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Original Article

The Effect of Processing on Chemical Constituents of *Agaricus* spp. Mushrooms

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Abstract

Agaricus spp. mushrooms are edible fungi of commercial and medicinal importance. Mushrooms convert nutritionally valueless substances into proteinous food with a very efficient bioconversion. Consumption of mushrooms, has increased substantially due to their delicacy, flavour, nutritional and medicinal value, being considered an excellent source of protein, which can contribute to the formulation of a balanced diet. Three species of *Agaricus* spp. mushrooms were used in this study: *Agaricus campestris* (L. ex Fr.), *Agaricus bisporus* (J.E. Lange) and *Agaricus blazei* (Murrill). This study aimed to examine the effects of blanching, soaking and manufacturing processes (sun drying, oven drying and canning) on some chemical constituents of mushrooms (soluble dry matter, protein and total sugars). The analyses were performed initially at conservation and they were repeated after 6, 12 and 18 months of preservation. Since all treatments caused reduction of the dry matter content, it can be concluded that the smallest decrease during the storage period was recorded by soaked and sun dried *A. blazei* (1.6 g 100 g^{-1} FM). The maximum soluble dry matter loss was recorded at blanched and canned *A. bisporus* (2.8 g 100 g^{-1} FM). Blanching treatment of preheated (sun dried and oven dried) samples, after storage period, led the protein content to slightly decrease, between 0.4 g 100 g^{-1} DM and 0.8 g 100 g^{-1} DM. The smallest decrease of total sugars during the storage period was registered by canned, untreated *A. campestris* with 1.2 g 100 g^{-1} DM and the largest decrease was registered by blanched oven dried *A. blazei* with 2.9 g 100 g^{-1} DM.

Keywords: *Agaricus bisporus, A. blazei, A. campestris,* blanching, drying, dry matter, total sugars

Abbreviations: CP - crude protein; DM - dry matter; FM - fresh matter; S1 - *Agaricus campestris* L. ex Fr.; S2 - *Agaricus bisporus* J.E. Lange; S3 - *Agaricus blazei* Murrill; T1 - untreated sample (control); T2 - blanching in 2% sodium chloride and 0.2% citric acid boiling solution for 2 min; T3 - soaking in 2% sodium chloride and 0.2% citric acid boiling solution for 10 min; TS - total sugars

Introduction

Since ancient times, mushrooms have been consumed by humans not only as a part of the normal diet, but also as a delicacy, because they have a highly desirable taste and aroma (Baysal *et al*., 2007).

An analysis of some common edible mushrooms showed that on fresh weight basis the moisture content is 89-91%, ash 0.97-1.26%, protein 2.78-3.94%, fat 0.25-0.65%, crude fibre 0.09-1.67%, carbohydrates 5.3-6.28% and energy value 24.4-34.4 kcal (Kapoor, 2004; Beluhan and Ranogajec, 2011; Glamoclija *et al.*, 2015). Glucose is present in very small quantity and like other vegetable, edible mushrooms contain fat in small amounts (Kapoor, 2004).

Edible mushrooms, like *A. bisporus* and *A. campestris*, are commonly used in cooking and in catering (Bernas *et al*., 2006). *A. bisporus*, white button mushroom, is the most popular mushroom in the world and is the most extensively cultivated edible mushroom with yields accounting for 70% of the total edible fungi (Shi *et al.*, 2012).

Worldwide, the nutritive and medicinal values of mushrooms, have long been recognized, as some of the edible mushroom species (*A. blazei* Murrill), also possess pharmacological properties (Lindequist *et al*., 2005; Oliveira Lima *et al*., 2011).

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Agaricus spp. mushrooms have a short shelf life compared to most fruit and vegetables. The fresh mushrooms lose their commercial value within a few days (for 5 to 10 days, depending on the storage condition), due to senescence water loss, microbial attack and browning (Jolivet *et al*., 1998).

Thermal processing is generally applied to extend shelf life of food products. However, it is well known that natural nutrients could be significantly lost during the thermal processing due to the fact that most of the bioactive compounds are relatively unstable to heat. But some studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment (Kim *et al*., 2000; Dewanto, *et al*., 2002). Radzki *et al*. (2015), stated that little attention has been paid to the impact of thermal processing on the chemical composition of edible mushrooms.

Thus, it is necessary to apply technologies to extend the shelf life of *Agaricus* spp. mushrooms. The method of preservation used is mainly determined by the final use of the product and the estimated storage time. Cooling is the most accepted preservation method to extend the shelf life of mushrooms for a short time (Singh *et al.*, 2010). Canning and salting are frequently used methods to preserve foods for a long time (Akram and Kwon, 2010; Fernandes *et al.*, 2012).

Dehydration is among the most popular methods for shelf-life extension of highly perishable foods. Convective drying is widely used; however, several disadvantages of this method have been reported: degradation of important nutritional substances due to relatively long drying times and high temperatures (Marfil *et al*., 2008; Vega-Galvez *et al*., 2012; Galoburda *et al.,* 2015), changes in product colour and texture (Kotwaliwale *et al*., 2007), decrease in rehydration ability due to shrinkage (Giri and Prasad, 2007).

Blanching is a common step in most processes, as it reduces microbial load, prevents enzymatic browning, induces contraction in size and air leaking, and generates a better product for further industrial operations. However, several drawbacks of blanching are weight loss (water, dry matter, nutrients), as well as colour and texture changes (Vanaclocha and Requena, 2003).

Mushroom fruiting bodies are rarely eaten raw and some kind of culinary processing is usually applied before eating. This may include cutting, blanching, soaking or boiling. Moreover, mushrooms which are intended to be stored for a period of time (dried, frozen, pickled or canned) are often subjected to blanching, soaking or boiling (Galgano *et al*., 2007; Yao and Ren, 2011; Ahmed and Ali, 2013). Several previous studies have reported that treatments of vegetables, especially those above mentioned, where elevated temperature is involved, may lead to substantial changes in the chemical composition of mushrooms (e.g. effect of blanching on protein content, enzyme activity and color changes reported by Deylami *et al.*, 2016; effects of porosity and thermal treatment on hydration of mushrooms reported by Paudel *et al*., 2016; frying induced severe losses in protein, ash, and carbohydrates content but increased the fat and energy, reported by Roncero-Ramos *et al*., 2017). Moreover, Radzki *et al*. (2016) reported that some thermal treatments, including boiling and blanching, determined the decrease of polysaccharides, proteins and phenolic content of processed *Pleurotus* spp. in comparison of the raw material, in detriment of their nutritional value.

This study aimed to evaluate the influence of treatment (blanching and boiling, in salt and citric acid solutions), preservation method (sun drying, oven drying and canning) and storage period of *Agaricus* spp. mushrooms, on the dry matter, protein and sugars content of preserved mushrooms. The comparative study was done on three *Agaricus* mushroom species, respectively *A. campestris*, *A. bisporus* and *A. blazei*.

Materials and Methods

Biological material

Agaricus campestris L. ex Fr. (S1), sin. *Psalliota campestris*, popularly called manure mushroom or champignons, sometimes confused with *Agaricus arvensis*, is a saprophyte edible fungi species of the Basidiomycota, from the *Agaricaceae* family and *Agaricus* genus.

Agaricus bisporus J.E. Lange (S2) is an edible basidiomycete mushroom native to grasslands in Europe and North America. It has two colour states while immature, white or brown, both of which have various names. When mature, it is known as portobello mushroom, often shortened to just portobello. When immature and white, this mushroom may be known as common mushroom, button mushroom, white mushroom, cultivated mushroom, table mushroom and champignon mushroom.

Agaricus blazei Murrill (S3), is a species of mushroom, commonly known as almond mushroom, mushroom of the sun, God's mushroom, mushroom of life, royal sun agaricus, jisongrong or himematsutake and by a number of other names. *A. blazei* is a choice edible, with a somewhat sweet taste and fragrance of almonds. The fungus is also well known as a medicinal mushroom, for its purported medicinal properties, due to research which indicates it may stimulate the immune system (Fang *et al.*, 2016; Frost, 2016; De Miranda *et al.*, 2017; Martins *et al.*, 2017; Roncero-Ramos and Delgado-Andrade, 2017).

Sampling and preservation treatments of Agaricus spp. mushrooms

The samples of mushrooms were obtained from 'Ciupercaria SRL', Cluj county, Romania, mushroom farm.

The shelf life of each *Agaricus* spp. mushrooms, were preserved using different processing methods: sun drying, oven drying and canning procedures.

Whole fresh fruiting bodies of *A. campestris*, *A. bisporus* and *A. blazei* were weighted and thoroughly washed in tap water until they were cleaned and then divided into three portions. Each part was subjected to one of the following pre-treatments to inhibit the enzymatic browning reaction before dehydration process:

(T1) Untreated sample (control).

(T2) Blanching in 2% sodium chloride and 0.2% citric acid boiling solution for 2 min.

(T3) Soaking in 2% sodium chloride and 0.2% citric acid boiling solution for 10 min.

All the treated samples were dried, either by air drying or oven dehydration.

Sun drying. Pre-treated samples of the studied mushrooms species were sun dried according to Suguna *et al*. (1995). Samples were spread in a single layer into trays (100×100×2.5 cm) and dried in indirect sun light for 10 hours/day. Overnight, until the next sun drying, the samples were stored in a dry and cold place. Sun drying was continued until the samples reached to constant weight.

Oven dehydration. Stainless steels trays (45×45×2 cm) were loaded by different mushroom samples and dried into an air ventilated oven at 70 °C for 2 hrs, then temperature was reduced to 50 °C until samples reached constant weight according to the method of Bhatti *et al*., (1989). All dried samples were packed in polyethylene bags and stored for 18 months at room temperature and analysed every six months.

Canning of mushrooms. Fruiting bodies of studied mushrooms, were washed in tap water until they were thoroughly cleaned and then blanched or soaked in boiling water containing 2% sodium chloride and 0.2% citric acid. The mushrooms samples were filled into glass container. The container was filled with a hot solution of 2% sodium chloride and 0.2% citric acid, then was preheated in water bath for 5 min at 100 °C, closed tightly and preheated in water bath at 100 °C for 30 min (Hassan, 2002) and sterilized at 121 °C for 20 min in an autoclave (Eby *et al*., 1977), then the samples were cooled at room temperature. The canned mushrooms were taken for chemical analysis immediately at conservation, after canning (zero time) and periodically at 6, 12, 18 months.

Chemical composition of mushrooms

Preparation of dry defatted meal. The mushroom samples were defatted with ice-cold acetone in a blinder. The defatted matter was air dried and stored at 4 °C until used.

Determination of total nitrogen and crude protein. The Kjeldahl procedure was used to determine the total nitrogen content in mushroom. This was performed by Rapid nitrogen apparatus Model Buch 426, FRG. The crude protein was then calculated by multiplying nitrogen content by 4.38 as a factor for fruit bodies (Crisan and Sands, 1978).

Extraction of soluble sugars. Soluble sugars were extracted according to Macrae and Zand-Mghdlam (1978) method. Finely ground defatted meal (2.0 g) was weighted into a 250 ml round bottom flask and treated with 40 ml of a 80% methanol. The suspension was boiled under reflex in a water bath at 98 °C for 2 hrs, after cooling the suspension was transferred to a centrifuge tube and centrifuged at 3000 rpm for 15 min. The residue was extracted twice more with 40 ml boiling aqueous methanol and the final residue washed with 40 ml of water. The combined extracts and washing were evaporated to dryness in vacuo below 50 °C. The residue was dissolved in 15 ml of water and treated with 2 ml of Carrez solution No. l and No. 2, then diluted to 20 ml with water (Carrez solution No. l contains 16.6 g of potassium ferrocyanide made up to 100 ml with water. Carrez solution No. 2 contains 21.9 g of zinc acetate dehydrate and 2 ml of glacial acetic acid made up to 100 ml with water). After shaking, the precipitated material was centrifuged off and the supernatant filtered through a small Whatman No. l filter paper. The filtrate was used for analysis.

Determination of total soluble sugars. Total soluble sugars were determined by the phenol-sulfuric acid method described by Dubois *et al*. (1956). An aliquot (1 ml) containing 10-70 μg carbohydrate reacted with phenol (1 ml, 5% phenol in water) and concentrated sulphuric acid (5 ml), then after 30 min the colour development was measured spectrophotometrically at 490 nm. The concentration of sugars was determined by using calibration curve constructed by solution containing known concentrations (10 to 50 $\mu g\ ml^{-1}$) of glucose.

Statistical analysis

The processing of the obtained results was made by analysing the polyfactorial variance, on each analysed character, and the statistical interpretation was made with the ANOVA program by the Duncan test.

Results and Discussion

The influence of pre-treatment, treatment and storage period on the soluble dry matter (DM) of Agaricus spp. mushrooms

The DM content of studied *A. campestris* mushroom, variate between 13.2-13.9 g 100 g^{-1} FM at untreated samples, between 9.6-10.2 g 100 g^{-1} FM at blanched samples and between 12.8-13.5 g 100 g^{-1} FM at soaked samples. These results are in agreement with those reported by Beluhan and Ranogajec (2011), Pereira *et al*. (2012) and Glamoclija *et al*. (2015) concerning the DM content of *A. campestris* mushroom, which varied between 11.8-14.9 g 100 g^{-1} FM.

The DM content of studied *A. bisporus* mushroom variate between 11.1-12.7 g 100 g^{-1} FM at untreated samples, between 8.6-9.2 g 100 g^{-1} FM at blanched samples and between 11.1-11.7 g 100 g^{-1} FM at soaked samples. According to Mattila *et al*. (2002), Reis *et al*., (2012) and Jaworka *et al*. (2015) the DM content of cultivated *A. bisporus* mushroom, varied between 7.3-13.2 g 100 g^{-1} FM.

The DM content of studied *A. blazei* mushroom varied between 12.7-13.4 g 100 g^{-1} FM at untreated samples, between 10.8-11.4 g 100 g^{-1} FM at blanched samples and between 12.5-13.2 g 100 g^{-1} FM at soaked samples. According to Tsai *et al*. (2008), Carneiro *et al*. (2013) and Cohen *et al*. (2014) the DM content of cultivated *A. blazei* mushroom, varied between 10.5-13.7 g 100 g^{-1} FM.

Following the DM variation during storage period, at sun dried pre-treated mushrooms (Table 1), we can see that at 6 and 12 months of storage the largest content of DM was recorded by *A. campestris* with 12.93 g DM 100 g^{-1} FM at 6 months and 12.43 g DM 100 g^{-1} at 12 months of storage. After 18 months of storage, the largest content of DM was recorded by *A. blazei* with 11.60 g DM 100 g^{-1} FM.

The smallest content of DM was recorded by *A. bisporus* with 8.13 g DM 100 g^{-1} FM at 6 months, 7.73 g DM 100 g^{-1} at 12 months and 6.53 g DM 100 g^{-1} FM after 18 months of storage.

The smallest decrease of DM content during storage period at sun dried mushrooms was recorded by soaked *A. blazei* mushroom with 0.8 g DM 100 g^{-1} loss (6.07%) at 6 months, 1.2 g DM 100 g^{-1} loss (9.11%) at 12 months and 1.6 g DM 100 g^{-1} loss (12.15%) at 18 months of storage. The largest decrease of DM content during storage period at sun dried mushrooms was recorded by blanched *A. bisporus* mushroom with 1.07 g DM 100 g^{-1} loss (11.63%) at 6 months, 1.47 g DM 100 g^{-1} loss (15.97%) at 12 months and 2.67 g DM 100 g^{-1} loss (29.02%) at 18 months of storage.

Following the DM variation during storage period (from 6 to 18 months), at oven dried pre-treated mushrooms (Table 2), the largest content of DM was revealed by *A. campestris* with 12.60 g DM 100 g^{-1} FM after 6 months, 11.80 g DM 100 g^{-1} FM at 12 months and 11.13 g DM 100 g^{-1} FM after 18 months. The smallest content of DM was recorded by *A. bisporus* with 7.70 g DM 100 g^{-1} FM at 6 months, 7.20 g DM 100 g^{-1} FM at 12 months and 6.23 g DM 100 g^{-1} FM after 18 months of storage.

The smallest decrease of DM content during storage period at oven dried mushrooms was recorded by soaked *A. campestris* mushroom with 0.93 g DM 100 g^{-1} loss (7.13%) at 6 months and 1.43 g DM 100 g^{-1} loss (10.97%) at 12 months. At 18 months of storage of oven dried mushrooms the smallest decrease of DM content was recorded by blanched *A. campestris* mushroom with 1.86 g DM 100 g^{-1} loss (16.71%). The largest decrease of DM content during storage period at oven dried mushrooms was recorded by blanched *A. bisporus* mushroom with 1.17 g DM 100 g^{-1} loss (13.19%) at 6 months, 1.67 g DM 100 g^{-1} loss (18.83%) at 12 months and 2.64 g DM 100 g^{-1} loss (29.76%) at 18 months of storage.

Following the DM variation during storage period, at canned mushrooms (Table 3), it can be seen that the largest content of DM was recorded by *A. campestris* with 12.40 g DM 100 g^{-1} FM at 6 months, 11.60 g DM 100 g^{-1} FM at 12

Table 1. DM content variation at sun dried mushrooms during the storage period (expressed in g DM 100 g^{-1} FM)

Variant	Initial DM value	Variant	DM value after 6 months	Variant	DM value after 12 months	Variant	DM value after 18 months
T1S1	13.93 a	T1S1	12.93 a	T1S1	12.43 a	T1S3	11.60 a
T1S3	13.50 ab	T1S3	12.40 b	T1S3	12.00 a	T3S3	11.57 a
T3S1	13.40 b	T3S1	12.40 b	T3S1	12.00 a	T1S1	11.43 a
T3S3	13.17 b	T3S3	12.37 b	T3S3	11.97 a	T3S1	11.10 a
T2S1	11.87 c	T2S1	10.87 c	T2S1	10.47 b	T2S3	9.93 b
T2S3	11.73 c	T2S3	10.73 c	T2S3	10.33 b	T2S1	9.63 bc
T3S2	11.43 c	T3S2	10.43 c	T3S2	10.03 b	T3S2	9.00 c
T1S2	10.20 d	T1S2	9.17 d	T1S2	8.73 c	T1S2	8.10 d
T2S2	9.20 e	T2S2	8.13 e	T2S2	7.73 d	T2S2	6.53 e
SD 0.48-0.54		SD 0.47-0.53		SD 0.60-0.68		SD 0.81-0.91	

Table 2. DM content variation at oven dried mushrooms during the storage period (expressed in g DM 100 g-1 FM)

Variant	Initial DM value	Variant	DM value after 6 months	Variant	DM value after 12 months	Variant	DM value after 18 months
T1S1	13.57 a	T1S1	12.60 a	T1S1	11.80 a	T1S1	11.13 a
T3S1	13.03 b	T3S1	12.10 ab	T3S1	11.60 a	T3S1	10.70 ab
T1S3	13.00 b	T1S3	12.07 ab	T1S3	11.50 a	T1S3	10.43 ab
T3S3	12.67 b	T3S3	11.57 b	T3S3	10.80 b	T3S3	10.13 b
T2S3	11.23 c	T2S1	10.30 c	T2S1	9.77 c	T2S1	9.27 c
T2S1	11.13 c	T3S2	10.10 c	T3S2	9.60 c	T2S3	8.83 c
T3S2	11.10 c	T2S3	9.93 c	T2S3	9.37 c	T3S2	8.67 c
T1S2	9.87 d	T1S2	8.93 d	T1S2	8.33 d	T1S2	7.80 d
T2S2	8.87 e	T2S2	7.70 e	T2S2	7.20 e	T2S2	6.23 e
SD 0.50-0.56		SD 0.74-0.83		SD 0.65-0.74		SD 0.74-0.84	

Table 3. DM content variation at canned mushrooms during the storage period (expressed in g DM 100 g^{-1} FM)

Variant	Initial DM value	Variant	DM value after 6 months	Variant	DM value after 12 months	Variant	DM value after 18 months
T1S1	13.23 a	T1S1	12.40 a	T1S1	11.60 a	T1S1	10.73 a
T1S3	12.83 ab	T3S1	11.90 ab	T3S1	11.40 a	T3S1	10.30 ab
T3S1	12.70 b	T1S3	11.87 ab	T1S3	11.30 a	T1S3	10.03 ab
T3S3	12.50 b	T3S3	11.37 b	T3S3	10.60 a	T3S3	9.73 b
T2S3	11.07 c	T2S1	10.10 c	T2S1	9.57 b	T2S1	8.87 c
T2S1	10.80 c	T3S2	9.90 c	T3S2	9.40 b	T2S3	8.33 c
T3S2	10.80 c	T2S3	9.733 c	T2S3	9.17 b	T3S2	8.27 c
T1S2	9.57 d	T1S2	8.73 d	T1S2	8.10 c	T1S2	7.40 d
T2S2	8.57 e	T2S2	7.60 e	T2S2	7.00 d	T2S2	5.83 e
SD 0.50-0.56		SD 0.71-0.80		SD 0.65-0.73		SD 0.73-0.83	

Table 4. Protein content variation at sun dried mushrooms during the storage period (expressed in g CP 100 g^{-1} DM)

Variant	Initial CP value	Variant	CP value after 6 months	Variant	CP value after 12 months	Variant	CP value after 18 months
T1S3	30.07 a	T1S3	29.47 a	T1S3	29.27 a	T1S3	29.10 a
T3S3	28.93 ab	T3S3	28.63 a	T3S3	28.47 a	T3S3	28.30 a
T1S2	28.43 bc	T1S2	28.20 ab	T1S2	27.90 ab	T1S2	27.77 ab
T3S2	27.43 c	T3S2	27.10 bc	T3S2	26.87 bc	T3S2	26.73 bc
T2S2	26.07 d	T2S2	25.80 cd	T2S2	25.57 cd	T2S2	25.43 cd
T2S3	25.63 d	T2S3	25.23 d	T2S3	25.00 d	T2S3	24.83 d
T1S1	23.33 e	T1S1	23.13 e	T1S1	22.90 e	T1S1	22.80 e
T3S1	22.00 f	T3S1	21.73 e	T3S1	21.47 e	T3S1	21.33 f
T2S1	19.72 g	T2S1	19.63 f	T2S1	19.23 f	T2S1	18.93 g
SD 1.25-1.41		SD 1.41-1.58		SD 1.45-1.63		SD 1.46-1.64	

Table 5. Protein content variation at oven dried pre-treated mushrooms during the storage period (expressed in g CP 100 g-1 DM)

Variant	Initial CP value	Variant	CP value after 6 months	Variant	CP value after 12 months	Variant	CP value after 18 months
T1S3	29.77 a	T1S3	29.67 a	T1S3	29.50 a	T1S3	29.33 a
T3S3	28.63 ab	T3S3	28.53 ab	T3S3	28.37 ab	T3S3	28.10 ab
T1S2	28.13 bc	T1S2	27.73 bc	T1S2	27.53 bc	T1S2	27.40 bc
T3S2	27.13 c	T3S2	26.73 c	T3S2	26.50 c	T3S2	26.33 cd
T2S2	25.80 d	T2S2	25.40 d	T2S2	25.20 d	T2S2	25.07 de
T2S3	25.33 d	T2S3	25.23 d	T2S3	25.10 d	T2S3	24.90 e
T1S1	23.03 e	T1S1	22.73 e	T1S1	22.57 e	T1S1	22.30 f
T3S1	21.70 f	T3S1	21.40 f	T3S1	21.13 f	T3S1	21.00 g
T2S1	19.47 g	T2S1	19.17 g	T2S1	18.90 g	T2S1	18.70 h
SD 1.24-1.40		SD 1.24-1.40		SD 1.24-1.40		SD 1.27-1.43	

months and 10.73 g DM 100 g^{-1} FM after 18 months of storage. The smallest content of DM was recorded by *A. bisporus* with 7.60 g DM 100 g^{-1} FM at 6 months, 7.00 g DM 100 g^{-1} FM at 12 months and 5.83 g DM 100 g^{-1} FM after 18 months of storage.

The smallest decrease of DM content during storage period at canned mushrooms was recorded by untreated *A. campestris* mushroom with 0.83 g DM 100 g^{-1} loss (6.27%) at 6 months, by soaked *A. campestris* with 1.30 g DM 100 g^{-1} loss (10.24%) at 12 months and by blanched *A. campestris* mushroom with 1.93 g DM 100 g^{-1} loss (17.87%) after 18 months of storage. The largest decrease of DM content during storage period at canned mushrooms was recorded by blanched *A. blazei* mushroom with 1.34 g DM 100 g^{-1} loss (12.08%) at 6 months, by blanched *A. bisporus* with 1.57 g DM 100 g^{-1} loss (18.32%) at 12 months and 2.74 g DM 100 g^{-1} loss (31.97%) at 18 months of storage.

Blanching treatment of preheated, sun dried and oven dried, samples, after storage period extended for 18 months, decreased the DM level of mushrooms with 29.02-31.97%.

The influence of pre-treatment, treatment and storage period on the crude protein (CP) of Agaricus spp. mushrooms

Protein is an important component of dry matter of mushroom. Proteins constitute more than half of total nitrogen and their content depends among other things, on the composition of the substratum, size of mushroom, harvest time and species of mushroom (Tsai *et al*., 2007).

The protein content of the initial material of the studied *A. campestris* mushroom, varied between 22.8-23.3 g 100 g^{-1} DM at untreated samples, between 27.8-28.4 g 100 g^{-1} DM at blanched samples and between 29.5-30.1 g 100 g^{-1} DM at soaked samples, results which are in agreement with those reported by Beluhan and Ranogajec (2011), Pereira *et al*. (2012) and Glamoclija *et al*. (2015), (18.6-38.9 g CP 100 g^{-1} DM).

In case of the initial material of *A. bisporus* mushroom, the protein content varied between 19-19.8 g 100 g^{-1} DM at untreated samples, between 25.4-26.1 g 100 g^{-1} DM at blanched samples and between 24.9-25.6 g 100 g^{-1} DM at soaked samples. Similar results were reported by Mattila *et al*. (2002), Reis *et al*. (2012) and Jaworka *et al*. (2015) who mentioned for cultivated *A. bisporus* a protein content which varied between 10.0-36.3 g 100 g^{-1} DM.

The protein content of studied *A. blazei* mushroom varied between 21.3-22.0 g 100 g^{-1} DM at untreated samples, between 26.7-27.4 g 100 g^{-1} DM at blanched samples and between 28.2-28.9 g 100 g^{-1} DM at soaked samples. The revealed protein content of the studied samples did not exceed the values reported by Tsai *et al*. (2008), Carneiro *et al*. (2013) and Cohen *et al*. (2014), (13.4-31.3 g CP 100 g^{-1} DM).

Following the protein variation during storage period, at sun dried pre-treated mushrooms (Table 4), it can be seen that during the storage period, the largest content of protein was recorded by untreated *A. blazei* with 29.47 g CP 100 g^{-1} DM at 6 months, 29.27 g CP 100 g^{-1} DM at 12 months and 29.10 g CP 100 g^{-1} DM after 18 months of storage. The smallest content of protein content was recorded by blanched *A. campestris* with 19.63 g CP 100 g^{-1} DM at 6 months, 19.23 g CP 100 g^{-1} DM at 12 months and 18.93 g CP 100 g^{-1} DM after 18 months of storage.

The smallest decrease of protein content during storage period at sun dried mushrooms was recorded by soaked *A. campestris* mushroom with 0.09 g CP loss 100 g^{-1} DM (0.45%) at 6 months. At 12 and 18 months, the smallest

decrease of protein was recorded by *A. blazei* mushroom, with 0.46 g CP loss 100 g^{-1} DM (1.59%) at 12 months and 0.63 g CP loss 100 g^{-1} DM (2.17%) at 18 months of storage. The largest decrease of protein content during storage period at sun dried mushrooms was recorded by untreated *A. blazei* mushroom with 0.6 g CP loss 100 g^{-1} DM (11.63%) at 6 months and 0.8 g CP loss 100 g^{-1} DM (2.66%) at 12 months. At 18 months of storage the largest decrease of protein content during storage period at sun dried mushrooms was recorded by *A. campestris* with 0.79 g CP loss 100 g^{-1} DM (4.01%).

Following the protein variation during storage period, at oven dried mushrooms (Table 5), it can be seen that at 6, 12 and 18 months of storage the largest content of protein was recorded by untreated *A. blazei* with 29.67 g CP 100 g^{-1} DM at 6 months, 29.50 g CP 100 g^{-1} DM at 12 months and 29.33 g CP 100 g^{-1} DM after 18 months of storage. The smallest content of DM was recorded by blanched *A. campestris* with 19.17 g CP 100 g^{-1} DM at 6 months, 18.90 g CP 100 g^{-1} DM at 12 months and 18.70 g CP 100 g^{-1} DM after 18 months of storage.

The smallest decrease of protein content during storage period at oven dried mushrooms was recorded by soaked *A. blazei* mushroom with 0.1 g CP loss 100 g^{-1} DM (0.34%) at 6 months, 0.27 g CP loss 100 g^{-1} DM (0.9%) at 12 months and 0.44 g CP loss 100 g^{-1} DM (1.48%) at 18 months of storage. The largest decrease of protein content during storage period at oven dried mushrooms was recorded by blanched *A. bisporus* mushroom with 0.4 g CP loss 100 g^{-1} DM (1.55%) at 6 months and by untreated *A. campestris* with both 0.57 g CP loss 100 g^{-1} DM (2.93%) at 12 months and 0.77 g CP loss 100 g^{-1} DM (3.95%) at 18 months of storage.

Following the protein variation during storage period, at canned mushrooms (Table 6), it can be seen that at 6, 12 and 18 months of storage the largest content of protein was recorded by untreated *A. blazei* with 29.37 g CP 100 g^{-1} DM at 6 months, 29.00 g CP 100 g^{-1} DM at 12 months and 28.67 g CP 100 g^{-1} DM after 18 months of storage. The smallest content of CP was recorded by blanched *A. campestris* with 18.90 g CP 100 g^{-1} DM at 6 months, 18.47 g CP 100 g^{-1} DM at 12 months and 18.17 g CP 100 g^{-1} DM after 18 months of storage.

The smallest decrease of protein content during storage period at canned mushrooms was recorded by untreated *A. blazei* mushroom with 0.13 g CP loss 100 g^{-1} DM (0.44%) at 6 months, by soaked *A. blazei* with 0.43 g CP loss 100 g^{-1} DM (1.52%) and with 0.77 g CP loss 100 g^{-1} DM (2.73%) at 12 months of storage. The largest decrease of protein content during storage period at canned mushrooms was recorded by soaked *A. campestris* mushroom with 0.24 g CP loss 100 g^{-1} DM (1.13%) at 6 months, by blanched *A. campestris* with 0.57 g CP loss 100 g^{-1} DM (2.99%) at 12 months and 0.87 g CP loss 100 g^{-1} DM (4.57%) at 18 months of storage.

The effects of blanching and soaking treatment of preheated, sun dried and oven dried samples, after the storage period extended to 18 months, led the protein content of samples to slightly decrease 3.95-4.56%. The loss of protein may be due to the leaching of these components into solution during thermal treatments and could be attributed to the canning process or to Millard reaction, which usually happens between reducing sugars and amino acids resulting in undesirable change in the colour of the sored products. Similar results were obtained by many researchers (Coskuner and Ozdemir, 1997; Kotwaliwale *et al.*, 2007).

The influence of pre-treatment, treatment and storage period on total sugars (TS) of Agaricus spp. mushrooms

Total sugars are important components of mushrooms' dry matter. Soluble sugars and polyols usually contributed to a sweet taste. Tseng and Mau (1999) found variation in the total sugars and polyols content of *A. bisporus* (from 286 mg g^{-1} DM to 319 mg g^{-1} DM), according to different strains.

The total sugars content of studied *A. campestris* mushroom varied between 10.8-11.2 g 100 g^{-1} DM at untreated samples, between 9-9.3 g 100 g^{-1} DM at blanched samples and between 15.7-16.2 g 100 g^{-1} DM at soaked samples. For the analysed samples of *A. bisporus* mushroom the total sugars content varied between 8.7-9.3 g 100 g^{-1} DM at untreated samples, between 7.1-7.3 g 100 g^{-1} DM at blanched samples and between 13-13.6 g 100 g^{-1} DM at soaked samples, while the total sugar content of studied *A. blazei* mushroom varied between 9.9-10.3 g 100 g^{-1} DM at untreated samples, between 8.2-8.5 g 100 g^{-1} DM at blanched samples and between 14.4-14.7 g 100 g^{-1} DM at soaked samples. All these obtained results are in line with those reported by Beluhan and Ranogajec (2011), Pereira *et al.* (2012) and Glamoclija *et al.* (2015) for the total sugars content of *A. campestris* mushroom (8.5-20.2 g 100 g^{-1}

Table 6. Protein content variation at canned mushrooms during the storage period (expressed in g CP 100 g^{-1} DM)

Variant	Initial CP value	Variant	CP value after 6 months	Variant	CP value after 12 months	Variant	CP value after 18 months
T1S3	29.50 a	T1S3	29.37 a	T1S3	29.00 a	T1S3	28.67 a
T3S3	28.20 b	T3S3	28.07 b	T3S3	27.77 b	T3S3	27.43 b
T1S2	27.87 bc	T1S2	27.70 bc	T1S2	27.30 bc	T1S2	27.10 b
T3S2	26.70 c	T3S2	26.50 c	T3S2	26.10 c	T3S2	25.80 cd
T2S2	25.37 d	T2S2	25.20 d	T2S2	24.83 d	T2S2	24.63 d
T2S3	24.90 d	T2S3	24.77 d	T2S3	24.37 d	T2S3	24.03 d
T1S1	22.77 e	T1S1	22.63 e	T1S1	22.33 e	T1S1	22.00 e
T3S1	21.27 f	T3S1	21.03 f	T3S1	20.73 f	T3S1	20.50 f
T2S1	19.04 g	T2S1	18.90 g	T2S1	18.47 g	T2S1	18.17 g
SD 1.24-1.40		SD 1.23-1.38		SD 1.21-1.36		SD 1.23-1.39	

Table 7. Total sugar content variation at sun dried mushrooms during the storage period (expressed in g TS 100 g^{-1} DM)

Variant	Initial TS value	Variant	TS value after 6 months	Variant	TS value after 12 months	Variant	TS value after 18 months
T1S3	16.20 a	T1S3	15.60 a	T1S3	14.90 a	T1S3	14.10 a
T3S3	14.73 b	T3S3	14.07 b	T3S3	13.30 b	T3S3	12.47 b
T2S3	13.60 c	T2S3	13.00 c	T2S3	12.37 c	T2S3	11.47 c
T1S1	11.23 d	T1S1	10.77 d	T1S1	10.33 d	T1S1	9.47 d
T3S1	10.33 e	T3S1	9.83 e	T3S1	8.70 e	T3S1	8.17 e
T1S2	9.33 f	T1S2	8.93 f	T2S1	8.37 e	T1S2	7.83 e
T2S1	9.33 f	T2S1	8.80 f	T1S2	8.20 e	T2S1	7.77 e
T3S2	8.53 g	T3S2	8.03 g	T3S2	7.10 f	T3S2	6.63 f
T2S2	7.30 h	T2S2	6.97 h	T2S2	6.43 g	T2S2	5.87 f
SD 0.38-0.43		SD 0.43-0.49		SD 0.62-0.70		SD 0.84-0.94	

Table 8. Total sugars content variation at oven dried pre-treated mushrooms during the storage period (expressed in g TS 100 g-1 DM)

Variant	Initial TS value	Variant	TS value after 6 months	Variant	TS value after 12 months	Variant	TS value after 18 months
T1S3	15.67 a	T1S3	14.70 a	T1S3	14.13 a	T1S3	13.10 a
T3S3	14.37 b	T3S3	13.27 b	T3S3	12.67 b	T3S3	11.90 b
T2S3	12.97 c	T2S3	12.67 c	T2S3	11.37 c	T2S3	10.50 c
T1S1	10.87 d	T1S1	10.33 d	T1S1	9.73 d	T1S1	8.90 d
T3S1	9.90 e	T3S1	9.27 e	T3S1	8.50 e	T3S1	8.00 e
T1S2	9.00 f	T1S2	8.70 f	T1S2	7.87 f	T2S1	7.17 f
T2S1	8.93 f	T2S1	8.10 g	T2S1	7.67 f	T1S2	7.07 f
T3S2	8.30 g	T3S2	7.60 h	T3S2	6.87 g	T3S2	5.43 g
T2S2	7.13 h	T2S2	6.43 i	T2S2	5.80 h	T2S2	5.30 g
SD 0.48-0.54		SD 0.49-0.56		SD 0.62-0.70		SD 0.76-0.86	

Table 9. Total sugars content variation at canned mushrooms during the storage period (expressed in g TS 100 g-1 DM)

Variant	Initial TS value	Variant	TS value after 6 months	Variant	TS value after 12 months	Variant	TS value after 18 months
T1S3	15.93 a	T1S3	14.80 a	T1S3	14.60 a	T1S3	13.73 a
T3S3	14.53 b	T3S3	13.17 b	T3S3	12.97 b	T3S3	11.97 b
T2S3	13.47 c	T2S3	12.83 c	T2S3	12.63 b	T2S3	11.57 b
T1S1	10.83 d	T1S1	10.57 d	T1S1	10.47 c	T1S1	9.60 c
T3S1	9.43 e	T3S1	9.33 e	T3S1	9.23 d	T3S1	8.33 d
T1S2	9.00 f	T1S2	8.77 f	T1S2	8.57 e	T1S2	7.63 e
T2S1	8.73 f	T2S1	8.53 f	T2S1	8.43 e	T2S1	7.50 e
T3S2	8.17 g	T3S2	7.93 g	T3S2	7.73 f	T3S2	6.63 f
T2S2	7.07 h	T2S2	6.80 h	T2S2	6.53 g	T2S2	5.83 g
SD 0.37-0.41		SD 0.32-0.36		SD 0.35-0.39		SD 0.63-0.72	

DM), by Reis *et al*. (2012), Pei *et al*. (2014), Stojkovic *et al*. (2014) and Jaworka *et al*. (2015) for the total sugar content of *A. bisporus* mushroom (5.8-14.9 g 100 g^{-1} DM), respectively by Tsai *et al*. (2008), Carneiro *et al*. (2013), Cohen *et al*. (2014) and Stojkovic *et al*. (2014) for the total sugar content of *A. blazei* mushroom (13.4-31.3 g 100 g^{-1} DM).

Following the total sugar variation during storage period, at sun dried pre-treated mushrooms (Table 7), it can be seen that during the storage period, the largest content of total sugars was recorded by untreated *A. blazei* with 15.60 g TS 100 g^{-1} DM at 6 months, 14.90 g TS 100 g^{-1} DM at 12 months and 14.10 g TS 100 g^{-1} DM after 18 months of storage. The smallest content of total sugars was recorded by blanched *A. bisporus* with 6.97 g TS 100 g^{-1} DM at 6 months, 6.43 g TS 100 g^{-1} DM at 12 months and 5.87 g TS 100 g^{-1} DM after 18 months of storage.

The smallest decrease of total sugars content during storage period at sun dried mushrooms was recorded by untreated *A. blazei* mushroom with 0.6 g TS loss 100 g^{-1} DM (3.70%) at 6 months and with 2.1 g TS loss 100 g^{-1} DM (12.96%) at 18 months. At 12 months of storage, the smallest decrease of total sugars was recorded by untreated *A. campestris* mushroom, with 0.9 g TS loss 100 g^{-1} DM (8.01%). The largest decrease of total sugars content during storage period at sun dried mushrooms was recorded by soaked *A. bisporus* mushroom with 0.5 g TS loss 100 g^{-1} DM (5.86%) at 6 months, 1.43 g TS loss 100 g^{-1} DM (16.76%) at 12 months and with 1.9 g TS loss 100 g^{-1} DM (22.27%) at 18 months of storage.

Following the total sugars variation during storage period, at oven dried mushrooms (Table 8), it can be seen that the largest content of sugars was recorded by untreated *A. blazei* with 14.70 g TS 100 g^{-1} DM at 6 months, 14.13 g

TS 100 g^{-1} DM at 12 months and 13.10 g TS 100 g^{-1} DM after 18 months of storage. The smallest content of TS was recorded by blanched *A. bisporus* with 6.43 g TS 100 g^{-1} DM at 6 months, 5.80 g TS 100 g^{-1} DM at 12 months and 5.3 g TS 100 g^{-1} DM after 18 months of storage.

The smallest decrease of total sugars content during storage period at oven dried mushrooms was recorded by blanched *A. blazei* mushroom with 0.3 g TS loss 100 g^{-1} DM (2.31%) at 6 months, by untreated *A. blazei* mushroom with 1.54 g TS loss 100 g^{-1} DM (9.82%) at 12 months and 2.57 g TS loss 100 g^{-1} DM (16.40%) at 18 months of storage. The largest decrease of total sugars content during storage period at oven dried mushrooms was recorded by blanched *A. bisporus* mushroom with 0.7 g TS loss 100 g^{-1} DM (9.81%) at 6 months and with 1.33 g TS loss 100 g^{-1} DM (18.65%) at 12 months of storage. At 18 months of storage, the largest decrease of total sugars content during storage period at oven dried mushrooms was recorded by soaked *A. bisporus* with 2.87 g TS loss 100 g^{-1} DM (34.58%).

Following the total sugars variation during storage period, at canned mushrooms (Table 9), it can be seen that at 6, 12 and 18 months of storage the largest content of total sugars was recorded by untreated *A. blazei* with 14.80 g TS 100 g^{-1} DM at 6 months, 14.60 g TS 100 g^{-1} DM at 12 months of storage and 13.73 g TS 100 g^{-1} DM after 18 months of storage. The smallest content of TS was recorded by blanched *A. bisporus* with 6.80 g TS 100 g^{-1} DM at 6 months, 6.53 g TS 100 g^{-1} DM at 12 months and 5.83 g TS 100 g^{-1} DM after 18 months of storage.

The smallest decrease of total sugars content during storage period at canned mushrooms was recorded by soaked *A. campestris* mushroom with 0.1 g TS loss 100 g^{-1} DM (1.06%) at 6 months and with 0.2 g TS loss 100 g^{-1} DM (2.12%) at 12 months. At 18 months of storage the smallest decrease of total sugars content during storage period at canned mushrooms was recorded by soaked *A. bisporus* with 1.23 g TS loss 100 g^{-1} DM (11.35%). The largest decrease of total sugars content during storage period at canned mushrooms was recorded by soaked *A. blazei* mushroom with 1.36 g TS loss 100 g^{-1} DM (9.35%) at 6 months and with 1.56 g TS loss 100 g^{-1} DM (10.74%) at 12 months. At 18 months of storage, the largest decrease of sugars content during storage period at canned mushrooms was recorded by soaked *A. bisporus* with 1.54 g TS loss 100 g^{-1} DM (18.85%). Similar results were obtained by many researchers for *A. bisporus* mushrooms (Bernas *et al.*, 2006).

The soaking treatment led to the smallest TS decrease for all studied samples. These results coincide with the data obtained by Sandhu and Poonam-Aggarwal (2001) who studied the effect of blanching and soaking on *A. bisporus* samples, and mentioned decrease of chemical constituents, such as TS, during storage. Dikeman *et al.* (2005) found that cooking may promote a loss of nutrients, including TS, and that is due to interaction among constituents. Moreover, Chiang *et al.* (2006) reported that even soluble sugars usually contribute to a sweet taste, the low content in canned mushrooms (below 5%) give a weak sweet perception.

Conclusions

The less loss of dry matter content, after 18 months of storage, was registered by soaked sun-dried mushrooms, ordered by species as follows: *A. blazei* < *A. campestris* < *A. bisporus*. Blanching treatment of preheated (sun dried and oven dried) samples led the protein content to slightly decrease, after 18 months period of storage. All treatment used led to decrease in tissues' total sugars content. Considering the TS loss, the applied treatments could be ordered as follows: blanching > untreated > soaking, while the studied species can be ordered as follow: *A. blazei* < *A. campestris* < *A. bisporus*. These findings suggest that at the production of *A. bisporus* mushroom strains for industrialization, to increase the quality of the obtained products, some of the *A. blazei* and *A. campestris* mushrooms strains characters should be taken into consideration. For *A. bisporus* industrialization we recommend to use the soaking treatment for canning.

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