***Stručni članak***

# *URTICA DIOICA*L.AS FUNCTIONAL FOOD SUPPLEMENT

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**Abstract:** *Urtica dioica* L. leaves and Soxhlet extract were used as supplement for obtaining functional bread. The aim of this study was to determine the effect of the addition of the tested plant material, as well as additives, on the physico-chemical and sensory quality of bread. Leaves were added in twoconcentration(5% and 1%) and Soxhlet extract was added in concentration of 5% and 10% of wheat flour mass in dough. Physico-chemical and sensory parameters,such as the height and volume of bread, mass of bread, fineness and elasticity of the bread pores, phenols and flavonoids were determine 24 hours after baking, The results showed significant differences between bread samples with and without *Urtica dioica* L. supplements. Also, the addition of extract does not impair the technological quality of bread, whichwas the case with the addition of leaves.Functional bread with significant content of biologically active compounds was obtained.
***Keywords****:functional food, functional bread, Urtica dioica L., leaves, extracts*

## 1. Introduction

Functional food is food whose biological characteristics positively affect our health and certain body functions. It has a role to provide the body with the necessary energy and nutrients, as well as to influence the preservation of health and disease prevention, and the improvement of certain organism conditions, balancing and improving the standard of living (Milner, 2000; Roberfroid, 2002). In its form, functional food is similar to conventional food, which is an integral part of our usual diet, but it has psychophysical benefits in addition to basic nutritional functions. It can improve the general condition of the organism, reduce the risk of various diseases, as well as help in the treatment of some diseases (Mark-Herbert, 2004; Menrad, 2003).

Interest in functional food is increasing, so new products are appearing and guidelines are being set for what awaits us in the future. In our country, this concept has only recently gained real momentum with the introduction of new products, primarily in the dairy industry, and

immediately afterwards in the confectionery industry. Health awareness is growing and healthy, functional food is increasing in demand, because it prevents disease and improves the quality of life.

The largest part of the world's population satisfies daily energy needs primarily from carbohydrates. The large presence (35-45%) of bakery products in the structure of the daily meal is conditioned by eating habits, and partly by the living standard of the population. In recent years, the demand for special types of bread and bakery products with certain additives has increased in our market as well.

*Urtica dioica* L. is a perennial herbaceous plant 30-150 cm tall (Fig. 1). The plant itself has a woody, cylindrical and branched rhizome. The stem is erect, unbranched or branched with short bristles or long glowing hairs. It has long, linear valves. There are usually two leaves per nodus, and less often three. The leaf has three to five nerves, it is densely serrated on the rim, sometimes even double. This plant grows in lowland areas up to those at altitudes up to 1800 m. It primarily inhabits lands rich in nitrogen in very different habitats such as: wetlands, forests, river valleys, pens, fields, gardens, orchards, along roads and in populated areas. It is almost cosmopolitan, anthropogenically widespread. It is considered a weed species that grows almost everywhere.

*Figure 1. Urtica dioica L.*

*(https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:260630-2)*

There are several different studies available that have examined the chemical composition of this plant. Different parts of the plant, such as the leaf, stem and root, were examined (Upton, 2013). The analysis determined the presence of various phenolic or polyphenolic compounds (Kraus and Spiteller, 1990; Carvalho et al., 2017; Otles and Yalcin, 2012; Farag et al., 2013), then fatty acids (Guil-Guerro et al., 2003; Bagci, 2002), pigments (chlorophyll and carotenoids) (Guil-Guerro et al., 2003; Olsen, 2015; Hojnik et al., 2007), amino acids (Yunuskhodzhaeva et al., 2014), minerals (Kara, 2009; Mahlangeni et al., 2016), sterols and their glycosides (Chaurasia and Wichtl, 1987), B vitamins and vitamin C (Upton, 2013). In addition to the mentioned classes, terpenoids and various other compounds were detected, i.e. various hydrocarbons and derivatives of the mentioned classes (Lapinskaya and Kopyt`ko, 2008; Akalin et al., 2013), while the content of essential oil in the leaves of this plant is extremely small (Gul et al., 2012).

Previous research has indicated a wide range of effects of this plant and its extracts. Different extracts obtained by applying different solvents and their mutual combinations, as well as by applying different extraction techniques, both conventional and unconventional, were examined. Studies have shown that *Urtica dioic*a L. and its extracts have antioxidant,

antimicrobial, analgesic effects, as well as effects on ulcers (Upton, 2013; Usman et al., 2012; Ghaima et al., 2013).For all the above reasons, the importance of *Urtica dioica* L. and its extracts is clear, as well as examining the possibility of using this plant material for the purpose of obtaining functional food.The results of previous studies suggest that leaf and extract of *Urtica dioica* L. may be used as flour (Adhikari et al., 2016) and as functional food additive in egg pasta (Marchetti et al., 2018) and also in functional bread (Đurović et al., 2019).

## 2. Materials and methods

The following raw materials were used in the experimental work:

1. Flour for sourdough and homemade pasta T-500, manufactured by "Danubius doo" from Novi Sad,
2. Kitchen salt, commercial product, manufacturer "Solana", Tuzla,
3. Fresh baker's yeast, producer "Budafok", Hungary,
4. Commercial additive "Sava Lux 500" Company Kršulj, Belgrade,
5. Tap water for drinking.
6. Further more, plant materials used in the experiments were:
7. Dried *Urtica dioica* L. leaf in bulk
8. *Urtica dioica* L. extract 1:10, obtained by Soxhlet extraction.

The conventional extraction methodused for the extraction of plant material was Soxhlet extraction, where a certain mass of the sample (10 g) was extracted with 150 mL of solvent (70% ethanol).

The bread was prepared according to the procedure prescribed by the standard AACC method (AACC, 2000). The raw material composition of the bread dough was as follows: flour 100 g, salt 2% (by weight of flour), yeast 2,5% (by weight of flour). Additional raw materials were added according to the experimental plan (Table 1.), whereby part of the flour was replaced with *Urtica dioica* L. leaves, i.e. part of the water with *Urtica dioica* L. extract.

*Table 1. Experimental plan*

| Sample | Extract (% by weight of flour) | *Urtica dioica* L. (% by weight of flour) | Additive (% by weight of flour) |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0.3 |
| 3 | 5 | 0 | 0 |
| 4 | 5 | 0 | 0.3 |
| 5 | 10 | 0 | 0 |
| 6 | 10 | 0 | 0.3 |
| 7 | 0 | 5 | 0 |
| 8 | 0 | 5 | 0.3 |
| 9 | 0 | 1 | 0 |
| 10 | 0 | 1 | 0.3 |

According to the plan of the experiment, the dough was mixed in a kneader made by Silver Crest, Germany, for 5 minutes. It was then shaped into a ball and left on the work surface, at room temperature, to ferment in the mass for 90 minutes. During fermentation, the dough was stirred after 60 minutes. The dough was then hand-shaped oblong and placed in greased bread molds (15 cm long, 5 cm wide and 7 cm high). The final fermentation was performed at a temperature of 30 ºC, for 60 minutes. Bread samples were baked in a laboratory oven for 15 minutes at 250 ºC. After baking, the breads were cooled for 1 h at room temperature and then stored for 24 h under controlled humidity and temperature conditions.

Changes in bread quality in the function of selected independent parameters were monitored based on the results of determining the physico-chemical and sensory characteristics of the finished product.The mass of bread was measured 1 h after baking and cooling, and then after 24 h.The volume of bread was estimated 24 h after baking, and was determined by the method of squeezing millet grains (Kaluđerski and Filipović, 1998).The specific volume of bread Vsp ($cm^3/g$) was determined as the ratio of the volume and weight of bread.Moisture content was determined by the gravimetric method according to *Ph.Jug.IV* (1984). The obtained results are expressed as a percentage of moisture content (% m/m).The ash content was determined by the gravimetric method according to *Ph.Jug.IV*(1984). The obtained results are expressed as a percentage of ash (% m/m).

The quality of the bread was graded organoleptically 24 hours after baking, whereby the fineness of the crumb pore structure and elasticity were evaluated by palpation technique. Descriptive grades of the mentioned bread crumb quality indicators are shown in total values and graded in table for grading the bread crumb value (Kaluđerski and Filipović, 1998) and marked as Value for Bread Crumb – VBC (minimum 0,0 and maximum 7,0). Sensory parameters of bread samples (color, smell and taste) were evaluated by descriptive sensory analysis.

Determination of total phenol content was by the Folin-Ciocalte method (Singelton and Rossi, 1965; Kahkonen et al., 1999). This method is based on spectrophotometric determination of the content of total phenols in the reaction mixture of the tested extract, distilled water, Folin-Ciocalteu reagent (mixture of phosformolybdate and phosphotungstic acid) and 20% sodium carbonate solution. Solvent used for the extraction was 70% ethanol. To 0,1 ml of extract was added 7,9 ml of distilled water, 0,5 ml ofFolin-Ciolcateu reagent, and 1,5 ml of 20% sodium carbonate. Comparative trial was prepared in the same way by adding 8 ml of distilled water. After incubation for one hour at room temperature, the absorbance was measured at a wavelength of 750 nm. The calibration curve of the standard gallic acid solution was used to determine the content of total phenols.The content of total phenols in the tested ethanol extracts was expressed in mg of gallic acid equivalent per 1 ml of extract (mg GAE/ml).

The content of total flavonoids in ethanol extracts was determined by colorimetric method according to Markham (1989). To 1 ml of sample, 4,0 ml of distilled water and 0,3 ml of 5% Na-nitrite were added. As a comparative trial, a mixture was used in which 1 ml of distilled water was added instead of 1 ml of sample. The mixture was incubated at room temperature for 6 minutes. After incubation, 0,3 ml of $AlCl_3x6H_2O$ was added to the reaction mixture and 5 minutes later another 2 ml of $1mol/dm^3$ NaOH. The resulting mixture was made up to volume with distilled water to a total volume of 10 ml. The absorbance of the sample was measured at a wavelength of 510 nm. The total flavonoid content in the analyzed samples was expressed as mg of catechin equivalent per ml of extract (mg CE/ml).

## 3. Results and discussion

As planned in the experimental work, 10 samples of bread of appropriate composition were baked. Also conditions of mixing, fermentation and baking were showed in table 2.

*Table 2. Conditions of mixing, fermentation and baking*

| | |
|---|---|
| Flour temperature (° C) | 22 |
| Mixing water temperature (° C) | 30 |
| Mixing duration (min) | 5 |
| Duration and rest (min) | 60 |
| Duration of II rest (min) | 30 |
| Duration of final fermentation (min) | 60 |
| Baking time (min) | 15 |

According toabove mentioned experimental plan and mixing, fermentation and baking conditions,the following results with basic statistic (AVG - average values and ±SD - standard deviation)showed in Table 3.

*Table 3. Values of physico-chemical and sensory quality of bread*

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AVG | ±SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bread weight (g) | 144,87 | 151,44 | 146,28 | 146,04 | 144,22 | 143,61 | 145,17 | 159,98 | 143,82 | 143,14 | 146,86 | 5,18 |
| Bread weight after 24 hours (g) | 144,80 | 151,07 | 145,77 | 145,27 | 143,26 | 143,00 | 145,12 | 159,62 | 143,27 | 142,23 | 146,34 | 5,28 |
| Dough yield (%) | 156,22 | 163,38 | 161,12 | 162,12 | 160,47 | 158,85 | 157,63 | 174,26 | 157,29 | 155,02 | 160,64 | 5,48 |
| Bread yield (%) | 144,87 | 151,44 | 146,28 | 146,04 | 148,14 | 145,29 | 145,17 | 159,98 | 143,82 | 143,14 | 147,42 | 5,00 |
| Losses during baking (%) | 7,26 | 7,31 | 9,21 | 9,92 | 7,68 | 8,54 | 7,9 | 8,19 | 8,56 | 7,66 | 8,22 | 0,85 |
| Losses during cooling (%) | 0,05 | 0,24 | 0,35 | 0,53 | 1,56 | 1,42 | 0,03 | 0,23 | 0,38 | 0,64 | 0,54 | 0,53 |
| Bread volume (ml) | 345 | 395 | 350 | 460 | 225 | 215 | 315 | 360 | 315 | 370 | 335,00 | 73,56 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Specific volume | 2,38 | 2,61 | 2,40 | 3,17 | 1,57 | 1,50 | 2,17 | 2,25 | 2,20 | 2,60 | 2,29 | 0,49 |
| Bread height (mm) | 63 | 73 | 71 | 79 | 50 | 51 | 63 | 65 | 62 | 70 | 64,70 | 9,18 |
| Moisture content (%) | 41,12 | 41,36 | 36,86 | 40,30 | 36,77 | 35,78 | 34,47 | 38,03 | 40,07 | 37,73 | 38,25 | 2,36 |
| Ash content (%) | 8,09 | 7,99 | 8,96 | 9,27 | 10,30 | 10,64 | 8,91 | 7,71 | 10,44 | 10,18 | 9,25 | 1,10 |
| Elasticity of the medium | Good + (3,2) | Very good - (3,8) | Very good - (3,8) | Very good + (4,2) | Satisfies (2,0) | Satisfies (2,0) | Good (3,0) | Very good - (3,8) | Good (3,0) | Very good - (3,8) | | |
| The fineness of the pore structure | Almost fine / a little rough (1,2) | Almost fine / a little rough (1,2) | Almost fine / a little rough (1,2) | Almost fine / a little rough (1,2) | Rough (0,5) | Rough (0,5) | Little rough + (1,1) | Little rough + (1,1) | Little rough + (1,1) | Almost fine / little rough (1,2) | | |
| Value for Bread Crumb – VBC | 4,4 | 5,0 | 5,0 | 5,4 | 2,5 | 2,5 | 4,1 | 4,9 | 4,1 | 5,0 | 4,29 | 1,03 |

Table 3. shows the results of the test obtained from flour, without additives (sample 1), with 0.3% of additives (sample 2). It can be concluded that the quality of bread is significantly improved by the addition of additives. The volume of bread, as well as the height are increasing, and as a confirmation of the improved quality of bread, values for the elasticity of the medium and the fineness of the pore structure were obtained. A clear indicator is the Value for Bread Crumb – VBC, obtained by the sum of their values, and for sample 2, with the addition of 0.3% additives, it is 5,0.

When added to the base dough *Urtica dioica* L. extract, in different percentages, with a change in other parameters (addition of additives), the following results were obtained: Bread in which *Urtica dioica* L. extract was added in the amount of 5 % of weight of flour, had a very good height and value of VBC. With the addition of 0.3% additives, the quality of bread improved significantly. Both the height and volume of the bread have increased significantly. The Value for Bread Crumb was very high (5,4). The sensory bread characteristics were evaluated with a very good grade. The color of the bread is light, equal. The smell and taste of bread are pleasant, harmonious and tempting for consumers.

When it comes to bread samples to which a larger amount of *Urtica dioica* L. extract was added (10 %), based on the obtained results, it was found that the quality of bread did not significantly improve with the addition of additives (0.3%). The elasticity of the medium and the fineness of the pore structure were rated very low, so that the value of VBC is 2,5 for both samples (sample 5 and 6). The color of the bread is slightly greenish, which was also expected, since the leaf itself is green. The smell and taste of bread are harmonious, they give a mild reaction to the palate. The results of sensory testing of the samples showed that this type of the obtained product is acceptable for consumers.

Furthermore, samples 7 and 8 prepared with the addition of 5% dry leaf of *Urtica dioica* L., and the addition of 5 % dry leaf and 0.3% additive, respectively, received a high score for the

elasticity of the medium and the fineness of the pore structure, respectively for VBC, but the sensory characteristics were rated low. The color was extremely green, and the smell and taste were bitter and almost unpleasant, which concluded that the product obtained in this way was unacceptable for consumers. When samples were prepared with a lower percentage of added *Urtica dioica* L. leaf (1%), sample 9 and sample 10 (1% leaf and 0,3% additive), the sensory characteristics of breads were evaluated with slightly higher scores. The taste was slightly bitter, and the color was light green to brown.

Bread volume is one of the key parameters that determine the quality of bread. It is especially important from the point of view of consumers themselves. The addition of dry leaves of *Urtica dioica* L. reduces the volume (samples 7, 8, 9 and 10), while the addition of the extract increases the volume of bread (samples 3, 4, 5 and 6). Also, as already described, with the increase of the leaf content, there is a darkening of the bread, as well as a more pronounced taste of *Urtica dioica* L. leaves. The extract reduces the influence of the leaves, but the best results are shown by the sample with 5% of the extract, where the extract itself does not negatively affect the technological quality of bread, as is the case with the addition of 5 % leaves to the bread. Further, considering the fact that in samples 5 and 6, as well as samples 9 and 10, equal amounts of *Urtica dioica* L. leaves were added, only in different forms (10 % liquid extract 1:10, or 1% dry leaf) it is concluded that for this type of product, the liquid extract is a more adequate form. This result is in accordance with a previous study in which similar percentages of extracts were used (Đurović et al., 2019).

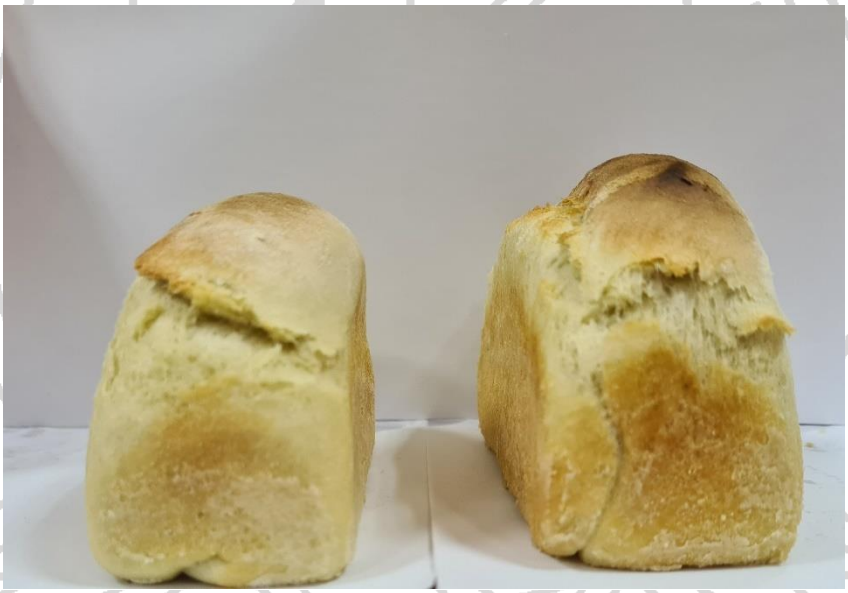

*Figure 2. Sample of bread with the addition of 5 % extract of Urtica dioica L., with (left) and without the addition of 0.3 % additive (right)*

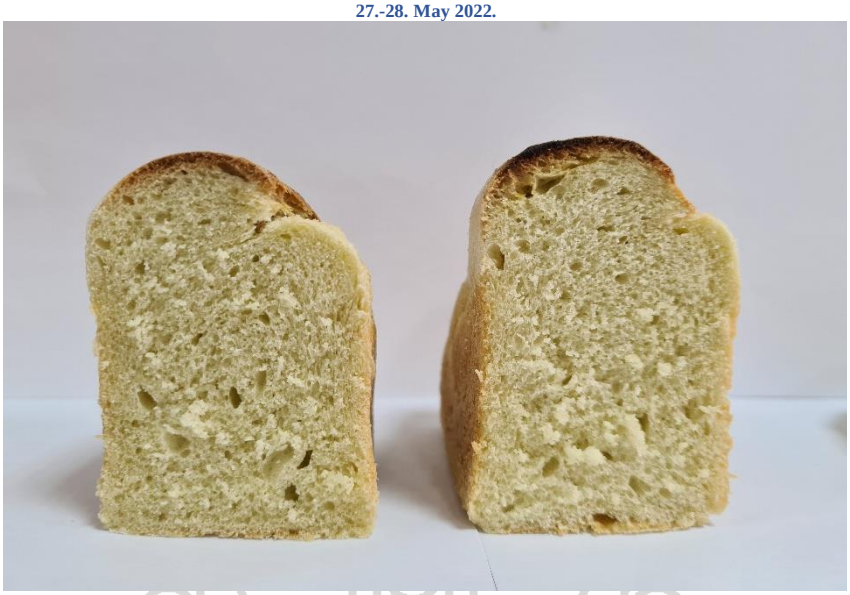

*Figure 3. Cross section of bread samples 3 and 4 made from the basic dough, with the addition of 5 % extract of Urtica dioica L. without additives (left), and with 03% additives (right)*

The content of total phenols in the tested ethanol extracts was in range of 194,6 and 199,7 mg GAE/ml, while the mid-range measured content of total flavonoids was 114,4 mg CE/ml.

Due to the presence of various phenolic or polyphenolic compounds, fatty acids, pigments, amino acids, minerals, sterols and glycosides, as well as vitamins, leaf of *Urtica dioica* L., as well as liquid extracts obtained by Soxhlet extraction, are biologically valuable compounds, so working with them for the purpose of obtaining functional bread is considered very justified. Every quantity of high-quality structures made from the *Urtica dioica* L., which is found in breads made in this way, makes them foods with a significantly enriched composition. This clearly shows that these types of products, under the conditions tested, are functional foods, and can form an essential part of our nutrition.

## 4. Conclusions

The practical application of *Urtica dioica* L. leaf (containing 5 and 1%) and it`s extract (5 and 10%) by making bread was investigated. The parameters of physical and chemical quality were examined, as well as the sensory characteristics of the obtained product.Results showed that the addition of an equivalent amount of *Urtica dioica* L. extract does not impair the technological quality of bread, as is the case with the addition of leaves, and that the obtaining functional bread is with significant content of biologically active compounds that can prevent human health.

## 5. Literature

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