Abstract: Nettle leaves is a good source of proteins, fibers, minerals and other bioactive compounds and it could be an ideal ingredient for improving the nutritional value of bread and bakery products. The quality evaluation of nettle leaves powder (Urtica dioica L.) and wheat flour (type 550) was performed and the effect of nettle leaves powder on the physico-chemical and sensory characteristics of wheat bread were studied. The nettle leaves powder was blended with wheat flour at different levels: 2%, 4% and 6% for preparing bread samples. The results showed a valuable increment in bread protein, ash and fiber content. The specific volume of the breads decreased as the level of nettle leaves powder increased due the dilution of gluten content in the blend and due to the interactions among fiber components, water and gluten. Nevertheless, substitution at 2%, 4% and 6%, gives parameter values at least as good as the control sample and produces acceptable bread, in terms specific volume and sensorial properties.

Keywords: bread, fiber, manufacturing technology, nettle leaves powder, protein.

Introduction
Nettles (genus Urtica, family Urticaceae) are of considerable interest as preservatives in foods for both human and animal consumption. They have also been used for centuries in traditional medicine (Kregiel et al., 2018). In Romania, as in the whole of Europe, North of Africa, Asia and North America, the nettle (Urtica dioica) is spread in uncultivated lands, in the plains, hillssides or mountains, on the edges of forests or roads, at the base of trees, being one of the many species of “spontaneous flora”. The whole plant is used as a diuretic, antihypertensive, anti-diabetic, hemostatic, anti-asthenia, antianemic, antispasmodic, antirheumatic and as a remedy for headaches and chills (Bnouham et al., 2002; Hnamouchi et al., 1999). Nettle is also used to treat spleen, renal and dermal disorders (Daoudi et al.,
The presence of valuable biologically important compounds such as proteins, vitamins, phenolic components, macro and microelements, tannins, flavonoids, sterols, fatty acids, carotenoids and chlorophylls contributes to the utilization of stinging nettle in different ways (Rafajlovska et al., 2013; Nica, et al, 2012; Kopyt'ko et al., 2012). The high nutritive values caused stinging nettle leaves to be included in the human consumption, as a tonic for strengthening the body, in the preparation of soups and various dishes and as a natural source of food flavouring (Wetherilt, 2003). A comprehensive analysis showed that nettle leaves harvested contained approximately 90% moisture, up to 3.7% proteins, 0.6% fat, 2.1% ash, 6.4% dietary fiber and 7.1% carbohydrates (Adhikari et al., 2016). On the other hand, nettle leaf powders contain on average 30% proteins, 4% fats, 10% fiber and 15% ash. Considering a higher level of protein in nettle powder, this species expected to supply higher concentrations of essential amino acids. Besides, it has a better amino acid profile than most of the other leafy vegetables (Rutto et al., 2013). Rutto et al. (2013) has reported relatively higher amounts of all essential amino acid content in Stinging nettle, except leucine and lysine. Nettle leaf powder has been incorporated in many recipes, for example, bread, pasta, and noodles dough that suggest it could be used as a protein-rich supplement (Adhikari et al., 2016).

The purpose of this study is the assessment of the quality control of bread obtained at three levels of added nettle leaves powder (2%, 4% and 6%).

Materials and methods

Procurement of raw materials

Nettle leaves (Urtica dioica) were collected from Alba, Romania. These were cleaned and washed so that the foreign particles are removed. The leaves were put inside the cabinet drier, for drying at 40°C for 24 hours, till the crispy texture was observed. Dried leaves were ground in a coffee grinder and sieved through the 80 size mesh making into a fine nettle powder (NP). The samples were placed in dark glass bottles were stored at 3-4°C in a refrigerator. The wheat flours, yeast and salt were purchased from the local market.

Baking test

Experimental breads were obtained from wheat flour blends containing 0% (100% wheat flour Type 550) and 2%, 4% and 6% of NP (as wheat flour replacement). The bread prepared from wheat flour without NF
substitution served as control. The bread dough was obtained in a laboratory mixer by kneading 1000 g flour, 15 g iodized salt, 25 g fresh yeast (Pakmaya Yeast Rompak, Romania) and with water 590 ml for control bread (BNP0%) and 612 ml, 630 ml, 645 ml respectively for bread supplemented with nettle powder (BNP2%, BNP4% and BNP6%). After kneading ca. 8 min, the dough was fermented at 30ºC for 60 min, then divided into 550 g portions and placed into non-stick baking trays. The dough was then proofed for 40 min at 35ºC and 85% relative humidity in a proofer (Zanolli Teorema Polis 3 PW, Italy), and baked immediately in a preheated oven (Zanolli Teorema Polis 3 PW, Italy), with top and bottom heat, at 220ºC, for 50 min. The oven was pre-steamed before and again after putting the bread in.

**Wheat flour, nettle leaves powder and bread chemical parameters**

The chemical characteristics were carried out according to AACC (2000) Approved Methods. Moisture (44-15.02), lipids (30-25.01), ash (08-01.01), crude fiber (32-07.01) and protein were measured using the Kjeldahl method (46-11.02), nitrogen to protein conversion factor was 5.7. Total carbohydrate (%) content was calculated as the difference: 100 - (moisture + ash + proteins + lipids + crude fibers) method reported also by Kasaye and Jha, 2015.

**Bread quality parameters**

Two hours after baking, the loaves were weighed, and bread volume was determined. The weight of the bread was taken using a digital balance. The bread volume was determined by the seed’s displacement method. The volume of seeds displaced by the loaf was considered as the loaf bread volume. Specific volume of bread was expressed as the volume / weight ratio (cm³/g) multiplied by 100 of finished bread.

**Sensory evaluation**

The sensory characteristics of bread were evaluated by 25 trained sensory panels. The panelists were asked to evaluate colour, aroma, taste, flavour, texture and overall acceptability of the samples on a 9-point hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely.

**Statistical analysis**
The results of three independent (n=3) assays performed with replicates each were expressed as means ± standard deviations. Data were analysed by one-way Analysis of Variance (ANOVA).

**Results and Discussion**

The mean values of chemical composition of wheat flour and nettle powder are presented in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wheat flour</th>
<th>Nettle leaves powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>13.79±0.37</td>
<td>3.70±0.08</td>
</tr>
<tr>
<td>Protein, %</td>
<td>11.20±0.27</td>
<td>29.74±0.33</td>
</tr>
<tr>
<td>Ash, %</td>
<td>0.57±0.03</td>
<td>17.67±0.09</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.23 ±0.13</td>
<td>2.75 ±0.12</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>0.69±0.45</td>
<td>8.37±0.54</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>72.52±5.47</td>
<td>33.77±3.72</td>
</tr>
</tbody>
</table>

Values expressed are means ± standard deviation;

The initial moisture content of the leaves was not measured. Bhaskar et al. (2016) has reported that previous studies have shown that the nettle plant contains relatively high level of moisture, for example, 89% (Rutto et al. 2013) and 84.4% (Mishra, 2007). The moisture content of wheat flour was 13.79% which is common with commercial wheat flour. After cabinet drying of leaves followed by grinding, the moisture content of the nettle powder was reduced significantly to 3.70% (Table 1).

High nutrient potential of nettle powder is due in large part to the high content of protein, ash and fiber. Different factors affect the chemical composition of nettle plants, such as the variety, genotype, climate, soil, vegetative stage, harvest time, storage, processing and treatment (https://www.pfaf.org; Augspole et al., 2017; Rafajlovska et al., 2013). Protein content of the ground wheat flour and powder nettle were 11.2%, and 29.74%, respectively. As compared to the conventional source of proteins, nettle powder contains 3.2 and 2.9 times greater amount of proteins as compared to wheat flours (Bhaskar et al., 2016). In a study of nettles by Rafajlovska et al. 2013, higher quantities of proteins were found in the leaves than in the stems and roots. The content of proteins in the leaves ranged from 16.08–26.89%, depending on the source of the sample (Adhikari et al., 2016).

Nettle powder has one of the richest sources of crude fiber (8.37%) (Table 1). The amount of crude fiber in the nettle powder is significantly
higher than most of the cereals and other plant foods, more than 10 times higher as compared to wheat flour. Published literatures showed the nettle powder has 9.08% crude fiber (Bhaskar et al., 2016; Kregiel et al., 2018). The level of crude fat is relatively low at 2.75%, but this value is still higher than wheat (1.23%).

Stinging nettle is rich in minerals. Compared with wheat flour, nettle powder has much higher total ash content (0.57% for wheat flour and 17.67% for nettle powder) (Table 1). Rutto et al. (2013) reported that the total ash content of nettle powder is 19.1% on a wet basis and Bhaskar et al., 2016 reported that the total ash content of nettle powder is 16.21% (db) being demonstrated by higher calcium level (169 mg / 100 g) and iron (277 mg / 100 g). USDA data showed the Nettle powder contains 4% calcium (db), 2.8% (db) potassium followed by phosphorus, magnesium and traces of iron, sodium and zinc (USDA, 2008). Based on this data, nettle powder is probably one of the richest sources of minerals among plant foods.

Nettle powder contained the lowest amount of carbohydrate (33.77%) as compared to wheat flour (75.52%). This study shows the nettle powder is much less glycemic as compared to the conventional sources of plant foods such as cereals. Carbohydrate levels in the nettle powder decreases with increase in the protein content, fiber, ash, and fat as shown in Table 1. The results agree to the report given by Palikhe (2012) Thapaliya (2010).

According to the results shown in table 2, significant reduction in specific loaf volume was observed at all the levels of NP supplementation (from 278.2 cm³/100g to 246.65 cm³/100g). Maximum reduction in loaf specific volume was observed in case of 6% NP supplementation level. This may be due to the dilution effect on gluten content with the addition of gluten-free flour to wheat flour that has been reported to be associated with specific loaf volume decreasing effect of composite flour. Hung et al. (2007) argued that the existence of dietary fibre diluted the protein and interfered with the optimal gluten matrix formation during dough mixing. According to literature, the addition of different materials rich in fiber, up to 7%, produced a volume drop which is proportional to the reduction of gluten content in the blend. Laurikainen et al., 1998 reported that above this value, bread volume decreases at a rate higher than the theoretical one, due to lower gluten protein content. Nevertheless, Izzo and Franck (1998) assert that the addition up to 20% of fibers, the volume downward trend is not significant, while at 40-50% the obtained bread is inadequate.

Table 2

| Chemical characteristics of bread supplemented with Nettle leaves powder |
|--------------------------|--------------------------|

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Characteristics

<table>
<thead>
<tr>
<th>Bread samples</th>
<th>BNP0%</th>
<th>BNP2%</th>
<th>BNP4%</th>
<th>BNP6 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific loaf volume, cm$^3$/100g</td>
<td>278.2±0.45</td>
<td>260.3±0.23</td>
<td>257.11±0.76</td>
<td>246.65±0.68</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>42.85±0.27</td>
<td>43.62±0.18</td>
<td>44.93±0.45</td>
<td>45.70±0.73</td>
</tr>
<tr>
<td>Protein, %</td>
<td>10.01±0.04</td>
<td>10.90±0.12</td>
<td>12.07±0.32</td>
<td>13.24±0.06</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.39±0.03</td>
<td>1.68±0.09</td>
<td>2.10±0.08</td>
<td>2.20±0.14</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.12±0.13</td>
<td>1.51±0.28</td>
<td>1.68±0.16</td>
<td>1.72±0.27</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>1.02±0.04</td>
<td>1.48±0.09</td>
<td>1.83±0.14</td>
<td>2.07±0.19</td>
</tr>
<tr>
<td>Total carbohydrate, %</td>
<td>43.61±1.46</td>
<td>41.81±0.89</td>
<td>41.39±0.55</td>
<td>41.07±0.27</td>
</tr>
</tbody>
</table>

Values expressed are means ± standard deviation;

Moisture content for bread increased on fortification with nettle powder from 42.85% in the case of the control sample to 45.70% for the bread with 6% added NP, which can be attributed to the high water binding capacity of the nettle powder. The protein content in wheat bread increased significantly (p<0.05) with the amount of substitution of NP. The bread fortified with 6% NP had significantly higher protein content, due to the high protein content of the nettle powder. Regarding the bread ash content and the crude fiber, in relation with the NP addition, there is a direct proportional increase between these parameters and the percentage of NP incorporated. By increasing the addition of Nettle powder from 2% to 6%, the fat contents increase slightly from 1.12% for BNP0% to 1.27% for BNP6%. Also, the crude fiber content increased from 1.02 to 2.07%. Growth of these compounds is almost 2 times compared to wheat flour. Both increments, in the ash and the lipids contents, are beneficial for health since nettle powder can provide important amounts of iron, zinc, magnesium, calcium, phosphorus, potassium (Amal et al., 2015) cobalt, nickel, molybdenum and selenium (Mihaljev et al., 2014).

Total carbohydrate decreased as the NP increased, due to the high content in protein, fat, crude fiber, lipid of the Nettle powder, resulting a final product enriched in all these bioactive compounds.

Table 3

Sensory evaluation of different bread samples

<table>
<thead>
<tr>
<th>Bread Samples</th>
<th>Colour</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP0%</td>
<td>8.57±0.40</td>
<td>7.97±0.12</td>
<td>7.83±1.34</td>
<td>8.66±1.45</td>
<td>8.37±0.88</td>
</tr>
<tr>
<td>BNP2%</td>
<td>8.46±0.23</td>
<td>7.81±1.14</td>
<td>7.77±1.22</td>
<td>8.38±1.68</td>
<td>8.12±1.03</td>
</tr>
<tr>
<td>BNP4%</td>
<td>8.39±0.12</td>
<td>7.77±1.06</td>
<td>7.70±1.10</td>
<td>8.08±1.75</td>
<td>7.98±1.23</td>
</tr>
<tr>
<td>BNP6%</td>
<td>8.31±0.61</td>
<td>7.56±0.48</td>
<td>7.37±1.37</td>
<td>7.79±1.63</td>
<td>7.75±1.47</td>
</tr>
</tbody>
</table>
The mean scores of sensory attributes ranged between 7.75 and 8.37 for the tested samples. Bread prepared with 100% of wheat flour scored maximum than the rest of the samples. Evaluating the influence of the samples with the addition of nettle powder, it is observed that the highest value was recorded for bread made with 2% NP while the lowest value was for bread made with 6% NP. There was an apparent decreasing trend for aroma and taste, probably due to the nettle powder. The sensory evaluation indicated that the sample fortified with 2% NP had the highest acceptability score. However, considering high nutritional value and health benefit, one can conclude that bread supplementation up to 4% nettle powder is optima.

Conclusions

The experiment demonstrated that it is possible to use nettle powder to partially substitute wheat flour in the elaboration of bread. From present results, it could be noticed that, the addition of nettle powder to wheat flour improved the protein, fibers and mineral content of the bread. The overall acceptability of the NP enriched breads was performed by sensorial analysis, revealing good organoleptic attributes for the samples up to 4% NP. Supplementation with 4% nettle powder could be adopted in wheat bread manufacturing without affecting quality adversely.

References


