CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM MONASCUS PURPUREUS FERMENTED DIFFERENT CEREAL SUBSTRATES

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ABSTRACT
Solid-state fermenting of cereals by Monascus is interesting strategy to produce cereals with more beneficial components. The objective of this study was to determine selected primary and secondary metabolites in cereals (rice, wheat, barley, sorghum, corn, buckwheat) fermented by Monascus purpureus and subsequently compare amount of these compounds with control sample (cereals without Monascus). In fermented cereals was determined higher protein, fat, reducing sugars, crude fiber and ash content with compare to non-fermented cereals. The antioxidant activity measured by DPPH assay, ABTS assay as well as reducing power assay was also higher in fermented Monascus cereals with the best results in rice (3.09 ±0.02; 62.9 ±2.24; 43.19 ±2.07 mg TEAC per g of dry weight). Sample of fermented rice contained the highest level of total polyphenols (15.31 ±3.62 mg GAE per g of dry weight), total flavonoids (1.65 mg QE per g of dry weight) and total phenolic acids (9.47 ±0.56 mg CAE per g of dry weight). In fermented cereals was also determined higher contact of reducing sugars (highest value in rice 246.97 ±7.96 mg GE per g), proteins (highest value in buckwheat 28.47 ±1.24%), ash (highest value in sorghum 2.74 ±0.08%) and fat (highest value in corn 4.89 ±0.03%) with compare to non-fermented samples. Results of crude fiber content of both – fermented and non-fermented cereals were balanced with similar values. Results of this study shown that Monascus purpureus fermented cereal substrates might be a potential sources of several bioactive compounds in food products.

Keywords: antioxidant activity; fat content; protein content; dietary fiber; Monascus

INTRODUCTION
Monascus purpureus is edible fungus widely used in solid state fermentation for centuries mainly in Asian countries (Srianta et al., 2016). Monascus spp. has been used as a common food additive and medicinal purposes for more than 1000 years. The genus Monascus encloses three main species (M. pilosus, M. purpureus and M. ruber) belonging to the family Monascaceae and to the class Ascomyceta (Pitt, 1997). Several secondary metabolites such as pigments, monacolins, γ-amino-butyric acid, dimerumic acid and citrinin have been identified (Cheng et al., 2011). Monascus fermented products have become very common, mainly because of the perception of Monascus food products as a “natural” therapy compared with a statin. Moreover, Monascus products are associated with a lowered risk of myalgia in comparison to statins and are consequently considered by consumers to be a safe option for the management of hypercholesterolaemia. Due to these cholesterol-lowering effects that have been demonstrated in several well-designed clinical trials, the European Union has accepted a health claim related to monacolin K from Monascus fermented products (mainly rice) and the maintenance of normal blood LDL cholesterol concentration (Childress et al., 2013). Monacolin K has a positive health promotion through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, a key enzyme of cholesterol synthesis in human body. γ-amino-butyric acid also play an important role in reducing the blood pressure (Srianta et al., 2014). These substances have a protection effect not only on cholesterol levels, but also on diabetes, cancer, osteoporosis, stroke, Alzheimer’s, and other dementias (Yang and Mousa, 2012). Monascus products have been used for the treatment of dengue virus infection. On the other side Monascus sp. produces metabolites which not only have health positive promotion, but also toxic for human body, mainly mycotoxin monascidin A characterized as citrinin (Blanc et al., 1995).

Monascus-fermented products are products of fermentation process by Monascus sp. through solid state fermentation or submerged fermentation methods. Solid-state fermenting process of cereals by Monascus can be biotechnological strategy that may produce bioactive compounds during fermentation (Lee et al., 2008; Srianta et al., 2014).
et al., 2014). Monascus-fermented products are applied for functional food, novel food ingredient to produce dairy products (bakery products, beverages) (Tseng et al., 2011). Currently, more than 50 patents concerning the use of Monascus pigments for food have been issued in Japan, the United States, France and Germany (Hajji et al., 2012).

The aim of this study was to determine bioactive compounds in selected cereals fermented with Monascus purpureus and subsequently compare obtained results with control sample without fermentation.

MATERIAL AND METHODOLOGY

Biological material

Wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), corn (Zea mays L.), rice (Oryza sativa L.), sorghum (Sorghum vulgare L.) and buckwheat (Fagopyrum esculentum L.) were purchased at a local market in Slovakia. Monascus purpureus MFTCCX 022/16 (Figure 1) and cereals fermented materials were obtained from private company Mycoforest, Slovakia. The fungi was inoculated onto potato dextrose yeast agar and incubated at 25 °C for 5 days. After a pure culture was obtained, Monascus purpureus was re-inoculated into potato dextrose yeast agar and mycelium was incubated at 27 °C for 7 days as the inoculum. Inoculum was then homogenized and inoculated into autoclaved cereals (120 °C, 1 hour) a rate of 7 mL/100 g-1. Monascus cereals were produced after the colonization of fungal mycelium for 14 days at 25 °C. Monascus cereals as well as uninoculated cereals, which were also autoclaved and used as controls were air-dried in an oven at 50 °C. Before the analyses samples were milling to powder (Perten 3100, Sweden).

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.5 g of cereals was extracted with 40 mL of 80% ethanol for 2 hours. After centrifugation at 4000 rpm (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). All analyses were realized in triplicate.

Dry mater, ash and protein determination

Dry matter, ash and protein were determined by the following standard AACC method 08-01. Nitrogen content was measured by the semi micro-Kjeldahl method. Nitrogen was converted to protein by using a factor of 5.7 for wheat and using a factor 6.25 for barley, buckwheat, sorghum, rice and corn.

Reducing sugars content

The reducing sugars content was determined by dinitrosalicylic acid colorimetric method according to the procedures described by Wang (2005). 0.5 g of sample was extracted with 80% of ethanol for 24 hours. After filtration 100 μL of extract was mixed with 800 μL dinitrosalicylic acid and mixture was heated at 90 °C for 5 minutes to develop the red-brown color. After cooling to room temperature 8 mL of distilled water was added and absorbance at 575 nm was measured (Jenway 6405 UV/Vis, England). Glucose (0.5 – 10 g.L-1; R2 = 0.998) was used as the standard, and the results were expressed in mg.g-1 glucose equivalent.

Crude fibre and total fat determination

Crude fibre content was determined using Fiber Analyzer (Ancom2000 USA) and total fat using Fat Extractor (Ancom XT15, USA) with methodic recommended by producer.

Antioxidant activity

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchez-Moreno et al., 1998) with slight modification. The extracts (1 mL) were mixed with 4 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 mg.L-1; R2=0.983) was used as the standard and the results were expressed in mg.g-1 Trolox equivalents.

Reducing power

Reducing power of samples was determined by the method of Oyaizu, (1986). One milliliter of sample extract was mixed with 5 mL PBS (phosphate buffer with pH 6.6) and 5 mL of 1% potassium ferricyanid in the test tube. Mixture was stirred thoroughly and heated in water bath for 20 minutes at 50 °C. After cooling, 5 mL of 10% trichloroacetic acid was added. 5 mL of mixture was pipetted into the test tube and mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride solution. Absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10 – 100 mg.L-1; R2 = 0.9974) was used as the standard, and the results were expressed in mg.g-1 Trolox equivalents.

ABTS radical cation decolorization assay

ABTS radical cation decolorization assay was determined by the method of Re et al., (1999) with slight modifications. ABTS (2,2’-azinobis[3ethylbenzthiazoline]-6-sulfonic acid) was dissolved in distilled water to 7 mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12-16 h) in the dark before use. The resultant intensely-coloured ABTS++ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.00 to give an absorbance value of ~0.70 at 734 nm. Two milliliters of ABTS solution was mixed with 0.98 mL of PBS and 0.02 mL of sample extract. Absorbance was measured spectrophotometrically (Jenway 6405 UV/Vis, England) at time intervals 6 minutes after addition of sample extract. Trolox (100 – 100 mg.L-1; R2 = 0.9991) was used as the standard, and the results were expressed in mg.g-1 Trolox equivalents.
Total polyphenol content

Total polyphenol content of extracts was measured by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. 0.2 mL of each sample extract was mixed with 0.2 mL of the Folin-Ciocalteu reagent, 2 mL of 20% sodium carbonate. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (1 – 150 mg.L⁻¹; \( R^2 = 0.998 \)) was used as the standard and the results were expressed in mg.g⁻¹ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of Quettier-Deleu et al. (2000). 2 mL of sample extract was mixed with 0.4 mL of 5% ethanolic solution of aluminium chloride. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.25 – 20 mg.L⁻¹; \( R^2 = 0.999 \)) was used as the standard and the results were expressed in mg.g⁻¹ quercetin equivalents.

Total phenolic acid content

Total phenolic acids content was determined using method of Farmakopea Polska (1999). A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL of 10% NaNO₂ +10% Na₂MoO₄, 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg.L⁻¹; \( R^2 = 0.999 \)) was used as a standard and the results were expressed in mg.g⁻¹ caffeic acid equivalents.

Statistical analysis

The basic statistical analyzes were realized in SAS programming packages (THE SAS SYSTEM V 9.2.). Correlation coefficients were calculated by CORR analysis (SAS, 2009).

RESULTS AND DISCUSSION

Dry matter, ash, reducing sugars, protein, fat and crude fiber content

Dry matter content (Table 1) ranged from 87.38% to 94.33% in control samples and from 91.52% to 93.59% in fermented samples.

Ash content (Table 1) in control sample ranged from 0.32% (rice) to 1.51% (buckwheat), but in Monascus fermented cereals amount of total ash was higher and ranged from 0.62% (rice) to 3.61% (buckwheat). Similarly results found in solid fermented rice Purvar et al. (2016), which determined significantly higher content in total ash with compare to control rice without fermentation. Vidyalakshmi et al. (2009) also compare fermented and non-fermented rice, and confirmed that solid state fermentation of cereals with Monascus increase total ash content. These authors determined in raw rice 0.66% of total ash, whereas in fermented rice determined amount of total ash 1.65%.

Reducing sugars content (Table 1) were in all control samples near to 20 mg GE.g⁻¹, but in fermented samples were higher and ranged from 35.39 (sorghum) to 246.97 mg GE.g⁻¹ (in rice). Increase of reducing sugars content is due to the starch utilized during growing of Monascus; during this process starch is broken down by amylase which starts to activate due to the increasing temperature during fermentation. Purvar et al. (2016) found in Monascus rice 113.2 mg GE.g⁻¹ of reducing sugars, while Vidyalakshmi et al. (2009) determined 253.9 mg GE.g⁻³ of reducing sugars in Monascus rice, which corresponds with our results in fermented rice. Cereals are very good substrate for fermentation medium due to the high starch content as a carbon source (C) and protein content as nitrogen source (N). Starch is the main carbon source in cereals which were hydrolyzed first prior to be transported into the mold cells (Sriantia and Harinojo, 2015). Proteins content (Table 1) ranged from 7.12% to 11.09% in control samples, while in fermented samples ranged from 14.18% to 28.49%. Purvar et al. (2016) published that in Monascus rise protein content increase to value 29.62%. Similar results also confirmed Vidyalakshmi et al. (2009), which detected in red rice 17.16% of protein, which is comparable with our findings – rice 14.18%. The utilization of rice carbohydrate by Monascus for its metabolism and production of the secondary metabolite namely the pigment has also resulted in an increased protein and crude fiber. In our study content of crude fiber decrease during fermentation (Table 1) which is with accordance to Purvar et al. (2016) findings. In their study amount of crude fiber in fermented rice decreased to amount 0.28%. On the other side Vidyalakshmi et al. (2009) observed increase of crude fiber in Monascus rice from 0.8 to 6.71%. Very interesting was to observe significant increase of fat content in fermented cereals with compare to control sample (Tab. 1). The highest increasing was determined in corn – 2.64 (in control sample) to 4.89% (fermented sample) and sorghum – from 2.08 (in control sample) to 4.55% (in control sample). Similarly Vidyalakshmi et al. (2009) detected increase of total fat in rice from 1.41% in control sample to 1.98% in fermented rice. Increase of fat content can be explain by Kennedy et al. (1999) which reported that Monascus on solid state fermentation produces mono unsaturated long-chain fatty acids of wide range from C₁₄ to C₂₄. Thirty-nine fatty acids were identified from Monascus fermented samples; twenty-two saturated fourteen monoenoic, two dioenoic and one ω-linolenic acid. Venkateswaran (2010) determined lauric, myristic, palmitic, stearic, oleic and linoleic acid in Monascus rice and sorghum. The same fatty acids determined in fermented corn, which contained also linolenic acid.

Antioxidant activity

Antioxidant activity of Monascus fermented cereals was higher by all used methods with compare to control samples. Radical scavenging activity (Table 2) in control sample ranged from 0.79 (sorghum) to 3.01 (rice) mg TEAC per gram of sample. Very high activity was detected in fermented rice, corn and wheat. Lee et al. (2008) determined increase of antioxidant activity by DPPH method of Monascus fermented soybean (26.4%) with compare to raw material (22.8%). Rajasekaran and Kalaivan, (2011) showed that fermented Indian rice had a strong activity by DPPH with the value 13.92 mmol TEAC.
per g, and also confirmed, that strong radical scavenging activity in vitro motivated the authors to investigate the biological significance of antioxidant activity of Monascus fermented cereals.

Reducing power (Table 1) of cereals ranged from 0.09 (barley) to 0.21 (buckwheat) mg TEAC per gram in control samples, while in fermented cereals was higher and ranged from 3.59 (sorghum) to 43.19 (rice) mg TEAC per gram of sample. Results showed that fermentation by Monascus increase very strong antioxidant activity. Rajasekaran and Kalaivan (2011) determined also strong reducing power in Indian Monascus rice, which lead these authors to investigate further in vivo by measuring lipid peroxidation and glutathione levels and superoxide dismutase and catalase activities. In their study confirmed that antioxidant activity of Monascus rice function through the induction of antioxidant enzymes and reduction hydrogen peroxide, quenching active singlet oxygen and by trapping and quenching radicals. Lee et al. (2008) determined increase of antioxidant activity by reducing power in fermented soybean (EC50 value 8.14 mg extract per ml) with compare to control non-fermented soybean (EC50 value 3.59 mg extract per mL). According to Mostafa and Abbady (2014) very important role as antioxidants in Monascus products play xanthomonascin A and B, glycurrubopunctatin, glyclymonascorubin, laccacia acid C and dimeric acid.

Antioxidant activity by ABTS radical cation decolorization assay (Table 2) ranged from 8.03 (barley) to 13.31 (rice) mg TEAC per g in control samples, while in fermented variants was higher and ranged from 15.31 (sorghum) to 62.89 mg TEAC per gram. By all used methods was the highest activity determined in fermented rice. Strong activity by ABTS assay in fermented rice bran published Cheng et al. (2016), which also showed possibility fermented rice bran used as a natural antioxidant agent due to its enhanced antioxidant activity.

**Total polyphenol, flavonoid and phenolic acid content**

Total polyphenol in cereals ranged from 0.14 (rice) to 1.33 (sorghum) mg GAE per gram in control samples, and from 2.34 (sorghum) in fermented cereals. Similarly like antioxidant activity amount of total polyphenols was strong increased in fermented cereals. Increase of polyphenols is probably due to release of these compounds from cell walls, which are destroy thanking enzymatic hydrolysis during fermentation. Razak et al. (2015) published that fermentation of rice bran by Monascus significantly increase amount of total polyphenols. In their study they compared control sample of rice bran (3.93 mg GAE per g) without fermentation with fermented rice bran (7.69 mg GAE per g) and also published that these compounds are better extracted with methanol (7.69 mg GAE per g) like with water (1.73 mg GAE per g). Yang et al. (2006) determined total phenolic content if Monascus dehulled rice in amount 40.39 mg per g which was significantly higher with compare to non-fermented control sample, with value 4.04 mg per g.

Strong correlation (p ≤ 0.05) was observed in our study between total polyphenol content and DPPH (p = 0.840), ABTS (p = 0.984) and reducing power (p = 0.977).

Total flavonoid content (Table 2) ranged from 0.01 (rice and corn) to 0.02 (sorghum and wheat) mg QE per g in control samples, while in fermented samples amount was higher and ranged from 0.53 (sorghum) to 1.65 mg QE per g (rice). Increase of total flavonoid content was very markedly, which correspond to Cheng et al. (2016) findings. In their work amount of flavonoid in rice bran increase four-times during fermentation. Huynh et al. (2014) published that during Monascus fermentation phenolic compounds are released and are obtained in soluble free form in the fermentation medium. This process contributes to the production of extracts and food products with a higher added value. For example Handa et al. (2014) determined that soybean fermentation by Monascus significantly increase genistein aglycone with compared to unfermented soybean. Phenolic aglycones have a higher antioxidant activity than their glycosides, which are dominant in non-fermented cereals and legumes. Strong correlation (p ≤ 0.05) was observed in our study between total flavonoid content and DPPH (p = 0.986), ABTS (p = 0.871) and reducing power (p = 0.867).

Total phenolic acid content (Table 2) in cereals ranged from 0.03 (rice) to 0.83 (sorghum) mg CAE per g in control samples, while in fermented samples amount was higher and ranged from 3.12 (sorghum) to 9.47 mg CAE per g (rice). Similarly like antioxidant activity, polyphenols and flavonoids Monascus fermentation can increase

**Table 1 Content of dry matter, ash, reducing sugars, protein, fat and crude fiber.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DM (%)</th>
<th>AC (%)</th>
<th>RSC (mg GE.g⁻¹)</th>
<th>PC (%)</th>
<th>FC (%)</th>
<th>CFC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>94.33 ±0.55</td>
<td>0.58 ±0.01</td>
<td>20.02 ±1.21</td>
<td>8.01 ±0.21</td>
<td>0.34 ±0.01</td>
<td>0.57 ±0.02</td>
</tr>
<tr>
<td>MFB</td>
<td>91.99 ±0.58</td>
<td>2.30 ±0.01</td>
<td>166.46 ±1.38</td>
<td>25.21 ±0.24</td>
<td>2.19 ±0.27</td>
<td>0.53 ±0.01</td>
</tr>
<tr>
<td>Rice</td>
<td>92.81 ±0.31</td>
<td>0.32 ±0.02</td>
<td>13.15 ±0.71</td>
<td>7.15 ±0.11</td>
<td>0.62 ±0.14</td>
<td>0.54 ±0.03</td>
</tr>
<tr>
<td>MFR</td>
<td>93.41 ±0.21</td>
<td>0.62 ±0.02</td>
<td>246.97 ±7.96</td>
<td>14.18 ±0.25</td>
<td>2.67 ±0.78</td>
<td>0.57 ±0.02</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>87.38 ±1.21</td>
<td>1.17 ±0.01</td>
<td>15.57 ±0.72</td>
<td>11.09 ±0.74</td>
<td>1.62 ±0.14</td>
<td>0.57 ±0.01</td>
</tr>
<tr>
<td>MFBw</td>
<td>92.32 ±0.68</td>
<td>3.48 ±0.31</td>
<td>74.64 ±0.71</td>
<td>28.49 ±0.23</td>
<td>3.34 ±0.44</td>
<td>0.56 ±0.02</td>
</tr>
<tr>
<td>Corn</td>
<td>93.27 ±0.55</td>
<td>1.28 ±0.11</td>
<td>13.96 ±2.11</td>
<td>8.81 ±0.17</td>
<td>2.65 ±0.24</td>
<td>0.55 ±0.03</td>
</tr>
<tr>
<td>MFC</td>
<td>93.54 ±1.02</td>
<td>2.52 ±0.01</td>
<td>67.35 ±7.58</td>
<td>19.27 ±0.11</td>
<td>4.89 ±0.03</td>
<td>0.53 ±0.01</td>
</tr>
<tr>
<td>Wheat</td>
<td>94.17 ±0.47</td>
<td>1.51 ±0.01</td>
<td>13.15 ±1.41</td>
<td>10.92 ±0.74</td>
<td>1.09 ±0.41</td>
<td>0.56 ±0.02</td>
</tr>
<tr>
<td>MFW</td>
<td>91.75 ±0.77</td>
<td>3.60 ±0.03</td>
<td>154.33 ±9.11</td>
<td>24.68 ±0.65</td>
<td>2.92 ±0.02</td>
<td>0.55 ±0.03</td>
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<td>Sorghum</td>
<td>91.52 ±0.88</td>
<td>1.46 ±0.03</td>
<td>13.15 ±0.73</td>
<td>8.88 ±0.83</td>
<td>2.08 ±0.06</td>
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<tr>
<td>MFS</td>
<td>93.59 ±0.05</td>
<td>2.74 ±0.11</td>
<td>35.39 ±3.05</td>
<td>17.97 ±0.14</td>
<td>4.55 ±0.22</td>
<td>0.51 ±0.01</td>
</tr>
</tbody>
</table>

phenolic acid content due to their release from plants cell walls during fermentation. Razak et al. (2015) showed that during Monascus fermentation increase amount of ferulic, syringic and sinapic acid in rice bran with compare to non-fermented bran. These authors also published, that structural breakdown of cell walls induced by fermentation may occur, leading to the liberation and/or synthesis of various bioactive compounds. For example, the substantial increase in the vanillic acid content with M. purpureus rice bran extracts which is attributed to the fact that ferulic and coumaric acids can be biologically transformed into smaller compounds such as vanillic acid. During fermentation, enzymes such as amylases, xylanases, and proteases, derived from the substrate and the fungi, contribute towards the modification of substrate compositions.

Strong correlation (p ≤ 0.05) was observed in our study between total phenolic acid content and DPPH (r = 0.952), ABTS (r = 0.943) and reducing power (r = 0.959).

**CONCLUSION**

The *Monascus purpureus* fermented cereals contained higher content of primary (protein, ash, reducing sugars, fat, dietary fiber) and secondary metabolites (polyphenols, flavonoids, phenolic acids as well as antioxidant activity) compare with to control non-fermented cereals. *Monascus* fermented cereals have a good potency as functional products. But further studies in the process fermentation and content of biologically active compounds are necessary in future and also toxicological properties, if any.

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