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STUDY ON TOTAL PHENOLIC CONTENT IN SOME ROMANIAN FOREST MUSHROOM SPECIES, BEFORE AND AFTER HEAT TREATMENT

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Abstract: *The purpose of this work was to study the phenolic compounds in ten forest mushroom species to see if and to what extent the thermal processing can modify the content of these compounds. The biological material consisted of ten forest mushroom species, colected from Suceava county area: Agaricus campestris, Morchella esculenta, Cantharellus cibarius, Armillaria mellea, Lactarius deliciosus, Boletus edulis, Pleurotus cornucopiae, Russula vesca, Sparassis crispa, Ramaria aurea. The experiment consisted in mushrooms thermal processing (boiling for 30 and 60 minutes, and roasting for 10 minutes at 160°C), followed by the analysis of Total Phenolic Content (TPC) which values were compared to the ones obtained from raw samples. As compared to raw material, the boiling process led to a significant increase of percent of TPC between 13.71% and 31.91% (after 30 min. of boiling) and between 12.33% and 61.36% (after 60 min. of boiling). As compared to raw material, the roasting of mushrooms for 10 minutes (at 160°C) led to significant increases of the phenolic compounds between 29.31% and 95.23% in all samples. High TPC values recorded by some species may be explained by the specific tissue structure of these species, which promotes a penetration and a more intense heat action on bound phenols.*

Keywords: *phenolic content, mushroom, boiling, roasting*

1. Introduction

Widespread in plants and present in many species, phenolic compounds highlight various properties, from antioxidant, anti-microbial, anti-inflammatory, anti-allergenic to cardio-protective and vasodilatory ones [1- 6]. There is a relationship between the consumption of phenolic-rich food products and a low incidence of coronary heart disease, atherosclerosis, certain forms of cancer and stroke [7-10].

According to Huang et al., 1992 [11], due to their antioxidant properties, plant phenolic compounds have potential benefits for health.

Plant species and varieties influence the content of phenols and of other metabolites [12- 16].

Lately, a number of scientific papers have revealed the presence of some mineral elements (Ca, Mg, P, Cd, Se), vitamins (B1, B2, B12, niacin, folates, C, D), flavonoids, lignans and phenolic acids in different species of cultivated mushrooms [17].

Also, have been reported some mushroom species used to prevent hypertension, hypercholesterolemia and even cancer [18, 19, cited by 20), due to the presence of chitin [21], and of beta glucans with β (1-3), β (1-4) and β (1-6) glycosidic linkages [22, 23 cited by 20].

The heat processing influences the concentration and quality of food nutrients

(carbohydrates, lipids, proteins), but there are less information, in this regard, on natural antioxidants and their activity.
Cooking induces changes in physiological and chemical composition, influencing the concentration and bioavailability of bioactive compounds in food, the thermal treatments decreasing the total phenolics in squash, peas and leek [24]. Dewanto *et al.*, *2*002a [25] showed that in sweet corn, cooking led to an increase in the level of phenolic compounds.
This paper studies the phenolic compounds in ten forest mushroom species in order to see if and to what extent the thermal processing can modify the content of these compounds.

2. Experimental

2.1. Research material and samples preparation

The biological material was represented by ten forest mushroom species, colected from Suceava county area: *Agaricus campestris, Morchella esculenta, Cantharellus cibarius, Armillaria mellea, Lactarius deliciosus, Boletus edulis, Pleurotus cornucopiae, Russula vesca, Sparassis crispa, Ramaria aurea.* All these species are edible and tasteful, being consumed with pleasure by people. From each species they have prepared control (raw material) and thermal processed samples.

2.2. Procedure and research methods

The experiment consisted in thermal processing (boiling and roasting), whose results were compared to those ones obtained from raw samples.
For boiling 30 minutes and 60 minutes, they have taken (for each procedure) 50 g mushrooms of each sample, which have been placed in a stainless steel vessel of two liters capacity. The boiling has done in one liter of tap water in the pot covered, for 30, respectively 60 minutes (timed from the moment when the water began to boil).
For roasting, 50 g of each sample were heated for 10 minutes at 160°C, in an electric oven.
In order to determine Total Phenolic Content (TPC), first an extract for each sample (raw or thermal processed) was obtained, weighing 10 g mushrooms, which were ground and subjected to extraction with a mixture methanol and water (80/20), by stirring, centrifuging and recovering the supernatant. The estimation of Total Phenolic Contents in extract was carried out through a colorimetric assay, by measuring its reducing capacity with Folin-Ciocalteu reagent [26, 27].
TPC was expressed as mg Gallic Acid Equivalent/100 g matter (mg GAE/100g). For this purpose, a standard curve was generated, representing the absorbance values of gallic acid standard solutions in relation to their concentrations [28].
Because during boiling, some of the cellular compounds can pass into the boiling water, TPC was dosed from both mushroom samples and boiling water, calculating the total amount for each sample.

2.3. Statistical analysis

The data of experiments, coming from four replicates of each determination, were statistically processed, using SAS Version 8.02 [29]. To analyze the significance of differences among samples, generalized linear model analysis was carried out. For multiple comparisons Duncan's multiple range test was used ($P<0.05$).

3. Results and discussion

In the Table 1 are rendered the values of Total Phenolic Content (TPC), in ten forest mushroom species.

Table 1

Comparative TPC mean values (±SD) in raw and thermal processed forest mushrooms

Test	**TPC (mg GAE/100 g)**			
Samples (Species)	Raw material	Boiled (30 min.)*	Boiled (60 min.)*	Roasted (10 min.)
Agaricus campestris	23.25±1.82 **C**	27.70±0.78 **BC**	29.67±1.23 **B**	35.82±0.72 **AB**
Morchella esculenta	18.43±1.07 **CD**	22.06±1.25 **C****	26.03±1.84 **BC**	29.56±0.88 **B**
Cantharellus cibarius	16.55±0.96 **D**	20.12±1.47 **C****	22.85±0.39 **C**	27.43±1.09 **BC**
Armillaria mellea	28.74±1.35 **B**	34.34±0.93 **AB**	37.23±0.96 **AB**	40.27±1.94 **A**
Lactarius deliciosus	19.88±0.78 **CD**	23.53±0.74 **C**	23.66±1.02 **C**	28.09±0.48 **B**
Boletus edulis	22.07±0.54 **C**	27.30±1.43 **BC****	27.49±0.45 **BC****	30.05±2.01 **B**
Pleurotus cornucopiae	22.38±1.36 **C**	24.89±2.01 **C**	25.14±1.58 **BC**	28.94±1.65 **B**
Russula vesca	24.66±0.97 **C**	28.04±1.44 **BC**	29.27±0.91**B**	33.67±1.03 **AB**
Sparassis crispa	17.39±1.04 **CD**	22.94±1.09 **C**	28.06±1.35 **BC**	33.95±0.57 **AB**
Ramaria aurea	20.12±0.89 **C**	26.15±0.53 **BC**	31.43±0.48 **B**	38.44±1.22 **A**

SD=standard deviation; *Sum of boiled sample and its boiling water; **Means with the same letters within a row or a column are not statistically diferent ($P<0.05$)

As seen from the table, in the raw material the values of TPC ranged between 16.55±0.96 (*Cantharellus cibarius)* and 28.74±1.35 mg GAE/100 g (*Armillaria mellea)*. Beetween these extreme values, there are some close ones (without significant differences among them) in five species (*Agaricus campestris, Boletus edulis, Pleurotus cornucopiae, Russula vesca* and *Ramaria aurea)* on one hand, and in other three species (*Morchella esculenta, Lactarius deliciosus* and *Sparassis crispa),* on the other hand.

As as result of **boiling process**, the TPC values showed modifications depending on boiling duration and species.

Thus, after 30 minutes the highest TPC value was registered by *Armillaria mellea*, followed by *Agaricus campestris, Boletus edulis, Russula vesca* and *Ramaria aurea*, with close values ($P<0.05$). The other species registered the least values and close between them.

After 60 minutes the highest TPC value was registered by *Armillaria mellea*, followed by *Agaricus campestris, Russula vesca* and *Ramaria aurea,* with close values, and by *Morchella esculenta, Boletus edulis, Pleurotus cornucopiae* and *Sparassis crispa. Cantharellus cibarius* and *Lactarius deliciosus* had the least values ($P<0.05$).

The **roasting process** caused the highest modifications of TPC values in all mushroom species.

The greatest TPC values was registered by *Armillaria mellea* and *Ramaria aurea,* followed by *Agaricus campestris, Russula vesca* and *Sparassis crispa* (with close values). The other four species registered the least values.

Comparing the TPC of mushrooms thermal processed with raw materials, one can observe a raising of TPC values after heating in all analyzed samples ($P<0.05$).

The table 2 highlights the mushrooms TPC increase percents after boiling and roasting.

Thus, compared to raw materials, ***boiling for 30 minutes*** led to a significant raising percent of TPC (except *Pleurotus cornucopiae*) beetween 13.71% (*Russula vesca*) and 31.91% (*Sparassis crispa*).

Compared to raw materials, ***60 minutes of boiling*** determined significant raising percent of TPC in all samples, the great values being registered in *Sparassis crispa* and *Ramaria aurea*, and the least one in *Pleurotus cornucopiae*. From 30 to 60 minutes of boiling were four species whose TPC values have not singnificantly modified (*Cantharellus cibarius, Armillaria mellea, Lactarius deliciosus* and *Boletus edulis.*

Table 2

TPC mean values raising percent in mushroom heated samples, compared to raw material

Samples (Species)	TPC raising percents		
	Boiled (30 min.)*	**Boiled (60 min.)***	**Roasted (10 min.)**
Agaricus campestris	19.14%	27.60%	54.00%
Morchella esculenta	19.70%	41.24%	60.40%
Cantharellus cibarius	21.57%	38.10%	65.74%
Armillaria mellea	19.48%	29.54%	40.12%
Lactarius deliciosus	18.36%	19.00%	41.30%
Boletus edulis	23.70%	24.56%	36.16%
Pleurotus cornucopiae	11.22%	12.33%	29.31%
Russula vesca	13.71%	18.69%	36.54%
Sparassis crispa	31.91%	61.36%	95.23%
Ramaria aurea	29.97%	56.21%	91.05%

*Sum of boiled sample and its boiling water

Investigating the effect of thermal treatment on corn, Xu and Chang, 2009 [30], found changes of phenols caused by liberating of free forms from bound phenols.

The results of the thermal treatment (boiling) applied to samples of the ten mushroom species, analysed in this work, are consistent with data reported by Choi et al, 2006 [31], which searching the effect of heat treatment (at 100°C and for 15 or 30 min.) on Shitake (*Lentinus edodes*) mushroom extracts, found the polyphenolic contents and antioxidant activities increased as heating temperature and time increased.

Although the increase in TPC mean values on heating could be attributed to the release of free phenolics from combinations, the differences between the studied species could be explained by the difference in structure between these species. For example, some species have their body composed by thin and wavy formations (*Sparassis crispa*) or by a number of vertical and cylindrical branches (*Ramaria aurea*), which increase the contact surface of the internal tissues with the heat agent, favoring a more intense heat action on cellular compounds, including bound phenols.

As to mushrooms ***roasting***, the highest raising percent was in *Sparassis crispa*, followed by *Ramaria aurea,* and the least one in *Pleurotus cornucopiae.*

The thermal treatment causes phytochemical degradation, oxidation, and Maillard reactions resulting in changes in antioxidant property [32]. By Lin et all [33], Maillard reaction products may protect phytochemicals from oxidation, and can maintain or even enhances the overall antioxidant properties of food products [34].

Heat treatment at 150°C for 40 min. liberated bound phenolics in citrus peels having as result a significant increasing of TPC after treatment [35].

Heating at 121°C for 30 min Shitake mushroom extracts, Choi et al, 2006 [31] found the free polyphenolic content increased by 1.9-fold compared to that in the extract from the raw sample.

4. Conclusions

The thermal processing of mushrooms, belonging to ten species colected from Suceava county area, significantly influenced their Total Phenolic Content (TPC).

Thus, as compared to raw material, the boiling process led to a significant increase in percent of TPC between 13.71% and 31.91% (after 30 min. of boiling) and

between 12.33% and 61.36% (after 60 min. of boiling).

Compared to raw material, the roasting of mushrooms for 10 minutes (at 160°C) evidenced in all samples, significant increases of the phenolic compounds between 29.31% and 95.23%.

It seems that heating at this temperature, for 10 minutes, led to an increase in the free phenols content by releasing bound phenols.

High TPC values recorded by some species may be explained by the specific tissue structure of these species, which promotes a penetration and a more intense heat action on bound phenols.

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