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Effect of *Zataria multiflora* Essential Oil on Histamine Production in Iranian Salted-Fermented Fish Sauce (Mahyaveh)

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Abstract

Background: Mahyaveh is an Iranian salted-fermented fish sauce which due to its high amount of protein has risk of histamine production. This study was carried out to determine effect of *Zataria multiflora* Essential Oil (EO) on histamine production in mahyaveh.

Methods: Dried anchovies (*Stolephorus* sp.), refined-salt and mustard seed (*Brassica juncea*) were purchased from the local market in Bandar Abbas, Iran. Three concentrations of EO including 0.1, 1, and 2% v/w were prepared by hydro-distillation of the air-dried powdered of *Z. multiflora* plant for 3 h, using British-type Clevenger apparatus. Histamine was determined by Enzyme Linked Immunosorbent Assay (ELISA). Mean values of histamine were compared using SPSS, Inc, Chicago, IL software (v. 16.0).

Results: Most of the samples showed increasing in the level of histamine when storage time was increased. At day 30, histamine level in all treatment samples containing 0.1, 1, and 2% of *Z. multiflora* EO were significantly lower than control group ($p<0.05$). However, in days 90 and 120, histamine level in all treatment groups had no significant difference ($p>0.05$) with control ones except 0.1% EO group. Analysis of four sensory items including color, odor, taste, and overall acceptance indicated that there was no significant difference between mean score of control and treatment groups. Overall acceptance scores in 0, 0.1, 1, and 2% EO were 6.33, 5, 6.33, and 5, respectively.

Conclusion: *Z. multiflora* EO could effectively serve as potential antimicrobial agent to inhibit histamine production in mahyaveh.

Introduction

In many Southeast Asian countries and other area that can easily access to the sea foods, protein sources are mainly provided by the consumption of fresh sea foods or fermented fish products. Fish sauce is a dark suspension of fish in water with high level of salt as well as the other

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additives that may be different based on the consumer preferences. This sauce is allowed to be conserved under sunlight in various times (Jiang et al., 2007; Jiang et al., 2014; Kuda and Miyawaki, 2010). Mahyaveh is one of the most widely consumed fermented products in Hormozgan province and some regions of Fars province, southern Iran, with the various production methods (Moradizadeh et al., 2011; Zarei et al., 2012). This product is typically composed of sardines (*Sardinella* sp.) or anchovies (*Stolephorus* sp.), salt, mustard (*Brassica juncea*), as well as water. Various spices such as cumin, coriander, fennel seeds, black pepper, and thyme can be added to this product. Hormozgan is a tropical province of Iran with hot and humid climate in which native people tend to consume spicy and chilli foods (Zarei et al., 2012).

Due to the high protein contents in fish, and on the other hand protein degradation by several enzymatic and microbial reactions would be done during fermentation, these products can be a potential source for biogenic amines (Jiang et al., 2014; Mah et al., 2002). Biogenic amines are some components based on nitrogenous substances that are present in meat, fish, and cheese products as well as in fermented foods (Fathi et al., 2014; Marcobal et al., 2005; Prester, 2011). Histamine (HM) is one of the main biogenic amines produced by histidine decarboxylation activity of several types of bacteria. This toxic compound is a major chemical hazard of sea food products that may lead to HM poisoning in consumers. The symptoms of HM poisoning include nausea, respiratory distress, hot flushes, hypotension, etc. in sensitive individuals (Lehane and Olley, 2000; Silva et al., 2011; Zarei et al., 2014). A hazardous level of HM for human health has been established as 500 mg/kg by Food and Drug Administration (FDA) (Mah et al., 2009; Tapingkae et al., 2010).

Several studies have been carried out on antimicrobial activities of plant Essential Oils (EOs) to improve food safety and quality (Ahmadi et al., 2014; Ercan et al, 2013; Moradi et al., 2014). *Zataria multiflora* (Persian thyme or Avishan-e Shirazi) is one of these spices that has been reported to possess antimicrobial activities against some microorganisms. It is a native Iranian plant, belonging to Labiatae family which its antibacterial effect has previously been proved (Akhondzadeh et al., 2007; Alipour- Eskandaniet al., 2009; Burt, 2004; Fazeli et al., 2007; Kordsardouei et al., 2013; Oussalah et al., 2006; Sharififar et al., 2007).

Considering high protein content as well as storage temperature of mahyaveh, HM production is too probable endangering food safety (Zarei et al., 2012). On the other hand, HM poisoning can represent as a serious health risk for sensitive individuals such as children, elderly, or sick people. Since mahyaveh is a traditional souvenir of this area in Iran, high HM level as a hazard is noticeable in non-native and tourist individuals that are not accustomed to consumption such high level of HM. Thus, this investigation was carried out to determine the effect of *Z. multiflora* EO on HM production in mahyaveh during different storage times.

Materials and methods

Raw materials

Dried anchovies (*Stelophorus* sp.), refined-salt, and mustard seed (*B. juncea*) were purchased from local markets in Bandar Abbas, Iran. Bandar Abbas is center of Hormozgan province of Iran that is located in south of the country and bordered by the Persian Gulf.

Preparation of Z. multiflora EO

The leaves of *Z. multiflora*, cultivated near Jiroft, Iran were collected in June 2015. *Z. multiflora* identity was confirmed by Herbarium Department from Faculty of Agriculture in Jiroft, Iran. The air-dried and ground herbal parts of the collected plant were submitted for 3 h to water-distillation using a British-type Clevenger apparatus (yield 4% v/w). After that, EO was dried under anhydrous sodium sulphate and then was kept at 4 $^{\circ}$C until next analysis steps (Sharififar et al., 2007).

Production of fish sauce

Preparation of mahyaveh samples was carried out based on Zarei et al. (2012) with small modifications. As shown in Fig. 1, first, 1600 g dried fish was weighed and divided into eight 200 g portions. Then, they washed thoroughly and put in a colander. Next, the anchovies were put in a plastic container and each one covered with 100 g salt and 500 ml boiling water, then the mixture was stirred to dissolve the salt. All experiments were carried out in triplicate included 0 (control), 0.1, 1, and 2% EO of *Z. multiflora*. For ripening, the containers were capped, kept under the sun for 30 days and mixed with spoon every two days of the first month. Then, the mixture was sieved and drained. After that, the brown liquid portion of fermented fish was mixed with scorched mustard powder. The final ripened products illustrated in Fig. 2, were again kept under sunlight until HM analysis at days 30, 60, 90, and 120.

HM analysis

After full shaking of the containers, each sample was taken into a plastic falcon tube. It was then tightly sealed with parafilm. They were immediately transported to the laboratory, kept at -80 freezer until analysis time.

Quantitative analysis of HM in the samples was performed by Enzyme Linked Immunosorbent Assay (ELISA) using HM detection kit (Neogen Corporation, USA). For preparation, the samples were thawed at room temperature and were shaken until homogenous. Then 2.5 g of the homogenous mixture was added to a clean falcon tube and distilled water was added to reach at final volume of 25 ml. Each sample was centrifuged at 5500 g under room temperature for 4 min to obtain clear supernatant. Sample extraction diluent buffer was prepared by adding a foil pouch of extract buffer concentrate of 10 mM PBS-tween to 1 L distilled water. Five ml prepared diluent buffer was added to 10 µl of fish sauce extract in a clean falcon tube. ELISA test procedure conducted according the manufacturers' instruction. Briefly, 100 µl conjugate was added to 100 µl of each control and diluted sample and mixed. After that, each 100 µl mixture was transferred to each antibody well and incubated for 10 min. In the next step, each well was thoroughly washed using diluted washing buffer. After that, 100 µl substrate was added to each well, incubated again for 10 min and then 100 µl red stop solution was transferred to each antibody well. Results were read by a microwell reader (BiotekElx 808) using a 630 nm filter.

Sensory evaluation

At 10th week storage of the mahyaveh samples, sensory analysis was carried out by 10 consumer trained panelists. Four different descriptions were employed to determine sensory properties including odor, color, taste, and overall acceptance using Visual Analogue Scales (VAS, 0-100 mm). This scoring method consisted of a 100 mm straight line anchored at the endpoints with minimal and maximal acceptance. The panelists were asked to give a judgment through insert a mark on the line for each sensory item. The final VAS scores were calculated through measuring in millimeter from the left edge end of the line to the marked point.

Statistical analysis

Statistical analysis was carried out based on normal confidence intervals and analysis of variance (one-way ANOVA) using SPSS, Inc, Chicago, IL software (v.16.0). The levels were considered significantly different at $p<0.05$.

Results

As shown in Table 1, most of the samples showed increasing in the level of the HM when storage time was increased. At the day 30, the HM level in all treatment samples containing 0.1, 1, and 2% of *Z. multiflora* EO were significantly lower than the control group ($p<0.05$). However, in days 90 and 120, HM level in all treatment groups had no significant difference ($p>0.05$) with the control ones except 0.1% EO group.

Analysis of four sensory items including color, odor, taste, and overall acceptance showed that there was no significant difference ($p>0.05$) between mean score of control and treatment groups (Fig. 3). Overall acceptance scores in 0, 0.1, 1, and 2% *Z. multiflora* EO were 6.33, 5, 6.33 and 5, respectively.

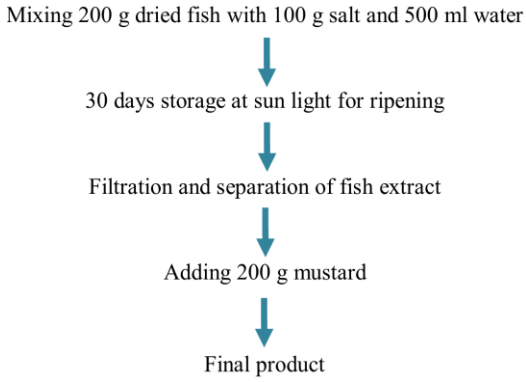

Fig. 1: Flow diagram of mahyaveh processing

Fig. 2: Final product sample of mahyaveh produced in this study

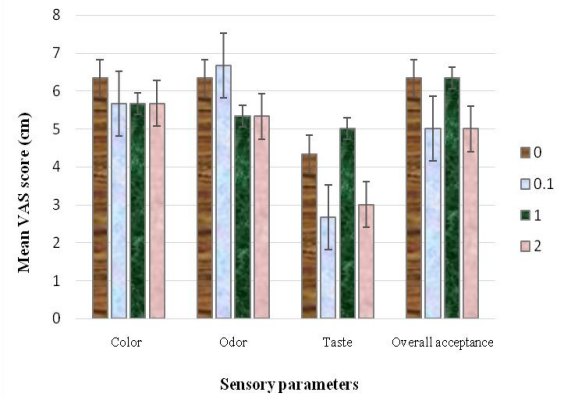

Fig. 3: Sensory parameters of mahyaveh samples contained various levels of *Z. multiflora* EO (0, 0.1, 1, as well as 2%) based on visual analogue scale

Table 1: Mean±standard deviation HM level (mg/kg) in control and treated groups contained *Z. multiflora* stored at different period of times

EO concentration (%)	30 days	60 days	90 days	120 days
0 (control)	16339.00±383.959 [a*]	14102.57±326.760 [a]	17030.85±993.819 [a]	17598.95±744.644 [a]
0.1	8705.15±833.311 [b]	5972.10±186.033 [b]	7764.12±254.979 [b]	9150.95±237.366 [b]
1	6542.90±507.119 [c]	7056.23±923.881 [b]	14721.30±365.578 [a]	22734.60±267.582 [a]
2	7127.95±127.950 [c]	14095.40±348.788 [a]	17381.02±279.729 [a]	19598.83±435.799 [a]

[*]Dissimilar letters in same column indicate significant diffrences

Discussion

In the present study, we found considerable and significant inhibitory effect of 0.1, 1, and 2% *Z. multiflora* EO on HM production level in mahyaveh as a popular fish sauce in some parts of Iran. In previous researches done on different types of foods, antimicrobial effect of Iranian *Z. multiflora* EO has been shown on some pathogens such as *Bacillus cereus* ATCC 11778 in barley soup (Alipour-Eskandani et al., 2009), *Staphylococcus aureus* in commercial soup (Akhondzadeh et al., 2007), and also *Escherichia coli* O157: H7 in minced beef (Noori et al., 2012). In this work, the reduction of HM level was observed in all treatment groups containing 0.1, 1, and 2% EO of *Z. multiflora* at 30th day. In a similar study, Moradizadeh et al. (2011) found that the content of Total Volatile basic Nitrogen (TVN) was significantly lower in mahyaveh samples contained garlic ingredient comparing to control group. Mah et al. (2009) showed that production of biogenic amine was reduced in media culture contained garlic extract. In the other researches, inhibitory effects of clove (Shakila et al., 1996) as well as, nuka, a main by-product of rice polishing (Kuda and Miyawaki, 2010) on HM producing were reported. Considering the previous similar published investigations which are in agreement with our findings, the reduction of HM level in mahyaveh by adding *Z. multiflora* EO observed in the present study, could be attributed to antibacterial function of this EO that is a native herb in southern Iran (Fazeli et al., 2007; Sharififar et al., 2007).

Based on Table 1, higher HM level was found in most treatment groups when storage time was increased. Similar finding was reported by Rabie et al. (2009) who revealed that free amino acid and biogenic amine content of an Egyptian fish sauce (Feseekh) was increased during fermentation and storage. Also, Jiang et al. (2007) showed that there was an increscent of bacterial counts till day 120 of fermentation. This phenomenon could be related to this fact that when fish fermentation period progresses, degradation increases and more amino acids, as precursor for HM production would be available for microorganisms. Until amino acid exists, production of HM would be continued (Ercan et al., 2013; Shukla et al., 2014). On the other hand, it could be proposed that in the early stage of fish fermentation production of HM was delayed because of EO components. But due to oxidative changes and deterioration reactions occurred during long fermentation, antibacterial effect of EO decreased resulted in higher HM accumulation (Cevallos et al., 2010). This issue highlighted the importance of using more preservative approaches (e.g. refrigeration storage of fish sauce) to reduce microorganisms activities probability and so degree of HM production.

The results of this study showed that overall acceptance scores in control and treatment groups were almost equal to some previous studies. Similar study by Moradizadeh et al. (2011) was reported that usage of garlic extract improved sensory properties of mahyaveh. Kordsardouei et al. (2013) stated that 0.05, 0.1, and 0.15% concentrations of *Z. multiflora*. EOs had not adverse effects on sensory properties of cakes. In another study, Noori et al. (2012) found no undesirable sensory properties in minced beef treated by *Z. multiflora* Boiss EO.

Conclusion

Our results indicated that minimum level of 0.1% *Z. multiflora* EO was sufficient to reduce HM level in mahyaveh and could be an effective additive in order to reduce HM risk in this product. But, more studies should be carried out to detect effects of some other processing parameters such as temperature, raw materials, etc. that may be affect HM accumulation in the fermented fish sauce.

Conflicts of interest

There are no conflicts of interest in this study.

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