CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

041802

The AOAC Research Institute hereby certifies the method known as:

Histamine Test

manufactured by

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 105-0003
Japan

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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METHOD NAMES
Histamine Test
Japanese チェックカラーヒスタミン (English: Check Color Histamine)

CATALOG NUMBERS
61341 - Histamine Test
60441 - チェックカラーヒスタミン / Check Color Histamine

60441

INDEPENDENT LABORATORY
Merieux NutriSciences
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366 Eagle Nest Dr., South Building
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APPLICABILITY OF METHOD
Target Analyte – Histamine

Matrixes – fresh raw tuna, frozen raw tuna, canned tuna in oil, canned tuna in water, mackerel, anchovy fish sauce

Performance claims – Quantification of histamine in the ranges 20-300 mg/kg and 10-150 mg/kg in raw and canned tuna, and in the ranges 160-2400 mg/kg and 80-1200 mg/kg in anchovy fish sauce using spectrophotometers with an optical path length of 1 cm or 2 cm, respectively.

Reference Method

***Official Methods of Analysis,* (2016), 20th Edition, AOAC INTERNATIONAL, Gaithersburg, MD, Method 977.13 (11)**

ORIGINAL CERTIFICATION DATE
April 23, 2018

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2025.

METHOD MODIFICATION RECORD
1. **January 2022 Level 1**

SUMMARY OF MODIFICATION
1. **Addition of Japanese kit name and catalog number.**

Under this AOAC *Performance Tested Methods*SM License Number, 041802 this method is distributed by:
1. **AS ONE CORPORATION**
2. **FUJIFILM Wako Pure Chemical Corporation**
3. **KENIS LIMITED**
4. **Nippon Bacterial Test Co., Ltd.**
5. **Weber Scientific**

Under this AOAC *Performance Tested Methods*SM License Number, 041802 this method is distributed as:
1. **Histamine Test**
2. **Histamine Test**
3. **Histamine Test**
4. **Histamine Test**
5. **Histamine Test**

PRINCIPLE OF THE METHOD (1)
Histamine Test is a colorimetric enzymatic assay for the quantitative analysis of histamine (13). Histamine dehydrogenase catalyzes the oxidation of histamine (13). This reaction in the presence of 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS) produces a colored tetrazolium salt that can be measured by absorption at 470 nm.

DISCUSSION OF RESULTS (1)

The Histamine Test is a rapid enzymatic quantitative assay kit for the determination of histamine in fishery product matrixes, including fresh and frozen raw tuna, canned tuna in oil and water, and anchovy fish sauce. The enzymatic reaction produces color that can be measured by a spectrophotometer. Most laboratories have a general spectrophotometer with 1-cm optical path length available. Moreover, a portable spectrophotometer, absorptiometer B or absorptiometer RGB with 2-cm optical path length is also recommended for its convenience and affordability. Here we report the validation study of the Histamine Test using both 1-cm and 2-cm optical path length spectrophotometers under the specific guidelines of the AOAC Research Institute *Performance Tested Method*^{SM} program.

The method range for a 1-cm optical path length spectrophotometer is 20-300 mg/kg for raw and canned tuna with 25-fold dilution or 160-2400 mg/kg for fish sauce with 200-fold dilution. The method range for a 2-cm optical path length spectrophotometer is 10-150 mg/kg for raw and canned tuna or 80-1200 mg/kg for anchovy fish sauce. In the linearity study, excellent correlations between the determined concentrations and the spiked concentrations were obtained in these ranges ($R^2 > 0.9997$). Moreover, the residual plots showed random patterns. Accordingly, the linearity in the range of the method was successively determined.

Selectivity tests demonstrated that there was no negative interference for 50 mg/kg histamine detection from the 12 biogenic amines tested in any of the matrixes (Table 1). These data suggested that the presence of other putrefactive amines will not cause false negative results. On the other hand, putrescine caused a slight positive interference in water and solid fish although no interference was observed in fish sauce. Agmatine yielded ca. 60-70% positive interference in water and solid fish, and ca. 40% positive interference in fish sauce (Table 1). These data are consistent with the previous report of substrate specificities of the histamine dehydrogenase (14). Positive interference in fish sauce is lower than solid fish due to the decreased enzymatic reactivity of agmatine by the 8-fold larger dilution. Indeed, agmatine interference may lead to an increase in the apparent histamine concentration. However, the following points shall be taken into account. Agmatine is produced from arginine by arginine decarboxylase associated with microbial food spoilage (5). It is well known that the arginine content is remarkably lower than histidine, a precursor of histamine, in Scombridae fishes. The previous report showed that a Yellowfin muscle sample contained 5950 mg/kg histidine and 10 mg/kg arginine (15). An Albacore muscle sample contained 6790 mg/kg histidine and 60 mg/kg arginine (15). The previous study similarly showed that the formation of agmatine is small compared with histamine in spoiled tuna. The data demonstrated that the decomposition of a tuna sample over three days generated 600-1300 mg/kg histamine, but agmatine was not detected (16). Therefore, agmatine interference in tuna appears to be practically negligible. If the fish species are different, agmatine interference may need to be considered, e.g. there is a report that agmatine in raw and salt-ripened anchovy (ca. 10-150 mg/kg) is a major amine (17). Regarding fish sauce, agmatine is considered a trace amine, e.g. agmatine in Korean anchovy fish sauce was below the detection limit, and four other fish sauce products whose main material was not described contained undetectable or 18 mg/kg agmatine (18). To be sure, the data in Table 7 showed that the four anchovy fish sauce products contained just 1.0-9.4 mg/kg agmatine although agmatine is known to be one of the major amines in anchovies (ca. 40-150 mg/kg, 17). The low agmatine level in fish sauce is likely caused by further conversion into putrescine by three enzymes viz agmatine deiminase, putrescine carbamoyltransferase and carbamate kinase in the fermentation process (4). However, the possibility that agmatine development can occur in anchovy fish sauce or other fish sauces produced under certain conditions needs to be taken into account due to high levels of arginine in anchovy species as mentioned above and arginine generation by proteolytic digestion in the fermentation process.

The matrix study demonstrated that the kit provides accurate and precise histamine quantification for all the evaluated matrixes. This kit offers several advantages, e.g. the easy sample preparation of extraction using 0.1 M EDTA·2Na (pH 8.0) with heating. The simple aqueous extraction, easy procedures and rapidity provide benefits over other histamine measurement methods, such as fluorescent method OMA **977.13** (11), high-performance liquid chromatography (HPLC) (12), and enzyme immunoassay (EIA). The good recovery (90.3-125.2%) and repeatability precision ($RSD_r < 10\%$) for all spiked matrixes revealed that the sample preparation and assay procedures produce acceptable results. Moreover, the determination of histamine using naturally contaminated frozen raw tuna afforded good agreement between the fluorescent method OMA **977.13** and the Histamine Test (Table 6). The determination of histamine using fish sauce also afforded good agreement between the HPLC method and the Histamine Test. Due to the higher dilution factor (200-fold) using sample treatment buffer, 0.1 M EDTA·2Na (pH 8.0), potential interference by many kinds of biogenic amines, high concentration of salt and low pH were avoided. These results also supported the reliability of the Histamine Test kit.

The claimed LOQ of the Histamine Test for raw and canned tuna using 1- and 2-cm optical path length spectrophotometers are 20 and 10 mg/kg, respectively. The claimed LOQ for anchovy fish sauce using 1- and 2-cm optical path length spectrophotometers are 160 and 80 mg/kg, respectively. The estimation of LOD and LOQ by the regression analysis of s_r versus mean histamine concentration and the validation study by spiking at the claimed LOQ levels of histamine for each matrix showed that the claimed LOQ are reasonable. These LOQ levels thoroughly cover the defect action levels of fishery products (50 mg/kg) and fish sauce (400 mg/kg) (1-9).

Generally, absorbance is directly proportional to the length of the light path, which is equal to the width of the cuvette. Hence, the 2-cm optical path length is advantageous for detection sensitivity over 1 cm, i.e. LOD and LOQ values obtained by the 2-cm cuvette are theoretically smaller than the values obtained by the 1-cm cuvette. However, there were tendencies that the data of the 1-cm cuvette spectrophotometer were slightly superior to the data of the 2-cm cuvette spectrophotometer in terms of R^2 values of regression analyses of the histamine level versus spiked histamine in matrix studies, LOD and LOQ. The main reason for this may be the number of significant digits that the spectrophotometers can display. The absorptiometer B with 2-cm cuvette used in this study can show only two decimal places, while the spectrophotometer with 1-cm cuvette can display three decimal places. This precision of the 1-cm cuvette spectrophotometer might bring about better results. However, even in that case, the use of absorptiometer B may be attractive due to its portability, simplicity and affordability. Absorptiometer RGB that is the successor model of absorptiometer B recently became available and it is expected to improve the accuracy of measurement since it displays results to 3 decimal places.

The claimed shelf life of 42 months (ca. 3.5 years) at 4°C was verified by real-time stability testing. The results proved that the kit is very stable throughout the testing period. Such long-term shelf life is likely attributable to the excellent heat stability of the enzyme in the kit (14) and lyophilized powder form of the reagents. Since the lots of four kits used in stability test were completely different, the lot-to-lot consistency study was also covered. As a result, it was also found that there were no observable lot-to-lot differences.

In the robustness study, the variation of extraction cleanup method (filtration and centrifugation), reaction temperature (35-39°C) and time (10-20 min) were examined. Regardless of the introduction of these minor changes, the mean recoveries were consistent (89.7-100%). The 3-way ANOVA indicated that both incubation temperature and time caused significant and marginal differences in the case of using the spectrophotometer with 2-cm and 1-cm cuvettes, respectively. While statistical differences were seen in incubation temperature and time, there were no practical differences observed in the mean recoveries compared to the nominal treatment conditions. The statistical difference is likely due to the fact that the standard deviations were very small, rather than any meaningful difference in mean results.

The independent laboratory showed very good precision in the matrix study, but it was not as remarkable as the method developer studies. Their s_r values ranged from 2.1 to 6.7 mg/kg. It is postulated that this could be due to longer than ideal time between centrifugation and sampling due to the treatment of 40 samples for each of the two spectrophotometers, or the pellet might have been disturbed when pipetting. Basically, homogeneous turbidity may not affect the results dramatically because the blank assay (Eb value) is deducted from the sample assay (Es value). However, if heterogeneous pellet is contaminated in the sample and the blank, the error of (Es – Eb) value seems to be non-negligible. Therefore, after centrifuging the sample, it is important to collect the homogeneous supernatant right away without disturbing the pellet. Filtration is also an effective means to obtain homogeneous samples.

Table 1 Results of selectivity study (1)

Optical path length, cm	Histamine, mg/kg	Compound (1000 mg/kg)	Results, mg/kg					
			water	Fresh raw tuna	Frozen raw tuna	Canned tuna in oil	Canned tuna in water	Anchovy fish sauce
1	0	Water	1.0	0.9	1.0	4.6	4.4	32.4
		Tyramine	1.4	0.9	1.1	4.4	4.4	33.4
		3-Methylhistamine	0.9	0.9	1.0	4.4	4.1	32.4
		L-Phenylalanine	0.9	0.9	0.9	4.4	4.1	31.4
		L-Histidine	1.0	0.9	1.0	4.3	4.9	32.4
		L-Tyrosine	0.9	0.7	0.9	4.2	4.7	32.4
		Tryptamine	1.1	0.8	1.0	4.5	4.5	32.4
		L-Tryptophan	1.1	0.9	1.0	4.3	4.3	31.4
		Cadaverine	2.0	1.1	1.3	4.9	4.4	32.4
		Putrescine	10.0	10.2	10.6	14.0	13.6	37.5
		Anserine	1.0	0.8	1.0	2.6	4.3	32.4
		Carnosine	1.0	0.8	1.0	4.3	4.3	32.4
		Agmatine	639.4	606.1	622.7	631.2	628.9	370.6
	50	Water	53.2	48.8	48.7	51.7	51.3	78.0
		Tyramine	54.9	48.3	48.2	53.2	52.7	78.0
		3-Methylhistamine	53.4	45.1	45.9	51.5	51.6	78.0
		L-Phenylalanine	53.3	48.5	48.6	51.6	51.3	77.0
		L-Histidine	52.2	50.9	48.8	51.5	51.2	80.0
		L-Tyrosine	48.8	48.8	47.2	52.2	51.7	77.0
		Tryptamine	52.0	50.1	48.3	52.6	52.7	80.0
		L-Tryptophan	52.9	51.3	47.1	51.7	51.7	77.0
		Cadaverine	53.8	48.8	48.0	51.5	51.7	79.0
		Putrescine	61.5	58.7	60.0	61.0	60.8	82.0
		Anserine	53.0	48.0	47.2	51.8	51.4	78.0
		Carnosine	53.1	52.7	47.4	52.0	51.7	77.0
		Agmatine	688.9	650.4	665.3	673.8	672.0	413.2
2	0	Water	1.2	2.0	1.0	1.2	2.4	52.4
		Tyramine	2.4	4.0	4.0	2.5	3.0	52.4
		3-Methylhistamine	2.4	2.0	2.0	1.2	3.0	52.4
		L-Phenylalanine	1.2	2.0	2.0	1.8	2.4	52.4
		L-Histidine	2.4	2.0	2.0	1.8	3.0	47.6
		L-Tyrosine	1.2	3.0	2.0	4.1	3.0	52.4
		Tryptamine	1.2	2.0	4.0	1.8	1.8	57.1
		L-Tryptophan	1.2	2.0	2.0	1.2	3.0	57.1
		Cadaverine	2.4	3.0	4.0	1.2	3.0	57.1
		Putrescine	14.5	15.0	7.0	14.0	15.2	57.1
		Anserine	1.2	3.0	3.0	1.2	3.0	52.4
		Carnosine	1.2	2.0	2.0	1.2	3.0	57.1
		Agmatine	733.3	632.0	622.0	689.9	609.1	390.5
	50	Water	54.2	52.0	51.0	51.5	49.7	104.8
		Tyramine	56.6	53.0	52.0	53.5	51.5	100.0
		3-Methylhistamine	53.0	51.0	51.0	52.7	49.1	100.0
		L-Phenylalanine	55.4	51.0	51.0	52.1	50.3	104.8
		L-Histidine	54.2	53.0	51.0	53.8	50.9	100.0
		L-Tyrosine	54.2	52.0	49.0	51.5	50.3	100.0
		Tryptamine	51.8	54.0	51.0	53.8	50.3	104.8
		L-Tryptophan	54.2	50.0	51.0	52.1	49.7	100.0
		Cadaverine	56.6	53.0	51.0	52.1	50.3	104.8
		Putrescine	65.1	67.0	65.0	63.3	63.0	104.8
		Anserine	55.4	54.0	52.0	53.3	51.5	104.8
		Carnosine	55.4	53.0	50.0	53.8	49.7	104.8
		Agmatine	777.1	719.0	750.0	765.8	703.0	438.1

Table 2. Results of matrix studies

Matrix	Initial histamine concn, mg/kg	Optical path length, cm	Fortification, mg/kg	Total Histamine, mg/kg	Number of replicates	Histamine Test Kit Results				
						Mean, mg/kg	s_r	RSD_r, %	Recovery, %	Bias, mg/kg
Fresh raw tuna	2.1	1	0	2.1	10	1.1	0.1	7.3	-[a]	-1.0
			20[b]	22.1	10	21.7	0.5	2.1	99.0	-0.2
			50	52.1	5	52.9	0.6	1.1	101.7	0.9
			100	102.1	5	103.0	0.2	0.2	101.0	1.0
			200	202.1	5	202.3	1.2	0.6	100.2	0.4
			300	302.1	5	302.4	0.5	0.2	100.2	0.5
		2	0	2.1	10	2.5	0.6	23.6	-[a]	0.4
			10[b]	12.1	10	11.6	0.6	5.5	97.0	-0.4
			25	27.1	5	28.4	0.0	0.0	105.3	1.4
			50	52.1	5	55.1	0.7	1.2	106.0	3.1
			100	102.1	5	106.4	1.0	1.0	104.4	4.5
			150	152.1	5	163.5	1.1	0.7	107.6	11.5
Frozen raw tuna	1.6	1	0	1.6	10	1.1	0.0	4.3	-[a]	-0.5
			20[b]	21.6	10	21.6	0.3	1.2	98.5	-0.3
			50	51.6	5	53.2	0.1	0.2	102.4	1.2
			100	101.6	5	103.1	0.5	0.5	101.2	1.2
			200	201.6	5	204.0	1.5	0.7	101.0	2.0
			300	301.6	5	302.6	1.0	0.3	100.2	0.6
		2	0	1.6	10	1.9	0.7	37.0	-[a]	0.3
			10[b]	11.6	10	10.8	0.8	7.2	90.3	-1.2
			25	26.6	5	29.4	0.9	2.9	109.2	2.5
			50	51.6	5	55.5	0.5	1.0	106.7	3.5
			100	101.6	5	105.0	0.7	0.6	103.0	3.1
			150	151.6	5	164.4	0.0	0.0	108.2	12.5
Canned tuna in oil	2.3	1	0	2.3	10	2.6	0.2	7.4	-[a]	0.3
			20[b]	22.3	10	23.6	0.4	1.7	107.4	1.6
			50	52.3	5	54.6	0.4	0.7	105.1	2.7
			100	102.3	5	107.5	0.7	0.6	105.4	5.5
			200	202.3	5	207.3	0.8	0.4	102.6	5.4
			300	302.3	5	304.7	0.6	0.2	100.9	2.7
		2	0	2.3	10	3.5	0.4	10.9	-[a]	1.2
			10[b]	12.3	10	14.9	0.8	5.5	124.7	2.9
			25	27.3	5	30.1	0.5	1.8	111.5	3.1
			50	52.3	5	56.7	0.5	1.0	109.2	4.8
			100	102.3	5	105.7	1.0	1.0	103.7	3.7
			150	152.3	5	158.5	2.2	1.4	104.3	6.6
Canned tuna in water	2.0	1	0	2.0	10	1.9	0.0	1.7	-[a]	-0.1
			20[b]	22.0	10	21.9	0.2	0.9	99.8	-0.1
			50	52.0	5	52.2	0.1	0.3	100.5	0.3
			100	102.0	5	103.2	0.8	0.8	101.2	1.2
			200	202.0	5	200.3	0.8	0.4	99.2	-1.6
			300	302.0	5	296.0	0.9	0.3	98.0	-5.9
		2	0	2.0	10	2.5	0.4	15.1	-[a]	0.6
			10[b]	12.0	10	12.6	0.6	5.0	105.4	0.6
			25	27.0	5	27.6	0.5	2.0	102.5	0.7
			50	52.0	5	52.1	0.9	1.6	100.3	0.2
			100	102.0	5	99.2	0.5	0.5	97.2	-2.8
			150	152.0	5	152.2	1.1	0.7	100.2	0.3
Anchovy fish sauce	36.0	1	0	36.0	10	53.7	1.8	3.3	-[a]	17.7
			160[b]	196.0	10	220.4	1.3	0.6	112.4	24.4
			400	436.0	5	466.6	2.9	0.6	107.0	30.6
			1000	1036.0	5	1082.6	11.8	1.1	104.5	46.6
			1600	1636.0	5	1686.7	8.2	0.5	103.1	50.7
			2400	2436.0	5	2480.3	17.3	0.7	101.8	44.3
		2	0	36.0	10	50.4	3.1	6.2	-[a]	14.4
			80[b]	116.0	10	145.2	4.8	3.3	125.2	29.2
			200	236.0	5	264.7	4.4	1.7	112.2	28.7
			500	536.0	5	563.0	0.0	0.0	105.0	27.0
			800	836.0	5	859.3	7.0	0.8	102.8	23.3
			1200	1236.0	5	1315.3	0.0	0.0	106.4	79.3

[a] Mean recovery is not relevant in the case of unspiked sample.

[b] The lower limits of the claimed method ranges. Ten replicates tests were carried out.

Table 6. Determination of histamine in naturally contaminated frozen raw tuna

Sample	Method	Number of replicates	Mean histamine, mg/kg	RSD_r, %	Relative recovery, %
A	AOAC Method[a]	3	1.6	14.2	
	Histamine Test	3	2.0[b]	34.6	-
B	AOAC Method[a]	3	21.4	0.8	
	Histamine Test	3	22.4	3.2	104.5

[a] AOAC Official Method **977.13**, [b] Below LOQ

Table 7. Determination of histamine in fish sauce made from anchovy

Products	Method	Number of replicates	Histamine, mg/kg	RSD_r, %	Relative recovery, %	Biogenic amines[a], mg/kg							
						AGM	PUT	PHE	SPD	TRY	SPM	TYR	CAD
A	HPLC	1	47.0			1.0	44.0	5.0	5.4	0.6	3.8	47.0	80.0
(pH4.9, 29% salt)	Histamine Test	3	42.1[b]	2.6	-								
B	HPLC	1	148.0			9.4	98.0	13.0	10.0	64.0	8.5	156.0	237.0
(pH5.3, 27% salt)	Histamine Test	3	147.9	0.9	99.9								
C	HPLC	1	288.0			5.4	192.0	29.0	10.0	146.0	6.7	126.0	294.0
(pH5.7, 17% salt)	Histamine Test	3	289.9	0.6	100.6								
D	HPLC	1	309.0			8.8	217.0	18.0	11.0	25.0	8.8	170.0	328.0
(pH5.7, 20% salt)	Histamine Test	3	289.7	0.1	93.8								

[a] AGM (agmatine), PUT (putrescine), PHE (phenylethylamine), SPD (spermidine), TRY (tryptamine), SPM (spermine), TYR (tyramine), CAD (cadaverine), [b] Below LOQ

Appendix Mackerel Data (1)

In response to a reviewer concern about the spiking procedure utilized in the matrix study, a temperature-abuse study was undertaken to demonstrate the relative recovery of naturally occurring histamine in fish. Since temperature-abusing raw tuna did not yield samples with histamine levels high enough, frozen raw mackerel (also in the Scombridae family of fish) was used. Mackerel was temperature-abused at 23°C to obtain low, medium, and high histamine levels within the range of the Histamine Test method. Table 10 shows the results of the study comparing the Histamine Test results to the AOAC **997.13** method. Relative recoveries ranged from 103.6 to 106.7 % and RSD_r values for the Histamine Test were as good or better than the AOAC method, except at the untreated level, which was below the LOQ of the Histamine Test. These results are in good agreement with the results from the spiked tuna matrixes (Table 2) and the incurred frozen raw tuna sample (Table 6). These data supported acceptance of the method for PTM certification and the matrix claim was clarified to "Scombridae fish species such as tuna, bonito and mackerel.

Table 10. Determination of histamine in naturally contaminated frozen raw mackerel (1)

Sample[a]	Method	Number of replicates	Mean histamine, mg/kg	RSD_r, %	Relative recovery, %
A	Histamine Test	5	1.3[c]	13.4	-
	AOAC Method[b]	3	1.1	3.5	
B	Histamine Test	5	22.1	0.4	103.6
	AOAC Method[b]	3	21.3	3.2	
C	Histamine Test	5	56.3	0.5	106.7
	AOAC Method[b]	3	52.7	1.7	
D	Histamine Test	5	131.8	0.8	106.0
	AOAC Method[b]	3	124.3	0.8	

[a] These samples were obtained by incubation at 23 °C for 24 h and stored at -20 °C.,
[b] AOAC Official Method **977.13**, [c] Below LOQ

REFERENCES CITED

1. Shimoji, K. and Bakke, M., Evaluation of the Validation Study of Histamine Test for the Determination of Histamine in Selected Fish Products, AOAC *Performance Tested Methods℠* certification number 041802.
2. Hungerford, J. M. (2010) *Toxicon* 56, 231-243.
3. Visciano, P., Schirone, M., Tofalo, R., & Suzzi, G. (2014) *Front. Microbiol.* **5**, 500.
4. Satomi, M. (2016) *Food Sci. Technol. Res.* **22**, 1-21.
5. Biji, K. B., Ravishankar, C. N., Venkateswarlu, R., Mohan, C. O., & Srinivasa Gopal, T. K. (2016) *J. Food Sci. Technol.* **53**, 2210-2218.
6. US Food and Drug Administration (2011) Fish and fishery products hazards and controls guidance, Ch 7, Scombrotoxin (Histamine) Formation, 4th Edition, pp 113-152.
7. European Food Safety Authority (2011) *EFSA J.* 9, 2393.
8. Codex (2011) Codex Standard for fish sauce, Codex Stan 302-2011.
9. Commission Regulation (EC) No 2073/2005 (2005) *OJ* L388, 1-26.
10. Commission Regulation (EU) No 1019/2013 (2013) *OJ* L282, 1-2.
11. *Official Methods of Analysis,* (2016), 20th Edition, AOAC INTERNATIONAL**,** Gaithersburg, MD, Method **977.13**, http://eoma.aoac.org/
12. Izquierdo-Pulido, M. L., Vidal-Carou, M. C., Marine-Font, A. (1993) *J. AOAC Int.* **76**, 1027-1032.
13. Sato, T., Horiuchi, T., & Nishimura, I. (2005) *Anal. Biochem.* **346**, 320-326.
14. Bakke, M., Sato, T., Ichikawa, K., & Nishimura, I. (2005) *J. Biotechnol.* **119**, 260-271.
15. Murata, Y., Henmi, H., & Nishioka, F. (1994) *Fish. Sci.* **60**, 473-478.
16. Nagayama, T., Tamura, Y., Maki, T., Kan, K., Naoi, Y., & Nishima, T. (1985) *Jpn. J. Toxicol. Environ. Health* **31**, 362-370.
17. Mohamed, S. B., Mendes, R., Slama, R. B., Oliveira, P., Silva, H. A., & Bakhrouf, A. (2016) *J. Food Nutr. Res.* **4**, 318-326.
18. Zaman, M. Z., Abdulamir, A. S., Bakar, F. A., Selamat, J., & Bakar, J. (2009) *Am. J. Appl. Sci.* **6**, 1199-1211**.**