Obtaining a Functional Product Through the Exploitation of Mushroom Flour in Pasta

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ABSTRACT
The present study focuses on the influence of adding mushroom flour on a certain assortment of pasta. The research comprises two major directions: the study of the raw materials used to produce pasta from a compositional point of view and the quantification of some biologically active compounds of interest; emphasizing the potential of using Boletus edulis mushroom flour in the composition of pasta. For this purpose, two types of pasta have been created, with different percentages of mushroom, 10% and 20%, but also a blank sample obtained in the same conditions, but without mushroom flour. To achieve the goal the following analyzes were conducted: proteins, total polyphenols, antioxidant activity, fat, humidity, ashes, acidity, increase in volume of the boiled pasta and customers’ preferences. By using acceptability test performed on the 9-point hedonic test has been established that the consumers preferred pasta enriched with 10% mushroom flour. The present results are therefore helping the food industry regarding product diversity, consumers being eager to consume these products which have nutritional value and functional proprieties

Keywords: pasta, mushroom, quality, polyphenols, antioxidant activity

INTRODUCTION
Pasta is an Italian word which describes a cooked, extruded and dried meal, its main ingredients being wheat flour or semolina and water. Pasta consumption is in a continuous growth for modern human nutrition. For example, in the United States, the consumption of pasta has increased from approximately 3 kilograms/person in 1965 to 9 kilograms/person by 1999 (Kadam et al., 2010), because pasta is easily transportable, manoeuvred and cooked (Tudorica et al., 2002), but also because of the appealing sensorial characteristics and the accessible price (Pop et al., 2014). Pasta is a rich source of complex carbohydrates, but also a source for small amounts of Sodium, lipids and proteins (Giese, 1992). Starting from the high consumption of pasta/person/year and their low amount of proteins and bioactive compounds, along with information which states that pasta is known for being a convenient matrix for supplementation (Burluc, 2003), is noticed the need to improve this product though the exploitation of mushroom flour.

Also known as „the food of gods”, nowadays, mushrooms gain more and more attention for being included in our daily consumption, because of their nutritional value and their pharmacological and medicinal value (Akyüz, 2010).

Regarding the pharmacological and medicinal action of mushrooms, they have been found
to present antimicrobial, antitumor and immunomodulation characteristics; they inhibit placental aggregation, reduce blood cholesterol concentration, prevent the development of heart disease and reduce the amount of blood glucose (Barros et al., 2007; Lindequist et al., 2005; Reis et al. 2012). Because of their nutritional value, mushrooms become appealing as a functional product but also as a rich source of proteins, fibers, fat acids, vitamins and other active biological compounds (Heleno et al., 2012; Nedelcheva et al., 2007). Moreover, besides their medicinal purposes, mushrooms also prove a significant antioxidant capacity. Among the antioxidant compounds, polyphenols have gained specific importance, because of the variety of biological action which includes the absorbance of free radicals and, amongst others LDL oxidation inhibition (Keles et al., 2011).

The aim of the present study is obtaining a functional product with a high nutritional value and consumer perception of such a product. Therefore, the obtained product will have a high nutritional potential and will supply a larger quantity of active biological compounds.

**MATERIALS AND METHODS**

**Materials**

The primary materials used were: rough wheat flour, *Boletus edulis* dried mushrooms and quality I chicken eggs. Three work alternatives were realized in the pilot station of USAMV. Control sample PM pasta without mushroom flour addition, PI pasta with 10% mushroom flour addition and PII pasta with 20% mushroom flour addition (Table 1).

The obtaining technological process presumed the operation of preparing the primary materials, dough preparation (Fig 1), lamination, cutting, drying (Fig 2).

**Methods**

In this study, a variable experiment was conducted to determine the specific effects of varying mushroom flour percentage on the physicochemical properties important for pasta quality. Also, acceptability test performed on the 9-point hedonic test was conducted to determine the most important qualities to consumers and to establish the optimal prototype for the industrial-scale production.

**Physicochemical analysis**

Moisture content, total protein, ash, titratable acidity and volume boiling were determined according to AOAC method (2000).

**Acceptability test performed on the 9-point hedonic test**

Hedonic testing of the samples was conducted in the Sensory Evaluation Laboratory of the Faculty of Food Science and Technology. Sensory profiling of pasta samples was performed by 30 panellists with age between 21-24 years. Samples of pasta (PM, P10%, P20%) were thermally processed before tasting. Fresh water was used to cleanse the palate between samples. The panellists evaluated all the pasta formulations for colour, odour, taste,
texture and overall acceptability using a 9-point hedonic scale, 0 being “dislike extremely” and 9 being “like extremely”.

**Evaluation of the antioxidant activity of extracts and quantification of total phenolics**

**The total phenolics assay**

In order to obtain the extract for the determination of total polyphenols and antioxidant activity, 1 g of flour material was extracted with 20 ml methanol using an ultrasonication bath for 10 min and then was centrifuged at 4000 rpm for 10 min. The extract was collected and stored at 18 °C for further analysis.

The content of total phenolic was determined following the Folin-Ciocalteu method (Singleton et al., 1999). An aliquot of 0.1 ml of extract was mixed with 6 ml of water and 0.5 ml of Folin-Ciocalteu reagent. After 4 min, 1.5 ml Na$_2$CO$_3$ solution (7.5%) was added and the sample was diluted to a final volume of 10 ml with distilled water. After incubation for 120 min at room temperature, the absorbance was read at 750 nm, using a Shimadzu UV-1700 PharmaSpec spectrophotometer, against the blank, in which the sample was replaced with methanol. The calibration curve was performed using different concentration of gallic acid standard ($r^2=0.9936$) and the results were expressed as mg GAE/100 g fresh weigh.

**Determination of 2,2-diphenylpicrylhydrazil radical scavenging capacity (DPPH)**

The DPPH scavenging activity assay was performed according to a method reported by Brand-Williams et al. (1995). A volume of 3.9 ml of methanolic DPPH solution was allowed to react in darkness, for 30 minutes with 10µl of sample and 90 µl of H$_2$O. The absorbance was measured at 515 nm against methanol. The antioxidant activity was calculated as follows:

\[
\% \text{ DPPH scavenging activity} = \left( \frac{A_o - A_f}{A_o} \right) \times 100, \quad \text{where} \quad A_o, \text{was the absorbance of DPPH solution and} \quad A_f, \text{the absorbance of the sample.}
\]

**The total lycopene content**

The total lycopene content of mushrooms was determined using a rapid spectrometric technique (Davis et al., 2003). Briefly, 0.6 g of mushrooms flour were weighed and added to a centrifuge tube that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% ethanol and 10 ml of hexane. The samples were centrifuged at 6000 rpm/15 min/4°C and then were added 3 ml of deionised water and mixed. The samples were left at room temperature to allow phase separation. The absorbance of the upper hexane layer was measured at 503 nm using hexane as blank (Shimadzu UV-1700 PharmaSpec spectrophotometer) and the total lycopene content was expressed as milligrams lycopene per kilogram.

**RESULTS AND DISCUSSIONS**

The addition of mushrooms flour in pasta has been made aiming the improvement of nutrition and sensorial quality of the final product.

The raw material used is premium quality and fits with the standard (STAS 90-88) in accordance to Tabel 2.

The humidity obtained was 12.5%, the acceptance condition of humidity of the wheat flour used in pastry is of maximum 14%. This flour has a high moisturizing capacity, because of the high percentage of damaged starch, a smaller period of dough development than that of common wheat flour and a higher tolerance when kneading. The acidity of the flour is an important parameter because it indicates the level of freshness. The wheat flour used must have an acidity level of maximum 2.8° acidity, therefore the flour used fits the freshness limit necessary in order to obtain a premium product. Taking into consideration the fact that the percentage of ash indicates the flour extraction level, the result obtained after a process of determination sets the used flour in the category of semi-white flour. The minimal amount of protein found in the wheat flour used to obtain the pasta is 10% and the optimal amount is 13%, which means that the flour used for determination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture [%]</th>
<th>Acidity [° acidity]</th>
<th>Ash [%]</th>
<th>Total protein [%]</th>
<th>Wet gluten [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>12.5</td>
<td>2</td>
<td>0.7667</td>
<td>12.37</td>
<td>34.72</td>
</tr>
</tbody>
</table>

Tab. 2 The physic-chemical characteristics of the flour
Boletus edulis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture [%]</th>
<th>Fat [%]</th>
<th>Ash [%]</th>
<th>Total protein [%]</th>
<th>Lycopene [mg/kg]</th>
<th>Total Polyphenols [mg/100 g]</th>
<th>Antioxidant activity [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mushrooms</td>
<td>12.52</td>
<td>3.063</td>
<td>5.939</td>
<td>30.32</td>
<td>7.21</td>
<td>173.3</td>
<td>14.47</td>
</tr>
</tbody>
</table>

meets this criterion, being close to the optimal value. The amount of wet gluten found in flour is an important factor because it mostly determines the technological properties of the flour. To obtain quality pasta the minimal amount of wet gluten of the flour must be of 28-30%. The used sample for this determination corresponds with the quality norms.

Taking into consideration the physico-chemical characterization of Boletus edulis mushrooms Table 3, we may assess that the obtained results can be correlated to those found in the specialized literature (Ouzouni et al., 2007): 12.52% humidity, 3.063% fat, 5.939% ash, 3.32% crude protein. The examined mushrooms are rich in lycopene, especially in mushroom flour, which makes their antioxidant activity to be a significant one.

Taking into account the proposed product in this paper is an innovative one, we may talk about other authors who tried to improve pasta using certain additives: Jambreci (2011), Nedeljković et al. (2014), Pop et al. (2014) and Man et al. (2016)

It has been realized with the scope of improving the nutritional quality of classical pasta, and the obtained results are explained in Table 4.

The three products versions, the PM, P I with 10% mushroom flour and P II with 20% mushroom flour have been analysed in regard to their composition and antioxidant activity.

For pasta conservation over an extended period of time, their humidity should be maximum 12%. All three samples correspond to the requests.

Because of this determination a growth in the amount of ash may be observed for P I by approximately 40% compared to the witness sample and a near doubling in the amount of ash for P II when compared to the same PM. The growth is a result to the addition of mushroom flour, which brings a considerable amount of mineral salts.

Depending on the type of pasta, their acidity fits the maximum limit found between 4-8 degrees acidity. The witness sample, without mushroom flour addition fits these limits. The high acidity of P I and II is explained though the addition of mushroom flour.

According to the standards concerning pasta, their amount of protein must be minimum 13%, so all three samples are eligible within this aspect. The high amount of protein in P I and II are a result of the mushroom flour addition which has a supplementary amount of protein substances.

The acceptance condition for good quality pasta is minimum 250% growths in volume after, so all three versions correspond from this point of view. P I and II have a growth in volume after boiling that is more reduces, because of the high amount of mineral substances and protein.

During boiling the smell was tested, which was a pleasant one, characteristic to the PM and with a pronounced mushroom taste for P I and more specifically for P II. After finished the boiling process the water was examined as well as the product’s behaviour. The boiling water was more intensely coloured in the case of the pasta with mushroom flour addition and the product looked characteristic with no seams, lumps, and with an adequate elasticity.

The antioxidant capacity of P I and II in which mushroom flour was added is sensitively higher because of the antioxidants presents in mushrooms, which are also present in the final product.

The Acceptability test performed on the 9-point hedonic test (Fig. 3) has been conducted on a sample of 23 persons, aged between 22 and 27 years old, using the method with the hedonic scale in 9 steps. In order to conduct the analysis the pasta were boiled, placed in plates and coded using a three digit code (111 –control sample PM, 222 –sample with 10% mushroom flour P1, 333 – sample with 20% mushroom flour P2). Testers had to judge to look, color, smell, taste and the general appreciation of the three types of product.

Regarding the look, the most appreciated by the testers was sample P2 with 10% addition of mushroom flour. The witness sample obtained
a 7.44 score, which means that it was positively appreciated, and sample P2 with 20% mushroom flour received a lower score, though not a negative one.

The colour of the pasta with 10% addition of mushroom flour has been appreciated with an average 7.9, which classifies it between tasty and very tasty. The witness sample without mushroom flour addition has also been appreciated positively in regards to the colour, while the sample with a 20 addition of mushroom flour obtained a lower score, but higher than when analysing the look.

When appreciating the smell, testers considered samples PM and P1 to have a pleasant to very pleasant taste, the average being between 5 and 6.

Concerning the taste, the pasta with 10% addition of mushroom flour obtained a significantly higher score that both other samples. However, the pasta with 20% addition of mushroom flour obtained a lower score, yet positive.

PM and P1 with 10% mushroom flour addition have been 100% appreciated by the testers. The percentage of testers who chose “I don't like it” for sample number 333 has been 9.62.

CONCLUSIONS

The conducted research shows that the pasta enriched with mushroom flour by 10%, respectively 20% have a higher protein concentration, of 3% and 4% in relation to PM. The antioxidant activity grows from 3.5% PM to 5.84% PI and 6.62% for P II. A growth in acidity may be noticed as well, with 7, respectively 15° acidity.

The acceptability PI test gained the largest score in comparison to PM and P II.

The present results are therefore helping the food industry regarding product diversity, consumers being eager to consume these products which have nutritional value and functional proprieties.

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