OPEN ACCESS Potravinarstvo Slovak Journal of Food Sciences Crossref Similarity Check

potravinárstvo® Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 12, 2018, no. 1, p. 292-298
doi: https://doi.org/10.5219/896
Received: 10 February 2018. Accepted: 13 March 2018.
Available online: 21 March 2018 at www.potravinarstvo.com

ISSN 1337-0960 (online)

BIOGENIC AMINES CONTENT IN THE FERMENTED ASIAN FOOD IN THE CZECH REPUBLIC

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ABSTRACT
The aim of this work was to study the typical fermented Asian food (miso paste, soy sauce, rice vinegar, kimchi and tempeh) to monitor their microbial quality and presence of biogenic amines in relation to time and temperature of storage. This study is focused on microbiological research in order to determinate presence of selected indicator groups of microorganisms during storage of individual products at three different temperatures, 8 °C, 23 °C, 30 °C. It was found that the highest increase of total viable counts was observed in products stored in 23 °C and 30 °C, especially in tempeh and miso paste. In soy sauce and rice vinegar were observed only very low amounts of microorganisms through the storage period. In the second part of the experiment, the biogenic amines were analyzed using high performance liquid chromatography. It was found that the levels of biogenic amines in tested products were low and does not affect human health.

Keywords: Asian fermented food, soy, microorganisms, biogenic amines, storage

INTRODUCTION
European consumption of soyfoods is similar to that of the United States, with meat and dairy alternatives comprising most of the soyfood sales. Throughout the rest of Europe, tofu is known, but it is not as popular as meat and dairy variants. As in the United States, soyfoods have become more of a mainstream food item, having crossed over from the natural products market to being widely available now in common supermarkets **(Riaz, 2006)**.

Tempeh is a traditional, fermented soyfood that is unique in its texture, flavor, and versatility. It is made from the whole soybean, which has been smashed, cracked, and boiled with added vinegar to reduce the pH. Tempeh contains about 19% protein, is richer in fiber than tofu, and is a significant source of vitamins and minerals **(Riaz, 2006)**.

Miso is a rich and flavorful paste made from either fermented and riped whole soybeans or soybeans in combination with wheat, barley, or rice. It contains enzymes and bacteria that can aid in digestion. It is high in protein, but it also contains high levels of sodium and should be consumed sparingly. Most of the miso sold today is pasteurized and refrigerated **(Fukushima, 1981; Chiou and Cheng, 2001; Riaz, 2006)**.

Soy sauce is the most well known and popular of the traditional soyfoods and is used extensively as a flavoring ingredient in most Asian dishes. When naturally processed, soy sauce is produced in a manner similar to that of miso. When made wholly of soybeans, the product is called tamari. If it is processed with a fermented wheat starter, the product is called shoyu. Much of the soy sauce sold today is not naturally fermented. Instead, it is made with hydrolyzed vegetable protein, sugar, color, and preservatives **(Riaz, 2006)**.

The soybean has long been embraced as a source of highquality protein from which a wide variety of foods can be made. The quality of soy protein is now considered as being essentially equivalent to animal protein. In fact, in 1999, the U.S. Food and Drug Administration approved a health claim for the cholesterol lowering effects of soy protein. Beyond heart disease, proposed benefits include reductions in the risk of breast and prostate cancer and osteoporosis. More speculative data suggest soyfoods may also positively affect kidney function and cognitive function and help to alleviate hot flashes in menopausal woman **(Riaz, 2006)**.

Soy protein supplies all nine essential amino acids and offers many functional benefits to food processors. Modern soy products such as soy flour, concentrates, isolates, and textured soy protein have been used for several decades as functional ingredients by the food industries in the United States and Europe. Natural fermentation process involved in the production of soy products make the process susceptible to microbial contamination from environmental sources and thus renders the process less or unhygienic. This also threatens the safety of this fermented food because of more contamination possibilities from environment containing pathogenic bacteria. Therefore, it is hard to control the quality of soya product when it is processed in an open environment **(Lee et al., 1996)**. Previous studies aiming to explore the microbiota of soy have reported the dominance of lactic acid bacteria (LAB) and species belonging to the genus *Bacillus* **(Chao et al., 2008; Sun et**

al., 2010). Lactic acid bacteria of the tofu play an important role in determining the overall flavor profile of soy food **(Liang et al., 2013; Li et al., 2014)**. The increased amount of decarboxylase positive microorganisms can accumulate production of biogenic amines **(Purevdorj et al., 2017)**.
Starter cultures in fermentation of soya product are *Aspergillus oryzae* and *Saccharomyces cerevisiae* and secondary cultures that are used in soya product are *Pediococcus halophilus*, *P. cerevisiae*, *Zygosaccharomyces rouxii*, *Candida* and *Enterococcus faecalis* **(Shurtleff and Aoyagi, 1976; Farnworth, 2008)**. The addition of selected starter cultures is one of the main tools able to prevent the formation of high levels of BA in fermented meat and dairy products **(Purevdorj et al., 2017)**.

However, an increase in total biogenic amines (BA), as undesirable substances in soy product, may be harmful **(Silla Santos, 1996; EFSA, 2011)**. Excessive intake of biogenic amines from foods can lead to various physiological and toxicological problems in humans such as nausea, sweating, migraine, respiratory distress, hot flushes, bright red rash, oral burning, heart palpitation, and hyperor hypotension **(Karovicova and Kohajdova, 2005; Guan et al., 2013)**. Biogenic amines may have more serious implication as its presence in foods may lead to death in certain cases. In addition, biogenic amines have also been reported to have correlation with the spoilage of food products **(Karovicova and Kohajdova, 2005; Tian et al., 2013; Li et al., 2014)**. However, currently there are no established standards or regulation for tofu or fermented soybean products to limit biogenic amines levels. In addition, biogenic amines can also be synthesized by spontaneous chemical reactions during extended fermentation **(Beneduce et al., 2010; Liu et al., 2011)**.

The analysis of presence of biogenic amines in miso paste by HPLC was realized at the university in Korea. In most of the samples, there were detected low concentration of BA; however some samples contained histamine and tyramine in amounts exceeding levels safe for human health. Variability in BA content can be caused by addition of other materials into miso paste (rice, barley). Contamination during production is mainly caused by bacteria such as *Bacillus subtilis* and *B. amyloliquefaciens*. These bacteria are able to produce BA, especially tyramine and spermine **(Belleme and Belleme, 2011; Byun et al., 2012).**

The aim of this work was to study the typical fermented Asian food (miso paste, soy sauce, rice vinegar, kimchi and tempeh) to monitor their microbial quality and presence of biogenic amines in relation to time and temperature of storage.

MATERIAL AND METHODOLOGY

Isolation and identification of the microorganisms:

Ten grams of the fermented Asian 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 Asian food was then subjected to routine microbiological analysis. The first analyses were executed in May 2017, before the new norm ISO Standard No. 21528−2 (2017) was introduced. The total microorganism counts were assessed according to ISO Standard No. 4833−1 (2013), the *Enterobacteriaceae* bacteria family according to ISO Standard No. 21528−2 (2004), the yeasts and moulds according to ISO Standard No. 6611 (2004) and halotolerant microorganisms (staphylococci) on mannitol salt phenol red agar after cultivation at 37 °C for 2 days according to **Chapman (1945)**. The selected colonies were isolated into BHI broth and cultivated 24 – 48 h at 25 °C (yeasts), 37 °C (*Enterobacteriaceae*, *Staphylococcus*) or 30°C (other microorganisms). Each soya product sample was microbial 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.

Preparation:

Lyophilised soya products were used for the biogenic amines (BA) and polyamines (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 soya product sample. The filtrated extract (filter porosity 0.45 µm) was then used directly for the derivatisation and determination of BA/PA content **(Dadáková et al., 2009; Buňková et al., 2013)** that followed.

Figure 1 Various types of miso paste (top left - shiro miso, top right – mugi miso, lower left - hatcho miso, lower right - genmai miso).

Biogenic amines 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 et al. (2009)**. 1,7−heptandiamine was used as the internal standard. Chromatographic separation (ZORBAX Eclipse XDB−C18, 50 9 3.0 mm, 1.8 µm; 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 soya product 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 contents in foodstuffs is very important. Presently, HPLC based methods are the most suitable for the analysis of fermented foods. The reliability and sensitivity of these methods render them useful as important techniques to determine the concentration of all biogenic amines in fermented food **(EFSA, 2011)**.

Statistical 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

Environment of soya products is an ideal media for the growth and survival of a variety of fungi and bacteria, particularly lactic acid bacteria.

Table 1 Viable counts (log $CFU.g^{-1}$) of the main microbial groups (first day) in the Asian soya food in the Czech republic.

	AGAR log $CFU.g^{-1}$		
Product	PCA	MSA	ENDO
K	–	3.69	–
KIM	6.83	–	2.70
MG	5.40	5.48	–
MH	4.70	–	–
MM	5.40	4.48	3.70
MS	4.70	–	3.70
TM	5.88	–	4.69
TN	3.58	–	7.05
TP	6.94	–	6.46
TS	–	–	3.69

At the beginning of the analysis, the colonies forming units of microorganisms ($CFU.g^{-1}$) were determined in all samples (Table 1): miso genmai (MG), miso mugi (MM), miso shiro (MS), miso hatcho (MH), tamari shoyu (TS), koikuchi shoyu (KS), komesu (K), Tempeh marinated (TM), Tempeh natural (TN), Tempeh party (TP), Usuchi shoyu (US), Shiro shoyu (SS).

Fermentation of soya is a complex process influenced by variety of factors, *e.g.* recipe and nutrient composition, temperature and fermentation time **(Chao et al., 2008; Li et al., 2014)**. The nutrient–rich environment of miso makes it an ideal media for the growth and survival of a variety of fungi and bacteria, particularly lactic acid bacteria.

The highest counts of bacteria in the "Miso shiro" samples were 7.24 log $CFU.g^{-1}$ at 23 °C after 15 days of storage. The amount of coliform bacteria was 6.11 log $CFU.g^{-1}$ under the same storage conditions. The amount of staphylococci was 5.34 log $CFU.g^{-1}$ at 30 °C and after 8 days of storage. After 50 days of storage, the amount of staphylococci did not exceed 3.00 log $CFU.g^{-1}$ at all temperatures (8, 23 and 30 °C). As for overall numbers of lactobacilli in relation to storage time, there is significant decrease from 3.00 log $CFU.g^{-1}$ to 1.69 log $CFU.g^{-1}$ ($p \leq 0.05$) at all storage temperatures. Yeast and moulds were only determined on the 57th day of storage at the temperature lower than 23 °C (2.17 log $CFU.g^{-1}$).

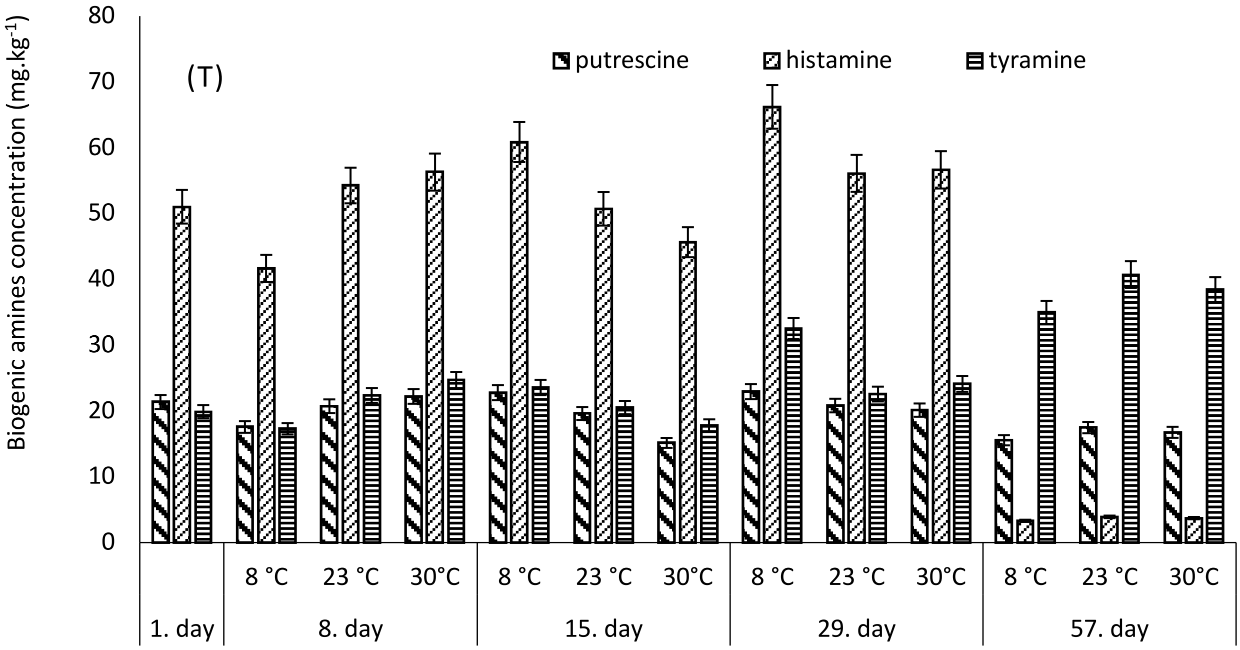
In "Miso mugi", total viable counts were 7.49 log $CFU.g^{-1}$ at 23 and 30 °C and 6.10 log $CFU.g^{-1}$ at 8 °C after 15 days of storage ($p \leq 0.05$). The highest amount of coliform bacteria was measured after 15 days of storage (7.11 log $CFU.g^{-1}$ at temperature ≤23 °C). The highest amount of staphylococci 5.47 log $CFU.g^{-1}$ was determined at 30 °C on the 8th day of storage. The yeast and mould growth on Chloramphenicol Yeast Glucose Agar was maximal at 23 °C on the 15th day of storage (4.84 log $CFU.g^{-1}$). At decreasing or increasing of the storage temperature, the amount of yeast and moulds in miso mugi dropped under 2.10 log $CFU.g^{-1}$ ($p \leq 0.05$). Lactobacilli were not determined in this product at any of the temperatures and observed durations.

As for "Miso shiro", the highest amount of bacteria was 6.13 log $CFU.g^{-1}$ and it was determined at 23 °C after the 15th day of storage. The highest amount of coliform bacteria (5.59 log $CFU.g^{-1}$) and yeast and molds (5.49 log $CFU.g^{-1}$) was identified at temperature 23 °C after 15th day of storage. In contrast, the highest amount of determined staphylococci was 3.78 log $CFU.g^{-1}$ at the same temperature (23 °C) after 57th day of storage. With farther prolongation of storage time, the amount of bacteria and moulds declined logarithmically on all of the selective diagnostic agars ($p \geq 0.05$). "Miso hatcho" had log $CFU.g^{-1}$ identical with bacteria amounts gained from miso shiro.

The maximal amount of living cells in "Miso genmai" was 7.91 log $CFU.g^{-1}$ and the amount of coliform bacteria was 7.11 log $CFU.g^{-1}$; both amounts were determined at 30 °C on the 15th day of storage. The amount of identified bacteria decreased along with decrease of storage temperature ($p \leq 0.05$). The highest value of determined amount of staphylococci was 5.60 log $CFU.g^{-1}$ at 8 °C after 8 days of storage. The highest amount of yeast and moulds was 5.18 log $CFU.g^{-1}$ at 30 °C after 15 days. Lactobacilli were not determined in any of the storage conditions.

In "Koikuchi shoyu", the total viable counts of bacteria were 2.69 log $CFU.g^{-1}$ at 8 °C after the 15th day of storage. Observed under the same conditions, the highest amounts of yeast and moulds were 2.00 log $CFU.g^{-1}$. No staphylococci or lactobacilli were detected in this sample.

"Kimchi" is a traditional product in Korean cuisine. The total viable counts of microorganisms on PCA were 9.13 log $CFU.g^{-1}$ at 30 °C on the 2nd day of storage. The highest values for lactobacilli were 8.34 log $CFU.g^{-1}$ after 4 days. The amounts of coliforms were the highest at 30 °C after the 4th day (4.77 log $CFU.g^{-1}$) and the highest amounts of styphylococci (3.93 log $CFU.g^{-1}$) were determined under the same conditions. he values of yeast and moulds were highest after the 5th day of storage – 3.00 log $CFU.g^{-1}$.



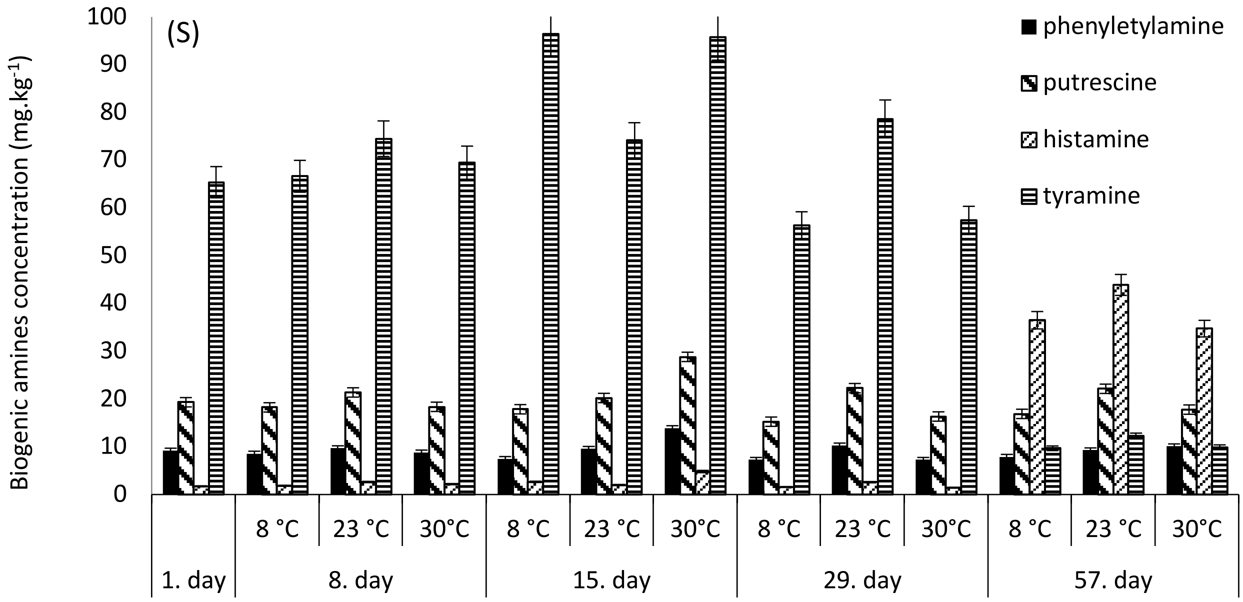


Figure 2 Biogenic amines content (histamine, tyramine, phenylethylamine, putrescine in Shoyu$^{(S)}$ and Tamari$^{(T)}$ samples (mg.kg^{-1}).

"Tempeh marinated" contained 9.00 log CFU.g^{-1} which was the highest amount of bacteria and it was determined at 30 °C after 4 days of cultivation. There were also high amounts of lactobacilli cells (8.89 log CFU.g^{-1}) in this product at 8 °C on the 5th day of storage. The highest amounts of coliform bacteria (8.00 log CFU.g^{-1}) and staphylococci (7.85 log CFU.g^{-1}) were measured at 30 °C on the 4th day of storage. As for yeast and moulds, the highest values reached 6.81 log CFU.g^{-1} and were determined at 23 °C on the 2nd day of storage. In "Tempeh natural", the highest amounts of bacteria, coliform microorganisms, yeast and moulds ranged from 9.28 to 9.48 log CFU.g^{-1} at 30 °C after the 5th day of storage. The maximal amount of lactobacilli (9.07 log CFU.g^{-1}) was determined at 8 °C after the 5th day of storage. Staphylococci values were the highest at 30 °C on the 4th day of storage (7.27 log CFU.g^{-1}). In "Tempeh party", the highest amounts of bacteria were determined at 30 °C after 4 days of storage (9.26 log CFU.g^{-1}). This product contained high amount of lactobacilli (9.12 log CFU.g^{-1}) at the storage temperature 23 °C on the 5th day. The highest amounts of coliforms (7.74 log CFU.g^{-1}) and staphylococci (7.44 log CFU.g^{-1}) were measured at 30 °C after 4 days. The highest amounts of yeast and moulds were 7.14 log CFU.g^{-1} at the temperature 23 °C on the 4th day.

"Tamari and Shoyu" contained the lowest amount of yeast and moulds (1.69 log CFU.g^{-1} at 15 °C after the 15th day of cultivation) out of all observed soy sauces.

As for "Komesu", there were identified these highest amounts: 2.00 log CFU.g^{-1} for mesophilic facultatively anaerobic bacteria and 2.30 log CFU.g^{-1} for yeast and moulds after 15 days of cultivation. There was no growth of staphylococci, lactobacilli and coliforms on selective diagnostic media.

Kim et al. (2012) reported that the aerobic plate counts of the samples varied from 3.00 to 7.59 log CFU.g^{-1}.

In the fermented foods, there is a possibility of biogenic amines presence because the number of decarboxylase positive microorganisms increases during ripening or storage **(Pleva et al., 2014)**.

Non starter isolates were chosen and identified from all of the determined numbers of bacteria and yeast by using matrix assisted laser desorption/ionization time–of–flight (MALDI): *Acinetobacter lwoffii, Aerococcus viridans, Arthrobacter oxydans, A. polychromogenes, A. scleromae, Bacillus cereus, Citrobacter koseri, Dermacoccus nishinomiyaensis, Enterobacter asburiae, E. cloacae, E. hormaechei* ssp. *hormaechei, E. kobei, Klebsiella oxytoca, Kosakonia cowanii, Micrococcus luteus, Ochrobactrum anthropi, Ochrobactrum* sp.*, Ochrobactrum tritici, Paenibacillus humicus, Pantoea agglomerans, Staphylococcus warneri.*

The results of executed microbiological analysis can be summarized as follows: the highest numbers of mesophilic facultative anaerobic microorganisms, enterobacteria, moulds and yeast in miso paste were observed particularly at storage temperature 30 °C. Compared to the other types of miso paste, miso genmai contained the highest number of mesophilic facultative anaerobic microorganisms ($p \leq 0.05$), whereas miso mugi contained the highest amount of enterobacteria ($p \geq 0.05$) and the highest amount of yeast and moulds was detected in miso shiro ($p \geq 0.05$). In kimchi, the highest amount of lactobacilli was determined at storage temperature 30 °C. In soy sauce, there was only a slight increase of facultative anaerobic microorganisms, yeast and moulds observed ($p \geq 0.05$), while tamari shoyu showed greater resistance against the growth of these microorganisms. The growth of facultative anaerobic microorganisms, yeast and moulds was noticed in rice vinegar komesu but only in a very small amount. Microorganisms can exhibit the ability to produce or degrade biogenic amines *in vitro* and they could be used as microbiological indicators to prevent BAs accumulation in food **(Butor et al., 2017)**.

Biogenic amine analysis

The results of chromatographic analysis of biogenic amines can be summarized as follows: in miso paste, only tyramine was determined (0.048 – 1.765 mg.kg^{-1}); other biogenic amines were not observed. However, **Yamamoto et al. (1980)** reported that miso products contained 21.0 – 169.5 mg.kg^{-1} of tyramine. **Shalaby (1996)** reported that fermented soybean products (miso) contained high levels of histamine (46.2 mg.kg^{-1}), putrescine (123.4 mg.kg^{-1}), cadaverine (63.4 mg.kg^{-1}), and tyramine (356.8 mg.kg^{-1}). Variations in the contents of biogenic amines in these commercial miso products could be attributed to variability in the ratio of soybean to other seeds used, the microbiological composition, and the conditions and duration of fermentation **(Chin et al., 1983; Nout et al., 1993)**. **Kung et al. (2007)** reported that 27.0 ±43.5 mg.kg^{-1} of tryptamine, 1.2 ±3.3 mg.kg^{-1} of putrescine, 31.6 ±45.3 mg.kg^{-1} of cadaverine, 16.4 ±40.4 mg.kg^{-1} of histamine, 12.1 ±4.8 mg.kg^{-1} of tyramine, 8.0 ±31.6 mg.kg^{-1} of spermine were detected in Miso products sold in supermakets, whereas 49.0 ±52.0 mg.kg^{-1} of tryptamine, 1.1 ±2.8 mg.kg^{-1} of putrescine, 9.1 ±2.8 mg.kg^{-1} of cadaverine, 7.7 ±26.5 mg.kg^{-1} of histamine, 15.8 ±10.2 mg.kg^{-1} of tyramine, 7.2 ±25.8 mg.kg^{-1} of spermine were detected in Miso products sold in retail markets in Taiwan. **Shukla et al. (2011)** reported that 0.2 ±0.4 mg.kg^{-1} of tryptamine, 7.7 ±14.8 mg.kg^{-1} of β–phenylethylamine, 4.8 ±5.5 mg.kg^{-1} of putrescine, 1.1 ±3.1 mg.kg^{-1} of cadaverine, 16.7 ±18.9 mg.kg^{-1} of tyramine, 4.5 ±12.6 mg.kg^{-1} of spermidine were detected in Miso products sold in supermarkets in Japan.

The analysed amounts of biogenic amines (putrescine, cadaverine, histamine, tyramine and spermidine) range from 0.058 to 3.048 mg.kg^{-1} in selected tested types of tempeh. In kimchi, there were detected only very low volumes of tyramine and spermidine, with their amounts ranging from 0.161 to 0.531 mg.kg^{-1}. In tamari **(Figure 2)**, the presence of putrescine was recorded in range 15.156 – 22.922 mg.kg^{-1}, 17.287 – 40.710 mg.kg^{-1} for tyramine, 1.293 – 7.538 mg.kg^{-1} for phenylethylamine, 0.701 – 1.875 mg.kg^{-1} for cadaverine and 3.306 – 66.208 mg.kg^{-1} for histamine, which also reached the highest values. **Toro–Funees et al. (2015)** described the amount of biogenic amines in Tempeh. Tempeh showed the highest contents of spermidine and spermine with 124 and 21 mg.kg^{-1}, respectively. These results are consistent with the ones reported by **Nishimura et al. (2006)** who found similar concentrations of these polyamines in tempeh and natto samples, but lower than the levels of 250 – 475 mg.kg^{-1} of polyamines reported by **Kim et al. (2012)** in natto.

The **Figure 2** indicates obvious trend of increasing the histamine value on the 57th day of storage ($p \leq 0.05$). Production this high can be caused not only by mentioned technology of processing the soy product but also by decarboxylase activity of microorganisms. However, these histamine concentrations should not endanger human health normally. In koikuchi shoyu, there was determined phenylethylamine, putrescine, histamine, tryptamine, cadaverine in amounts ≤3.00 mg.kg^{-1}; however, tyramine was detected in range from 9.686 to 96.503 mg.kg^{-1}. The low values of putrescine and tyramine were noticed in komesu, ranging in values 0.987 – 4.858 mg.kg^{-1}. **Pachlová et al. (2017)** reported that the content of biogenic amines (such as tyramine, putrescine, histamine, and phenylethylamine) was monitored during storage of cheese. The results showed an increasement in biogenic amine concetration depending on the time of ripening in all batches of model samples.

CONCLUSION

In the first part, this research focused on fermentation process, problematics of biogenic amines in typical Asian fermented products. The best–known and the most sold fermented products (miso paste, soy sauce, tamari shoyu, koikuchi shoyu, rice vinegar, tempeh and kimchi) were chosen for testing. The products underwent microbial analysis with goal to find out numbers of observed indicator groups of microorganisms (facultative anaerobic mesophillic microorganisms, enterobacteria, staphylococci, yeast, moulds and lactic acid bacteria). The studied samples were kept at various storage temperatures for a certain period of time, with intention to simulate conditions of environment where those products are stored in Asian countries. Lastly, the products were analysed using high-performance liquid chromatography to evaluate amounts of biogenic amines and to identify them. The most significant concentration of biogenic amines was determined in koikuchi shoyu (96.50 mg.kg^{-1} of tyramine) and in tamari (66.21 mg.kg^{-1} of histamine).

The achieved results of this research show that there are various representations of chosen indicator groups of microorganisms in analysed Asian fermented foods stored at temperatures 8 °C, 23 °C and 30 °C. Different numbers of colonies of observed microorganism groups and biogenic amines were probably caused by used ingredients and technological process of production. It is important to pay attention to contents of individual types of foods and to their microflora, especially in relation to their storage, and thus ensure their longevity and health harmlessness.

Modern soy products and their proteins provide all nine essential amino acids and offer many functional health benefits. In addition to an assortment of vitamins and minerals, like all plant foods, the soybean contains numerous biologically active nonnutritive substances. However it needs be emphasized that prolongation of the soy products storage increases the risk of increase of microorganism amounts or cumulation of biogenic amines respectively.

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Acknowledgments:

This work was supported from the Internal Grant of Tomas Bata University in Zlín (No. IGA/FT/2018/009) and the Grant Agency of the Czech Republic (GAČR No. 17−09594S).

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