. The role of YPF was clearly highlighted in the fifth stage of the Mixolab curve, were it was noticed a decrease of the maximum consistency during stage five (C5) and the difference of the points C5 and C4 proportional with the increase level of YPF addition, indicating an improvement of the shelf life of the finished bakery products.

3.3. Effect of yellow pea flour on bread quality

The results on bread physical characteristics are shown in Figure 2. It can be noticed that by increasing the level of YPF substitution, bread loaf volume, elasticity and porosity decreased up to 30% for the sample with YPF addition. However, the decrease is not significant ($p > 0.5$) for the sample with 5% and 10% addition. These results are in agreement with the results obtained by M. MAGALA & al. [32].

![Figure 2. Physical characteristics of bread samples](image)
Image analyses of the bread crumb structure shown in Figure 3 are in agreement with bread physical characteristics, namely its porosity. With the increasing of the level of YPF addition, more and large cells could be noticed due to the fact that the gluten content decreased and it could not retain the gas formed during fermentation which coalesced during baking.

![a) control sample](image1)
![b) bread with 5% pea flour](image2)
![c) bread with 10% pea flour](image3)
![d) bread with 15% pea flour](image4)
![e) bread with 20% pea flour](image5)

**Figure 3. Crumb structure of bread samples with yellow pea flour (YPF) at various levels: (a) 0%; (b) 5%; (c) 10%; (d) 15%; (e) 20%.

The texture properties of bread with different YPF addition levels are shown in Table 2. It can be noticed that the highest value for the hardness value was obtained for the bread with 20% YPF addition. This could be due to the pea protein which had a significant effect on the value increase of this parameter according to A. MOHAMMED & al. [33]. However, compared to the control sample, all the bread textural properties with YPF addition were higher.

<table>
<thead>
<tr>
<th>Textural parameters</th>
<th>The levels of replacement with yellow pea flour (%) of wheat flour</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 %</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>16.22±0.22</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.69±0.11</td>
</tr>
<tr>
<td>Elasticity</td>
<td>0.82±0.15</td>
</tr>
<tr>
<td>Gumminess (N)</td>
<td>11.19±0.17</td>
</tr>
<tr>
<td>Chewiness (N)</td>
<td>9.62±0.09</td>
</tr>
</tbody>
</table>
From the sensory point of view, the best results were obtained for the bread sample with 5% YPF addition, respectively 10% YPF addition. The increase of substitution level of YPF addition up to 20% led to the worsening of the bread sensorial properties, in especially the appearance, taste and colour.

4. Conclusion

The use of yellow pea flour as a partial substitute for wheat flour in recipes of bakery products can be successfully used. The products obtained presented a higher nutritional value providing health benefits to the consumers. Pea flour contained a double amount of protein and almost four times higher mineral amount than wheat flour, the main raw material for bakery products. Even if dough rheological properties and bread physical characteristics have grown worse with the increased level of yellow pea flour addition, the bread with 5% and 10% YPF substitution was well received by the consumers and therefore a substitution level up to 10% may be used in order to improve the nutritional quality of bakery products. Also according to the Mixolab data the yellow pea flour addition in wheat flour improves the shelf life of the finished bakery products due to the decrease of C5-4 value which indicates a lower starch retrogradation. Having in view the fact that starch retrogradation can be associated with bread staling phenomena it is possible through these values to predict the bread behavior during storage.

5. Acknowledgements

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