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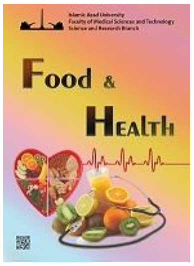

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Feasibility study of reducing histamine and heavy metal in Iranian fermented fish sauce by adding Mahdavi wheat bran

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ABSTRACT

Mahyaveh is a traditional fish sauce that is produced in the southern part of Iran and main problem related to this product is the very high histamine content. This study investigated the chemical properties of mahyaveh, in the presence of wheat bran. The effects of Mahdavi wheat bran investigated on the reduction of histamine in mahyaveh, as "Southern Iran Fermented FishSauce". Fish sauce samples used in this study consisted of control samples and the treated samples containing 10% and 20% of wheat bran. The histamine and heavy metals concentrations in the mahyaveh samples were determined by an enzymatic assay kit and graphite furnace atomic absorption, respectively. The pH and water activity of fish sauce samples decreased during the 45-day fermentation period (2.07% and 8.13%). While the amount of TVB-N experienced a meaningful increase in the presence of wheat bran (4.95%), the protein content significantly decreased ($p<0.05$) (3.83%). The iron and mercury concentration range of samples (1.3-6.2 mg/kg and 0.2-0.5 mg/kg) were below maximum permissible limits of WHO/FAO (43 mg/kg and 0.6 mg/kg) but cadmium concentration range of samples (0.6-2 mg/kg) and histamine concentration range of samples (832.21-950.40) were above maximum permissible limits of WHO/FAO (0.2 mg/kg) and FDA maximum permissible limits of (50 mg/kg), respectively. The high levels of histamine in mahyaveh samples decreased significantly ($p<0.05$) by adding wheat bran (13.3%). Moreover, wheat bran had a considerable reductive effect on the concentrations of heavy metals naming iron (Fe), cadmium (Cd), and mercury (Hg) (77.96%- 66.66% and 50%, respectively). Consequently, the major safety problem related to mahyaveh consumption is the very high histamine content and heavy metals contamination.

1. Introduction

Fermented fish sauce is a clear brown liquid with a salty taste and a mild fish flavor, and is mainly used as a seasoning (1,2). Different types of fish sauce are produced and consumed all around the world, namely, garum in Rome, botargue and ootarides in Italy and southern Greece, garos and aimeteon in Greece, (3), nample in Thailand, koami and ounago in Japan, patis in Philippines (4) and mahyaveh in Iran (5, 4). Mahyaweh is a fermented fish sauce that is prepared on the southern coast of Iran using Sardines (*Sardinella sp.*) or Anchovies (*Stelophorus sp.*), salt, *Eruca sativa*, and water. It is usually prepared by mixing dried fish with salt and warm water. The mixture is then fermented in clay pots exposed to sunlight at room temperature for 30 days. Then various spices are added to the mixture and the fermentation process continues for 15 days to create the proper taste and aroma in the product (5). Although fish sauces are rich sources of nutrients, they may also contain anti-nutritional compounds such as biogenic amines and heavy metals (1, 6, 7). From a toxicological and immunological point of view, histamine is the most important biogenic amine and the cause of scombroid fish poisoning and seafood intolerance (8, 9, 10, 11). Releasing excessive amounts of histamine was reported in mahyaveh samples collected from the southern coastal regions of Iran (5). In addition to histamine, fish and seafood are exposed to heavy metal contamination (12). Fish is the main ingredient of fish sauce so mahyaveh can be contaminated with heavy metals (13, 14). In prior studies, some treatments have been applied for histamine and heavy metals reduction in fermented fish

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sauce (1, 6, 15). To the best of our knowledge, no study has been carried out to reduce histamine and heavy metals by adding wheat bran to fermented fish sauce. Wheat bran is a low cost and valuable source of dietary fiber, minerals, vitamins, and bioactive compounds such as phenolic acids, arabinoxylans, alkylresorcinol, and phytosterols which has been suggested to prevention cardiovascular diseases and colon cancer . Wheat Bran can be used as a functional ingredient for production of baked cereal products, beverages and condiments (16). This study is aimed at exploring the effects of wheat bran on chemical properties of mahyaveh and screening the potential of histamine and heavy metals-reduction to improve the safety and quality of fermented fish sauce.

2. Material and methods

2.1. Material

Raw materials used in this study were Sardin fish (*Sardinella sindensis*), coriander, fennel, cumin, barley as well as Mahdavi wheat bran (86.3% of the sample remained in the opening sieves 250 mm), and coarse sea salt obtained from Hormozgan, histamine assay kit 'Check color Histamine', L-histidine hydrochloride monohydrate and histamine dihydro-chloride were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Sulfuric acid, sodium hydroxide, hydrochloric acid, boric acid, and nitric acid were supplied by Merck (Darmstadt, Germany).

2.2. Methods

2.2.1.Mahyaveh production

The following diagram shows the production steps of Mahyaveh (Fig. 1). According to the local recipes, dried sardines are washed and ground (500g) and heated at 80°C for 15 minutes. After placing heated fish in a clay pot, 1.5 liters of water and 500 grams of salt are added. The container is then placed at room temperature for 30 days to ferment, then is filtered and the spices including coriander (100g), mustard (100g), barley (150g), fennel (100g), and cumin (100g) are added. Then the clay pot is put in the ambient temperature for 15 days to complete the fermentation process. Similarly, treated samples are provided with 10 and 20 percent of wheat bran.

2.2.2.Determination of pH, moisture percentage, aw, and salt content

The pH of Mahyaveh samples was measured using a standard pH meter (Mettler, Switzerland) by placing the probe into each of the sample preparation. For moisture analysis fish sauce (approximately 3g) was dried using a hot-air oven (Memmert, Germany) at 105 °C until a constant weight was achieved (17). Water activity (a_w) values of fish sauce were measured by using a Novasina a_w meter. The tests were performed on the samples with three replications. The salt content in the samples was measured by titration according to the modified Volhard method and AOAC 935.47 (18).

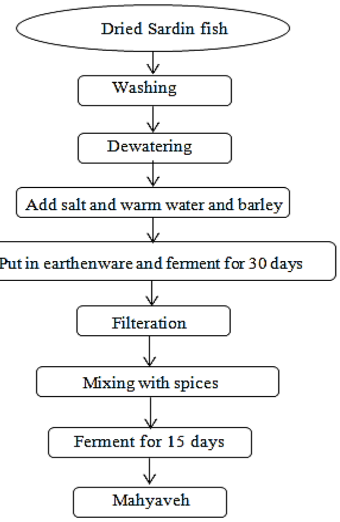

Fig 1. Flowchart of Mahyaveh sauce production

2.2.3.Determination of total volatile basic nitrogen and Crude protein

Crude protein was determined by the Kjeldahl method using a 6.25 Kjeldahl conversion factor (19). The total volatile basic nitrogen (TVB-N) was determined through direct distillation into boric acid using a Kjeldahl-type distillatory. The ammonium borate was titrated with 0.1 N of H_2SO_4 solution (20).

2.2.4.Histamine determination

The histamine concentration in the mahyaveh was determined using an enzymatic assay kit (Check Color Histamine, Kikkoman Co., Noda, Japan), through histamine dehydrogenase. 1.5–5 µl from each sample was put into 96 well microplates, and then up to 0.15 ml with distilled water (DW). Additionally, 0.025 ml of buffer solution and 0.025 ml of coloring reagent containing a tetrazolium salt (WST-8) and an electron donor (1-methoxy PMS) was added, then 0.025 ml of the histamine-dehydrogenase solution was added and incubated at 37 °C for 15 min. Finally, absorbance was measured at 490 nm before and after the enzymatic reaction (21).

2.2.5.Heavy metals determination

To determine the heavy metals, each sample was homogenized using a food blender. Centrifuging of mixtures was performed at 8000 X g for 10 min to get treated fish sauces. The supernatant of each sample was mixed with conc HNO3 (10 ml) in a 200 ml beaker. The beaker was covered with a watch glass and remained overnight to dissolve its.

HNO_3 (10 ml) in a 200 ml beaker. The beaker was covered with a watch glass and remained overnight to dissolve its contents. Then the beaker was heated on a hot plate at boiling temperature to obtain a clear solution. The solution was then cooled and transferred to a 50 ml volumetric balloon and diluted with distilled water. Heavy metals were determined using graphite furnace Atomic Absorption, (model-analyst AA400, PerkinElmer) (6, 12).

2.2.6.Statistical analysis

Experiments were performed in triplicates, and the significant differences among the means were analyzed using One way- ANOVA and LSD post hoc test at $p<0.05$ (SPSS, version 22, 2016).

3. Results and discussion

3.1. Changes in pH of mahyaveh

The pH value of mahyaveh is a very critical factor in the quality determination. The pH of mahyaveh samples was in the range of 6.05-6.26 and there was a significant difference in the pH value of different mahyaveh samples ($p<0.05$) (Fig. 2). The same as this trend, Kilinc et al. (22) reported the range of 4.98-6.38 for pH values of salt-fermented fish sauces.

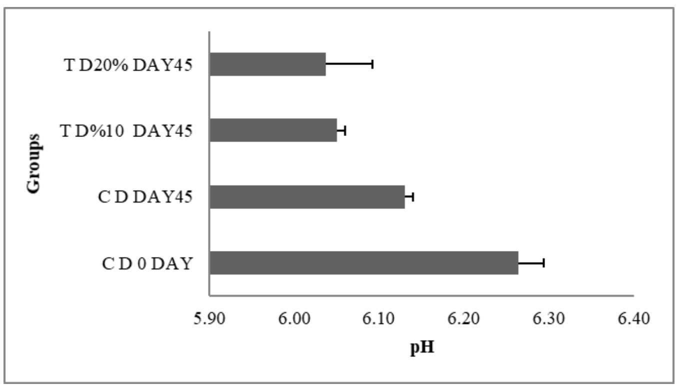

Fig. 2. pH value changes in mahyaveh samples (TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation), Superscripts in each column with different letters are significantly different ($p<0.05$).

Fig. 2 shows after 45 days of fermentation the pH values of control samples decreased compared with the control on the first day. Also, a downward trend was observed in the treated samples on the 45th day in the presence of wheat bran as compared with the control on the 45th day. This pH reduction in treated samples (from 6.13 and 6.05 to 6.04) depends on the wheat bran percentage, higher percentage renders a higher decrease in pH (1.30%-1.46%). The presence of higher carbohydrate compounds in treated samples that contain wheat bran may increase lactic acid production during fermentation by microorganisms and reduce the pH values of these products (22). Contrary to our results, Mueda (23) reported an increase in pH value during fermentation. It seems that the decomposition of raw fish during storage increased the pH value from neutral to basic pH in their study.

3.2. Evaluate aw and salt content

The moisture content of fermented fish sauce is a key factor for prime quality and strongly affects the durability of food products (24). The changes of water activity (a_w) of the mahyaveh samples during fermentation can be seen in Fig. 3.

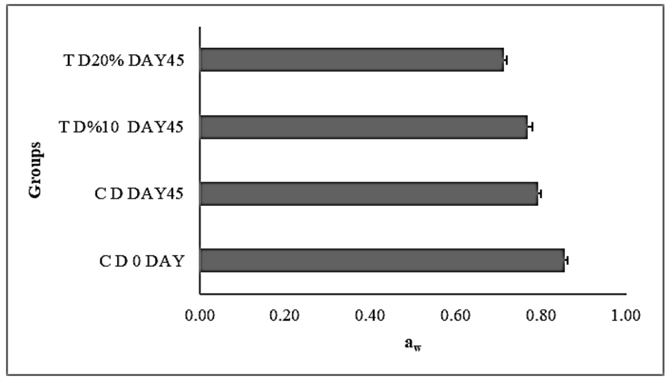

Fig. 3. Aw value changes in mahyaveh samples (TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation), Superscripts in each column with different letters are significantly different ($p<0.05$).

Mahyaveh samples water activity (a_w) was in the range of 0.71-0.86. Fig. 3 shows a significant ($p<0.05$) decrease of a_w in all samples during fermentation (8.13%), especially the treated samples (2.53%-10.12%). This reduction may be related to the water interaction in degradation and the hydrolysis of protein and carbohydrate during ripening and lactic acid bacteria's fermentation (25). The reduction observed in a_w of treated samples depends on the amount of wheat bran. Higher percentages of wheat bran render a higher decrease in a_w. Wheat bran is a concentrated source of insoluble fiber that binds with water molecules and decreases the accessibility of water and a_w (26). Inconsistent with the present study, Kilinc et al. (22) claimed that a_w of Sardine fish sauce had a slight decrease in the values during fermentation. However, these observations disagree with the findings of Dagadkhair et al. (24) who reported that the moisture content of Indian spiced fish sauces gradually increased during 60 days of fermentation. Salt is an important ingredient in the fish sauce because it helps fermentation to progress and prevents the growth of bacteria that cause spoilage (27). The mean NaCl concentrations in tested samples were in the range of 7.12% to 7.18% (Fig. 4). In line with our results, Zarei et al. (5) reported that mahyaveh samples originated from five different locations in the Southern part of Iran have 7.48-17.1% NaCl. Our results revealed no significant difference in the salt content between different mahyaveh samples (p>0.05). Similar to our study, Mueda (23) revealed no significant change in the salt content of fish sauce during fermentation (p>0.05) but the values were higher than our results within the range of 18.51-19.74%. Contrary to our results, Puat et al. (27) pointed out that Indonesian fish sauces contain 26.66% to 27% NaCl.

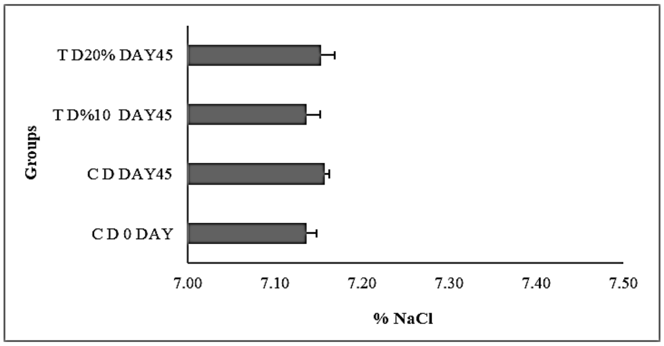

Fig. 4. NaCl concentrations in mahyaveh samples (TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation), Superscripts in each column with same letters are not significantly different (p>0.05).

3.3. Total volatile basic nitrogen and Crude protein

The total protein content of mahyaveh samples during fermentation can be seen in Fig. 5. The protein contents of mahyaveh samples were in the range of 10.53±0.31% to 11.24±0.24% which significantly decreased during fermentation (p<0.05) (2.58%).

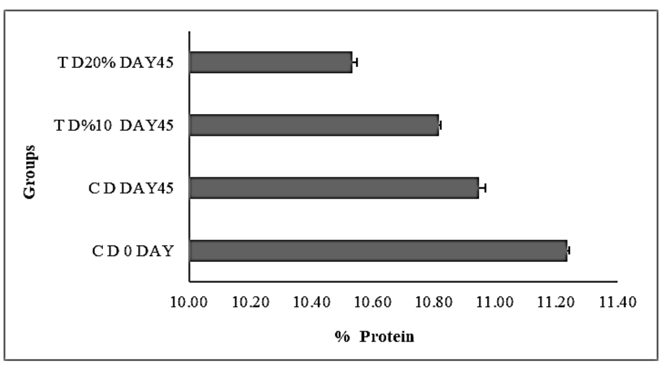

Fig. 5. Protein percentage in mahyaveh samples (TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation). Superscripts in each column with different letters are significantly different (p < 0.05).

This reduction in protein content could be explained by the remarkable increase in the growth of Lactic acid bacteria, especially in the presence of wheat bran during fermentation. The proteolytic system of Lactic acid bacteria comprises cell-wall bound proteinase and various intracellular peptidases that degrade the protein into shorter peptides and amino acids (28). The protein content obtained in this study is in agreement with the result of salt fermented fish sauce from anchovy in the range of 12.65-13.58% (23). This finding is also similar to the observation of Kilinc et al. (22) who observed the protein percentage of Turkey sardine fermented sauces was within the range of 11.72-13.5%. The TVB-N index in fish Sauce shows the intensity of sauce proteolysis by bacterial spoilage, autolytic enzymes, amino acid deamination, and nucleotide destructive reactions (23). The TVB-N content of mahyaveh samples was in the range of 235.72- 248.16 mg/100g and TVB-N index significantly increased during fermentation (p<0.05) (Fig. 6).

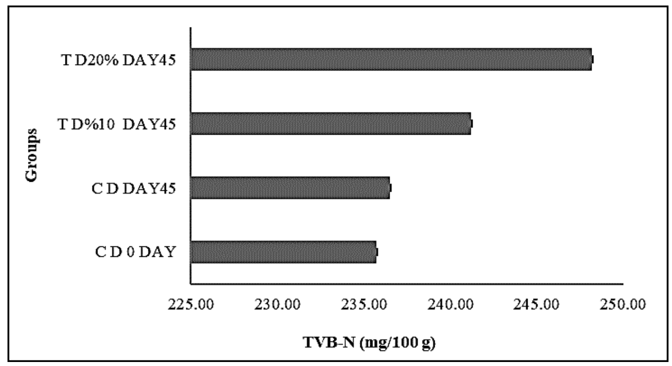

Fig. 6. TVB-N content in mahyaveh samples (TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation). Superscripts in each column with different letters are significantly different (p<0.05).

This finding is in agreement with Faisal et al. (29) who reported breakdown of protein and increase of total volatile nitrogen value in fish sauces. Zarei et al. (5) also reported a high amount of TVB-N in mahyaveh samples but they found a higher level of TVB-N in their samples (mean 309.8 mg/100g). Contrary to our studies, Kuda et al. (30) reported less amount of TVB-N in mackerel-nukasuke (41-76 mg/100 g). Our results cleared that the TVB-N levels increased significantly in the treated samples compared to the control (p<0.05) (1.98%-4.95%). It seems that the presence of wheat bran may enhance the growth of bacteria producing endogenous catalytic enzymes.

3.4. Histamine content assay

Histamine as a biogenic amine is formed in sea foods by microbial decarboxylation of the histidine or by transamination of aldehydes and ketones by amino acid transaminases (11). To produce histamine, the presence of amino acids, microorganisms with amino acid decarboxylases, and a proper condition for the growth and activity of a specific microorganism are necessary (7, 31). Histamine is the most important biogenic amine in fermented fish sauces. As shown in Table 1, the amount of histamine in the control sample after 45 days of fermentation was 960.61 mg/kg while food and drug administration (FDA) declared 50 mg/kg as a permitted level of histamine in seafood products (5). The mean concentration of histamine found in the mahyaveh samples in this study was higher than FDA maximum permissible limits. Also, Zarei et al. (5) found that histamine was the main biogenic amine in the Iranian fish sauce. However, they reported higher concentrations of histamine (with an overall mean of 2662 mg/kg) in mahyaveh. In this study, a significant reduction in histamine content of treated samples contained wheat bran was observed compared to the control (p<0.05) (13%-13.36%). Wheat bran is one of the richest sources of fiber and contains high numbers of non-starch polysaccharides (NSPs). Arabinoxylan, cellulose, and beta-glucan are the main NSPs that are present in wheat bran (12, 32). It seems that interaction between fiber content in wheat bran and histamine could decrease histamine content in mahyaveh. Consequently, this complex cannot be absorbed or easily hydrolyzed by the human intestinal tract and it will be excreted from the body with no serious changes (26). This observation is in agreement with the finding of Kuda and Miyawaki (33) who reported that the addition of the nuka-paste (20%, w/v) to sardine fish sauce reduced the histamine content to about 600-700 mg/L from 1200 mg/L.

3.5. Heavy metal concentration assay

Some heavy metals don’t have any toxic effects (such as manganese, zinc, iron, cobalt, molybdenum) and are even essential in small amounts, but are dangerous for the health in large amounts. On the other hand, our living cells do not need some heavy metals such as lead, cadmium, and mercury, even in small amounts. The accumulation of these toxic metals in the human body over time causes various diseases (15, 34). Since fish and seafood are exposed to heavy metal contamination and are consumed by humans as food, they may transmit toxic heavy metals to the human body (12, 35). Fish is the main ingredient of fish sauce so mahyaveh can be contaminated with heavy metals (13, 14).

Table 1. Histamine and heavy metal concentrations in mahyaveh samples.

Histamine and heavy metal concentration (mg/kg)	Groups			
	Treatment 20% Day 45	**Treatment 10% Day 45**	**Control Day 45**	**Control Day 0**
Histamine (mean± SD)	832.21± 1.29^{d*}	835.65±4.43^{c}	960.61 ± 0.92^{a}	950.40 ± 2.25^{b}
Fe (mean± SD)	1.3 ± 0.2^{d}	1.900000±0.40^{c}	5.9 ± 0.15^{ab}	6.2 ± 0.25^{a}
Hg (mean± SD)	0.2 ± 0.03^{c}	0.3 ± 0.01^{bc}	0.4 ± 0.05^{ab}	0.5 ± 0.03^{a}
Cd (mean± SD)	0.6 ± 0. 05^{d}	0.9 ± 0. 1^{c}	1.8 ± 0.08^{ab}	2 ± 0.01^{a}

***(**TD 20% Day 45: treatment samples contain 20% wheat bran on 45th day of fermentation, TD 10% Day 45: treatment samples contain 10% wheat bran on 45th day of fermentation, CD Day 45: control samples on 45th day of fermentation and CD Day 0: control samples on1st day of fermentation). Superscripts in each column with different letters are significantly different (p<0.05).

Iron (Fe) is an essential mineral for living cells, but excessive iron absorption might cause some chronic diseases, including diabetes, cancer, and cardiovascular disease (36). Table 1 shows that the Fe concentration of mahyaveh samples was in the range of 1.3- 6.2 mg/kg. The results cleared that adding wheat bran had a significant effect on the Fe concentration of treated mahyaveh samples (p<0.05). In the presence of 20% wheat bran, Fe concentration reduced from 5.9 mg/kg in control samples to 1.3 mg/ kg in treated samples (77.96% reduction). The mean concentration of iron found in all mahyaveh samples in this study was below the WHO/FAO maximum permissible limits (43 mg/kg) (37). Tiimub et al. (38) reported that the iron levels ranged from 44 mg/kg in Catfish to 53 mg/kg in Tilapia (38). Dissimilar to the present study, Alturiqi and Albedair (39) reported higher concentrations of iron in Saudi Arabia sardine ranging from 141.38 to 250.23µg/g. Cadmium (Cd) is found as an endocrine disturbing element that causes breast and prostate cancer in the human. Cadmium also causes damage in the kidney, hypertension, tumors, poor reproductive performance, and hepatic dysfunction. Table 1 shows the significant changes in Cd concentrations during the 45 days of the fermentation period (p<0.05). In the presence of 20% wheat bran, Cd concentrations reduced from 1.8 mg/kg in control samples to 0.6 mg/kg in treated samples (66.66%). The mean concentration of cadmium detected in the present samples was above the WHO/FAO maximum permissible limit of 0.2 mg/kg as stated in Table 1 (36). This is inconsistent with the study of Yi and Zhang (40), who reported the highest concentrations of Cd (0.115 mg/kg) in yellow-head catfish. Also, Dobaradaran et al. (41) reported that Cd mean values of two fish species of the Persian Gulf range from 0.17 to 0.26 mg/kg. This reduction is consistent with the study of Sasakia et al. (6) who, observed a decrease in Cd concentrations of fish sauce in the presence of tannin (0.39 mg/100 ml to 0.03 mg/100 ml). Mercury (Hg) is considered as a carcinogenic element and causes fetus defects in humans (42). Table 1 reveals that Hg concentration of mahyaveh samples was in the range of 0.2- 0.5 mg/kg. The results cleared that adding wheat bran had a significant effect on Hg concentration of treated mahyaveh samples (p<0.05). In the presence of 20% wheat bran, Hg concentration reduced from 0.4 mg/kg in control samples to 0.2 mg/kg in treated samples (50%). The mean concentration of mercury detected in all samples was below the WHO/FAO maximum permissible limit of 0.6 mg/kg (37). This finding is in agreement with the range of Hg concentration in Iranian tuna fish reported by Shabani et al. (43) (1.29-0.3 mg/kg). Dissimilar to the present study, Alturiqi and Albedair (39) reported less concentration of mercury in Saudi Arabia sardine ranging from 0.014 to 0.055µg/g. Table 1 cleared that adding wheat bran had a significant effect on heavy metal (Fe, Cd, and Hg) concentration of treated mahyaveh samples in comparison with the control sample ($p<0.05$). A scientific reason for these decreases is the chelating ability of phytic acid in wheat bran. Around 90% of phytic acid in the form of myoinositol Hexa phosphate is concentrated in the external covers in the aleurone layer of grain that is known as bran (44). Most of the minerals in wheat bran form complexes with phytic acid and produce phytate compounds. It seems that adding wheat bran to mahyaveh sauces produces phytate complex with fish heavy metal. In human body phytate complex won't be absorbed and will excrete from the body (44).

4. Conclusions

The present research has studied the effects of wheat bran on the reduction of histamine and heavy metals in mahyaveh. The concentrations of iron and mercury detected in mahyaveh samples were below the maximum permissible limits as per the WHO/FAO guideline standards comparison but cadmium mean concentration of mahyaveh samples was above the permissible limit. Adding wheat bran to mahyaveh can reduce the Fe, Cd and Hg contents to lower levels. Another safety problem related to mahyaveh is the high levels of histamine. The mean concentration of histamine was higher than the FDA maximum permissible limit for seafood products. The addition of wheat bran to this product reduced the histamine content significantly. These results cleared that wheat bran, a byproduct of the wheat milling process, could be utilized for the histamine and heavy metal reduction in mahyaveh.

Author contributions

NM conceptualized the study, conducted the experiments, designed the methodology, statistical analyses of the data, reviewed and edited the article to be published. MS conceptualized the study, conducted the experiments, curated data, supervised the experiments, provided visualization and resources, carried out formal analysis, and wrote the original draft.

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