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The monitoring of biogenic amines in the raw food

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ABSTRACT
The aim of this work was to evaluate microbial quality and the presence of biogenic amines in raw bars. This study was focused on microbiological research in order to determine the presence of selected indicator groups of microorganisms depending on the composition of raw food. Identification of microorganisms was carried out by MALDI-TOF MS. In the second part of the experiment, biogenic amines and polyamines were analyzed using high performance liquid chromatography with UV/VIS detection. An increased incidence of mold has been reported in the samples, which is associated with a risk of mycotoxin production. After identifying microorganisms, it was found out that genera *Micrococcus*, *Bacillus* and *Staphylococcus* were the most represented. The highest concentration of biogenic amines (tyramine 42.2 ±4.8 mg.kg^{-1}; putrescine 54.0 ±2.9 mg.kg^{-1}) was found in a sample containing the vegetable component. The average concentration of biogenic amines in the tested raw bars was <30 mg.kg^{-1} and therefore they do not pose a serious health hazard to a consumer.

Keywords: raw food; biogenic amines; UHPLC; microorganisms

INTRODUCTION
Raw foods consist mainly or entirely of raw uncooked food. In the Czech Republic, we can also see the name "live diet", and supporters of this trend are called "vitarians" **(Cunningham, 2004; Červenka, Brožková and Fišerová, 2016)**. Food is considered raw unless it has undergone a heat treatment of more than 48 °C. Generally, there is no single specific temperature in the literature, but the range is between 38 – 48 °C **(Cunningham, 2004; Červenka, Brožková and Fišerová, 2016)**. Food meets raw food parameters unless it is refined, pasteurized, treated with pesticides or otherwise processed industrially. Instead, this diet approves a variety of food modifications like mixing, lyophilization, soaking and germination. Raw diet is based on eating a plant-based diet, especially fruits, vegetables, nuts and seeds. Cereals and legumes are also allowed, but usually they are first soaked **(Cunningham, 2004)**.

Among the positive effects of eating raw food is the high intake of fiber, minerals and water-soluble vitamins **(Craig, 2009)**. The controversial effects of eating raw food are reducing the risk of cancer, especially women-specific types of cancer **(Lanou and Svenson, 2011; Tantamango-Bartley et al., 2013)** when mortality decreases compared to people, who consumed animal-based food **(Orlich et al., 2014)**. Raw food has a positive effect on the composition of the intestinal microflora, which exhibits a protective effect **(Glick-Bauer and Yeh, 2014)**. **Ling and Hänninen (1992)** describe a significant decrease in the activity of some precarcinogenic enzymes formed by the intestinal microflora when the raw foods were eaten for a week. Consuming raw food also seems useful in terms of intake of protective nutrients and photochemicals and also of minimizing intake of substances that are involved in many chronic diseases **(Dewell et al., 2008)**.

Limiting the animal fat in the diet decreases the intake of saturated fatty acids and cholesterol. These aspects lead to a lower incidence of cardiovascular disease. At the same time, HDL cholesterol is also reduced, as well as insufficient intake of vitamin B12 **(Dewell et al., 2008; Koebnick et al., 2005)**. In the long term, insufficient intake of vitamin B12, iron, zinc, essential fatty acids and essential amino acids is considered to be the major drawback of this nutritional trend. The largest natural sources of vitamin B12 are meat, offal, seafood, eggs, milk and dairy products **(Watanabe, 2016)**.

Deficiency of B12 may result in megaloblastic anemia, which causes interruption of the cell division process. Clinical manifestations are consequently fatigue, weakness, paleness and decreased muscle activity **(Aslinia, Mazza and Yale, 2006)**. Another type of anemia associated with the raw diet is sideropenic anemia, which is associated with reduction of iron in the blood **(Sahovic, Vukobrat-Bijedic and Sahovic, 2012)**. The best sources of iron in the raw diet are mainly nuts (cashews, almonds, hazelnuts) and legumes (lentils, beans, peas), which are first recommended to be soaked or germinated. In the long term, intake of raw foods leads to weight loss, but also to underweight in many cases. Increased consumption of raw

food is therefore usually associated with low values of BMI **(Koebnick et al., 1999; Craig, 2009)**. Eating raw food results in low intake of protein, calcium and vitamin D. Low density of bone tissue and increased risk of osteoporosis **(Fontana et al., 2005)** are often manifested in people following this diet. **Ganss, Schlechtriemen and Klimek (1999)** reported an increased incidence of tooth enamel erosion. Its decay is associated with excessive consumption of fruit, which contains easily fermentable sugars. Eating raw foods leads to insufficient intake of polyunsaturated fatty acids necessary for normal function and further development of the brain, especially in children and adolescents who are still growing **(Fonseca-Azevedo and Herculano-Houzel, 2012)**.

Eating raw food is also associated with worse digestibility of plant proteins, correlated with reduced nutrient utilization due to the presence of antinutrients. Antinutrients act on the activity of some enzymes, vitamins and minerals. In legumes and cereals, lectins, protease inhibitors, saponins and phytic acid are found to be destroyed only by heat treatment of foods **(Soetan and Oyewole, 2009)**. Protease inhibitors, which were found in soybeans and peanuts, prevent proteolysis and subsequent protein utilization. The body reacts to the resulting amino acid deficiency by producing pancreatic proteases. In adolescents, these substances can cause stop in growth and further development **(Kvasničková, 1998)**.

Also, it is importat to report increased risk of food intoxications, which stems from inadequate heat treatment of foods **(Cunningham, 2004)**.

Biogenic amines are one of the substances involved in the food quality. They are low molecular weight organic nitrogen compounds. Biogenic amines exist in living organisms, where they fullfil a number of metabolic and physiological functions **(Silla-Santos, 1996; Košmerl, Šućur and Prosen, 2013; Cunha, Lopes and Fernandes, 2016)**. Biogenic amines are essential for all humans. But in high concentrations, they may cause health problems. Histamine and tyramine belong among the most toxicologically relevant biogenic amines **(Shalaby, 1996; Buňková et al., 2013)**. The most common manifestation of the occurrence of biogenic amines are respiratory problems, nausea, palpitations, irregular heartbeat, erythema, swelling and headaches **(Santos et al., 2003; Li et al., 2013)**.

The maximal limit permitted by European legislation is defined only for histamine. According to European Commission of Regulation (EC) nu. 2073/2005, the maximum histamine content in fish and fishery products is set at less than 100 $mg.kg^{-1}$. A number of biogenic amines in foods of plant origin have been described by some authors **(Halász et al., 1994; Nishibori, Fujihara and Akatuki, 2007; Pleva et al., 2018)**. However, according to available literature, the determination of these substances in raw food has not been carried out yet.

Scientific hypothesis

Biogenic amines can be present in raw bars and their content is variable.

MATERIAL AND METHODOLOGY

Isolation and identification of the microorganisms:

Ten grams of the fermented raw food sample (Figure 1) was weighed out, aseptically removed and put into 90 mL of sterile physiological solution that was subsequently homogenised for 10 min (using a stomacher). The raw bars were then subjected to routine microbiological analysis. The total microorganism counts were assessed according to **ISO 4833−1 (2013)**, the *Enterobacteriaceae* bacteria family according to **ISO 21528−2 (2017)**, yeasts and moulds according to **ISO 6611 (2004)** and halotolerant microorganisms (staphylococci) according to **Chapman (1945)** on mannitol salt phenol red agar after cultivation at 37 °C for 2 days. The selected colonies were isolated into BHI broth and cultivated for 24 – 48 h at 25 °C (yeasts), 37 °C (*Enterobacteriaceae*, *Staphylococcus*) or 30 °C (other microorganisms). Each raw bar product sample was microbially analysed 3 times. Identification of the microorganisms was performed via the MALDI−TOF MS method using a Bruker Autoflex Speed (Bruker Daltonics, Bremen, Germany) and the Biotyper 3.1 database (Bruker Daltonics) after preliminary classification of isolates into individual microorganism groups. Visualisation of the protein profiles was performed via mMass 5 **(Strohalm et al., 2010)**. The individual identifications were performed in at least two independent experiments in two parallels **(Pleva et al., 2018)**.

Figure 1 Various types of raw bars. Note: (top left – raw sesame bar, top right – raw stick with cashew, left bottom – raw chocolate florentines, bottom right – raw apple ball).

Table 1 Composition of the product.

code	product	composition of the product
B1	Raw balls tropical mix	dates, almonds, dried mango, dried pineapple, almond paste, uncooked cocoa beans, raw syrup of agave, orange peel, ethereal orange oil
B2	Raw balls coconut	coconut grated, raisins Sultana, dates, sunflower seed
B3	Raw balls Jamaica	dates, unroasted cocoa beans, almonds, ground vanilla, spices
B4	Raw chocolate marokánka	dates, figs, raw cashews, almonds
B5	Raw bars with cashew	cocoa powder, agave syrup, orange peel, almonds, walnuts, dates, raisins, pecans, sunflower seeds, pumpkin seeds, coconut, apples, ground cinnamon, ground cardamom, Himalayan salt pink
B6	Raw sesame bars	cashews, raisins, sunflower seeds
B7	Raw vegetable bars	date, sesame
B8	Raw vegetable bars	Brazil nuts, dried tomatoes with sea salt, garlic, onion, Sultana raisins, hemp seeds, Roman cumin, marjoram, chilli minced
B9	Raw apple balls	raisins, sunflower seeds of the core, apple pulp powder, cinnamon
B10	Raw bars with red beet	dates, raisins Sultana, sunflower seed, beet powder, extra virgin olive oil, lemon essential oil
B11	Raw cocoa balls	raisins, dates, cocoa, coconut, chia seeds, sunflower
B12	Raw protein bars with banana	dates, banana, rice protein, coconut
B13	Raw protein hazelnut bars	dates, hazelnuts, rice protein (heat-unprocessed protein from whole-grain brown rice, rice oligodextrin, stevia, xanthan gum, sea salt, pectin), sunflower seeds, raw cocoa mass, chia seeds
B14	Raw apple bars	date, activated sunflower seed, dried apples, Sultana raisins, cinnamon
B15	Raw plum bars	dates, cashew, beetroot, plums, cocoa beans, poppy

Table 2 Viable counts (log CFU.g^{-1}) of the main microbial groups (first day) in raw bars in the Czech republic.

	log CFU.g^{-1}							
Sample	**VRBA**	**MSA**	**MRS**	**SB**	**M17**	**CHYGA**	**RCA**	**BHI**
B1	3.9 ±0.3	3.8 ±0.2	3.2 ±0.1	3.7 ±0.4	2.8 ±0.1	3.6 ±0.1	2.6 ±0.4	3.8 ±0.3
B2	3.5 ±0.3	5.2 ±0.1	3.5 ±0.2	-	3.9 ±0.5	2.3 ±0.1	5.0 ±0.2	3.9 ±0.3
B3	7.3 ±0.4	7.6 ±0.3	2.7 ±0.1	4.1 ±0.2	5.0 ±0.2	3.5 ±0.1	5.3 ±0.1	9.2 ±0.4
B4	3.6 ±0.2	3.0 ±0.1	3.0 ±0.2	3.7 ±0.2	3.5 ±0.3	3.3 ±0.1	6.1 ±0.2	2.9 ±0.2
B5	3.0 ±0.3	2.9 ±0.2	-	3.2 ±0.3	6.4 ±0.2	3.6 ±0.3	3.3 ±0.3	3.3 ±0.1
B6	4.3 ±0.5	3.6 ±0.2	4.8 ±0.3	-	5.1 ±0.2	3.0 ±0.1	3.4 ±0.2	5.2 ±0.2
B7	6.7 ±0.4	3.2 ±0.3	3.9 ±0.3	3.2 ±0.1	3.7 ±0.4	3.6 ±0.2	5.0 ±0.2	7.5 ±0.2
B8	3.7 ±0.3	3.2 ±0.2	4.6 ±0.4	4.0 ±0.3	2.6 ±0.1	3.3 ±0.2	5.7 ±0.2	3.5 ±0.1
B9	3.4 ±0.3	3.4 ±0.1	-	-	3.7 ±0.2	2.4 ±0.1	-	3.7 ±0.2
B10	4.2 ±0.5	5.3 ±0.4	6.7 ±0.2	-	4.4 ±0.1	-	-	3.4 ±0.4
B11	2.7 ±0.3	4.0 ±0.2	6.3 ±0.3	3.0 ±0.7	6.1 ±0.4	3.2 ±0.2	4.9 ±0.3	3.6 ±0.2
B12	2.9 ±0.4	4.0 ±0.2	-	-	3.4 ±0.2	4.0 ±0.1	-	4.2 ±0.3
B13	3.6 ±0.3	3.7 ±0.3	3.8 ±0.1	3.5 ±0.2	3.7 ±0.3	2.5 ±0.1	2.3 ±0.2	3.7 ±0.1
B14	3.3 ±0.2	4.7 ±0.4	-	-	4.8 ±0.1	-	-	4.6 ±0.2
B15	3.0 ±0.3	4.1 ±0.1	-	-	4.1 ±0.2	3.6 ±0.2	-	4.4 ±0.3

Preparation:

Lyophilised raw bar products were used for the biogenic amine (BA) and polyamine (PA) analysis. Triple extraction of BA and PA from the lyophilised samples was carried out using a perchloric acid solution (0.6 mol.L^{-1}). Three independent extractions were performed on each raw bar sample. The filtrated extract (filter porosity 0.45 μm) was then used directly for derivatisation and a following determination of BA/PA content **(Dadáková, Křížek and Pelikánová, 2009; Buňková et al., 2013)**.

Biogenic amine detection by HPLC:

The concentrations of eight present biogenic amines, such as histamine (HIM), tyramine (TYM), phenylethylamine (PHE), tryptamine (TRY), putrescine (PUT), cadaverine (CAD), spermine (SPE) and spermidine (SPD), were analysed via high performance liquid chromatography (HPLC) (LabAlliance, USA and Agilent Technologies, Agilent, Santa Clara, California, USA) after derivatisation using dansylchloride. The dansylchloride sample derivatisation procedure was performed according to **Dadáková, Křížek and Pelikánová (2009).** 1,7−heptandiamine was used as the internal standard. Chromatographic separation (ZORBAX Eclipse XDB−C18, 50 9 3.0 mm, 1.8 lm; Agilent Technologies) and detection (spectrophotometric λ = 254 nm) were performed according to **Buňková et al. (2013)**. Each extract was derivatised twice after cultivation, and each derivatised mixture was applied to the column twice. Each raw bar sample was analysed 12 times (3 extractions, 2 derivatisations, 2 applications to the column). Detection limits for the individual amines were in the range 0.24 – 1.39 mg.kg^{-1}. Given the significance of biogenic amines to human health and food safety, monitoring their content in foodstuffs is very important. Currently, HPLC based methods are the most suitable for the analysis of fermented food **(Pleva et al., 2018)**. The reliability and sensitivity of these methods render them useful as important techniques to determine the concentrations of all biogenic amines in fermented food **(EFSA, 2011)**.

Statistic analysis

The obtained experimental data were analysed using Statistical software Unistat 6.5 (Unistat, London, UK). The significance level of all statistical tests was set at p <0.05. The Kruskall-Wallis and Wilcoxon tests were used to evaluate the data obtained.

RESULTS AND DISCUSSION

Microbial analysis

Raw bars are ideal media for the growth and survival of a variety of fungi and bacteria.

The results of the microbial analysis are given in Table 2. The amount of microorganisms cultured in BHI ranged from 2.9 to 9.2 log CFU.g^{-1}. Although there is no hygienic limit for this type of product in the current legislation, the log boundary of 6.0 CFU.g^{-1} is considered to be safe

(Červenka, Brožková and Fišerová, 2016). This hygienic limit was not crossed by all tested samples, except for samples B3 (9.2 log CFU.g^{-1}) and B7 (7.5 log CFU.g^{-1}). The number of yeasts and moulds (CHYGA) ranged from 2.3 to 4.0 log CFU.g^{-1}. A similar result was obtained by **Červenka, Brožková and Fišerová (2016)**, who reported the amount of moulds in raw foods ranging from 1.8 to 3.7 log CFU.g^{-1}. Yeasts and moulds occurred in almost all samples, despite the fact that the antimycotic agent hexamidine at a concentration of 50 mg.L^{-1} was applied to B9, B10, B12, B14 and B15 samples. An increased number of moulds can be caused by contamination of feedstocks or by used processing technology. Later, isolated moulds were microscopically identified as *Aspergillus* and *Penicillium*, which are responsible for the production of mycotoxins. Many authors have reported an increased occurrence of mycotoxins, especially ochratoxin A and aflatoxins, in dates **(Ragab, Ramadan and Abdel-Sater, 2001; Azaiez et al., 2015)** or in raisins **(Azaiez et al., 2015)**, which form a substantial part of the raw bars. Mycotoxins were also found in other raw materials, that raw bars are made of. For example mainly in figs **(Azaiez et al., 2015)**, dried plums **(Engel, 2000; Azaiez et al., 2015)**, but also peanuts **(Hoeltz et al., 2012; Schwartzbord and Brown, 2015)**, cashews **(Milhome et al., 2014)**, coconut **(Saxena and Mehrotra, 1990)** and sunflower seeds **(Jiménez et al., 1991)**.

The number of coliform bacteria (VRBA) ranged from 2.7 to 7.3 log CFU.g^{-1}. However, an increased number of coliform bacteria was observed in samples B3 (7.3 log CFU.g^{-1}) and B7 (6.7 log CFU.g^{-1}). The presence of coliform bacteria can be caused by fertilization of bio food with faecal matter, insect vector transport or by contaminated water. **Červenka, Brožková and Fišerová (2016)** reported that the content of coliform bacteria was in the interval from 1.9 to 4.4 log CFU.g^{-1} in samples of raw food but, in two samples, such increase was not noticed due to the antimicrobial effect of young barley. In their study, **Brožková et al. (2016)** presented the contents of coliform bacteria in raw materials for the production of raw bars, such as hazelnuts (2.9 log CFU.g^{-1}), goji (2.8 log CFU.g^{-1}), cashew (<1 log CFU.g^{-1}), chia seed (<1 log CFU.g^{-1}) and linseed (5.9 log CFU.g^{-1}). Although the microbicidal effect of essential oils was described in citruses **(Oikeh et al, 2016)**, these compounds did not have a significant effect on the number of coliform bacteria in B1 and B10 samples. The number of staphylococci (MSA) was found to range from 2.9 to 7.5 log CFU.g^{-1}, with the highest concentration being detected in samples B3 (7.5 log CFU.g^{-1}), B10 (5.3 log CFU.g^{-1}) and B2 (5.2 log CFU.g^{-1}). Enterococci (SB) were recorded in 8 samples with numbers ranging from 3.0 to 4.13 log CFU.g^{-1}. Streptococci (M17) and lactobacilli (MRS) were observed in all samples, the number of streptococci ranged from 2.6 to 6.4 log CFU.g^{-1} and the number of lactobacilli (MRS) ranged from 2.3 to 6.1 log CFU.g^{-1}.

Out of 15 samples cultivated on 8 selectively diagnosed soils, 68 species of bacteria and yeast were isolated and identified by the MALDI-TOF MS method. The following microorganisms were identified: *Acinetobacter pittii* (B6, B10), *Bacillus cereus* (B4, B10, B12, B15), *Bacillus safensis* (B9, B14, B15), *Bacillus thuringiensis* (B4), *Cronobacter sakazakii* (B6), *Enterococcus casseliflavus* (B15), *Micrococcus luteus* (B1, B5, B6, B7, B8, B10, B11, B12, B13), *Pseudomonas oryzihabitans* (B6, B10), *Rhodotorula mucilaginosa* (B12), *Serratia fonticola* (B3), *Serratia marcescens* (B1, B3), *Staphylococcus aureus* (B3), *Staphylococcus hominis* (B1, B13), *Staphylococcus pasteuri* (B9), *Staphylococcus warneri* (B2, B4, B7).

Biogenic amine and polyamine analysis

The selected results of the chromatographic analysis of biogenic amines and polyamines are summarized in Figure 2. These biogenic amines were detected: PEA (8.14 − 37.78 mg.kg^{-1}), HIM (2.14 − 18.92 mg.kg^{-1}) and TYM (1.98 − 42.23 mg.kg^{-1}).

The highest concentration of PEA was observed in samples B4 (37.8 ±2.4 mg.kg^{-1}) and B9 (35.0 ±2.3 mg.kg^{-1}) and the lowest was in B13 (8.1 ±0.8 mg.kg^{-1}) and B3 (8.2 ±1.6 mg.kg^{-1}). In case of HIM, the highest concentration was detected in samples B1 (18.9 ±1.2 mg.kg^{-1}) and B13 (11.9 ±2.0 mg.kg^{-1}), on the other hand, the lowest was in B3 (2.1 ±0.8 mg.kg^{-1}) and B2 (2.9 ±1.1 mg.kg^{-1}). The highest concentration of TYM was observed in samples B8 (42.2 ±4.8 mg.kg^{-1}) and B4 (31.7 ±3.4 mg.kg^{-1}), the lowest was in samples B6 (2.0 ±0.5 mg.kg^{-1}) and B14 (3.3 ±1.6 mg.kg^{-1}). Based on the statistical analysis, statistically significant differences ($p \leq 0.05$) were found in the BA content of individual raw bars.

PEA is a natural component of cocoa beans **(Halász et al., 1994; Silla-Santos, 1996)**. PEA (<20 mg.kg^{-1}) was reported in non-cured cocoa beans, however, higher concentrations were detected in roasted beans. Higher concentrations are related to the decarboxylation of phenylalanine to PEA as a result of roasting **(Halász et al., 1994)**. No increased concentrations of PEA were recorded in samples containing cocoa beans or cocoa powder. The measured HIM concentration was below 20 mg.kg^{-1}. The highest TYM content was recorded in samples containing dried tomatoes (B8), bananas (B12) and plums (B15). **Halász et al. (1994)** also reported an increased incidence of TYM in tomatoes, plums and bananas.

The total polyamine content ranged from 6.88 to 28.32 mg.kg^{-1}, PUT from 8.31 to 53.95 mg.kg^{-1}, SPD from 0.76 to 11.23 mg.kg^{-1} and SPM from 9.24 to 30.73 mg.kg^{-1}.

The highest concentration of CAD was observed in samples B8 (28.3 ±3.8 mg.kg^{-1}) and B7 (25.7 ±2.4 mg.kg^{-1}), while the lowest was in B6 (6.9 ±1.1 mg.kg^{-1}) and B2 (10.1 ±0.8 mg.kg^{-1}). The most of PUT was contained in samples B8 (54.0 ±2.9 mg.kg^{-1}) and B4 (39.4 ±2.6 mg.kg^{-1}) and the lowest concentration was recorded in samples B3 (8.3 ±0.6 mg.kg^{-1}) and B14 (10.1 ±1.7 mg.kg^{-1}). The highest volumes of SPD were observed in samples B6 (11.2 ±0.9 mg.kg^{-1}) and B7 (10.3 ±0.9 mg.kg^{-1}) but the lowest SPD content was in samples B12 (0.8 ±0.3 mg.kg^{-1}) and B10 (2.8 ±0.2 mg.kg^{-1}). In case of polyamine SPM, the highest volumes were detected in samples B3 (30.7 ±2.4 mg.kg^{-1}) and B6 (26.3 ±1.8 mg.kg^{-1}), the lowest were in samples B10 (9.2 ±0.4 mg.kg^{-1}) and B9 (10.8 ±0.7 mg.kg^{-1}).

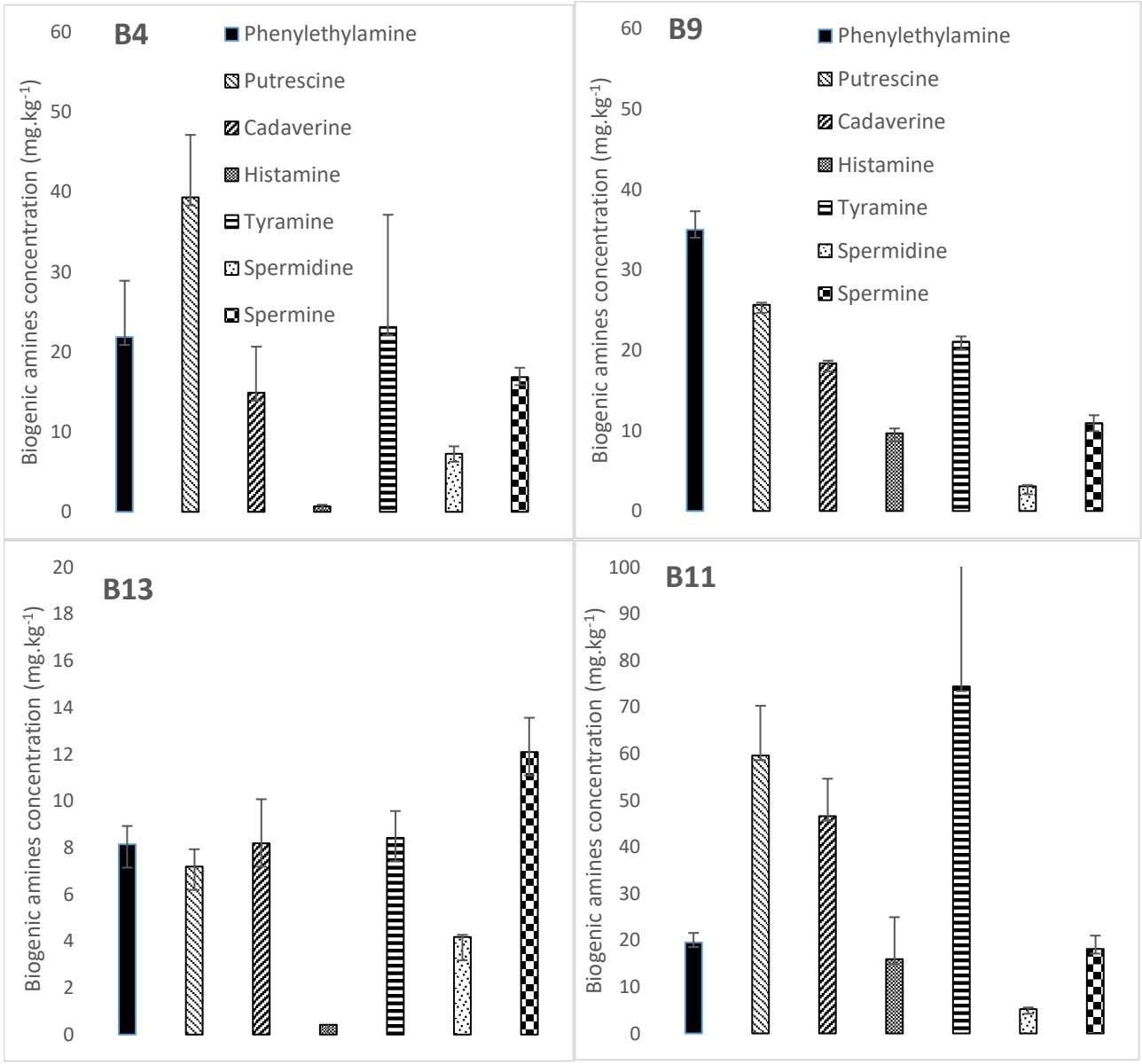

Figure 2 Biogenic amines content in selected raw bar samples (B4, B9, B11 and B13) (mg.kg^{-1}).

Nishibori, Fujihara and Akatuki (2007) reported the amount of polyamine SPM to be 13.6 mg.kg^{-1} in almonds and 24.1 mg.kg^{-1} in cashews. Results from this study correspond with our results because the samples B3 (almonds) and B6 (cashews) contained the highest SPM concentration in the raw bars. The highest measured PUT content was in sample B8 (54.0 ±2.9 mg.kg^{-1}) containing the vegetable component. However, this result is different compared with results achieved by **Nishibori, Fujihara and Akatuki (2007)** who reported lower amounts of PUT in tomato (5.9 mg.kg^{-1}), raisins (0.1 mg.kg^{-1}), garlic and onion (each 2.3 mg.kg^{-1}).

CONCLUSION

The first part of this study concerned the characteristics of raw food, its microbial quality and the problematics of biogenic amines. 15 types of raw bars with various content composition were selected for this experiment (Table 1). These foodstuffs were subjected to a microbial analysis with a goal to find indicator groups of microorganisms (facultative anaerobic mesophilic microorganisms, enterobacteria, staphylococci, yeasts, moulds, and lactic acid bacteria). The highest concentration of biogenic amines was recorded in the sample of the raw bar containing vegetable components with this product containing, beside others, a biogenic amine tyramine in concentration 42.23 mg.kg^{-1} and a polyamine putrescine in concentration 53.95 mg.kg^{-1}. More than a half of the samples did not exceed the limit of concentration of biogenic amines 15 mg.kg^{-1}; two thirds of the samples did not exceed the limit 20 mg.kg^{-1}. Identification of present microorganisms proved that the most represented genus were *Micrococcus*, *Staphylococcus* and *Bacillus*, which a decarboxylase activity was observed in. Taking this fact into account, it is important to consider the content of individual biogenic amines in the tested samples. The achieved results of this study show that raw bars contain various microorganisms according to their content composition. It is necessary to pay attention to the content of individual types of foodstuff and their microflora, especially in relation to human health. Even though it was not a primary goal of this study to focus on presence of

moulds, the occurrence of mycotoxigenic genus *Aspergillus* and *Penicillium* in the studied samples is alarming. The presence of mycotoxins is very probable in these products and that is why it would be suitable to focus the studies of raw bars this way. The amounts of biogenic amines in the tested samples were not high. However, it is important to consider a "cocktail effect" of these substances and to consume raw bars in moderate amounts.

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