IDENTIFICATION OF BIOGENIG AMINE IN SALAD DRESSINGS

Laura Carmen APOSTOL[1], Ancuta Elena PRISACARU[1], Sorina ROPCIUC[1], Cristina DAMIAN[1]

e-mail: laura.apostol@fia.usv.ro

Abstract

The presence of biogenic amine in food represents a quality indicator of acceptability. The intake of foods containing biogenic amines can present a health hazard through the direct toxic effect of these compounds. The biogenic amine contents in four samples of sauce used for salad enrichment were determined using HPLC. From the sampled fresh open after purchase, just two were identified with traces of biogenic amines. Of the nine biogenic amines under study, six of them were identified after 7 days after -sauces were open and stored in refrigeration: putrescine (1%), spermidine (1%), spermine (1%), tryamine (4%), phenylethylamine (72%) and histamine (21%). The total content for the identified amine in each sauce was 150.45 mg/g with a range from 2.55 mg/g to 112.75 mg/g. After 14 days in three sauces was identified one type of biogenic amine namely: putrescine, spennidine and phenylethylamine.

Key words: quality indicators, refrigeration, sauces

The sauce is a semi-solid food not normally consumed by consumers; it is used to add flavor, moisture, and visual appeal to another dish. Depending on the purpose, sauces can be strongly flavored, hot and spicy, or even sweet to be served with a dessert (Chong Y.Q., 2016).

In the case of commercials sauces, once opened, the freshness and flavor will slowly deteriorate. The sauce is still safe to use beyond this time but the quality may not be best all the time. Therefore, after opening it is recommend the sauce refrigeration (Tiersky E., 2013). Refrigeration helps the flavor and quality characteristics to remain at their peak for a longer period. In general, a change in flavor and/or color is the first indication that a sauce is beginning to lose its freshness. The proposed chemical indices used for the identification of the early signs of sauces alteration are: pH, water activity and the biogenic amine content (Weaver C.M. *et al*, 2013).

In the last period a wide variety of foods such as vegetables, wines, cheese, fish, and meat have been subjected to researchs in order to identified biogenic amines.

The biogenic amine occurrence is the result of the enzymatic decarboxylation of the precursor amino acids although microorganism activities (Baston O., Barna O., 2010). The main factors that acts on the biogenic amine formation are the availability of the amino acid substrate, the level of decarboxylase activity, and the specific bacterial strain(s) present (Linares D.M. *et al*, 2009). The quantitative production of biogenic amines is usually reported to be temperature and time dependent (Zaman M.Z. *et al*, 2009).

The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine, and agmatine. From the biogenic amine usually analyzed, polyamines spermine and spermidine are natural amines produced by the body (Biji K.B. *et al*, 2016). Histamine and tyramine are considered as the most toxic and food safety relevant, and fermented foods are of particular biogenic amine concern due to associated intensive microbial activity and potential for their formation (Naila A. *et al*, 2010).

According to European Food Safty Authority the amount of biogenic amine in fermented foods for which no adverse health effects were observed varies between 50 and 600 mg/kg (*table 1*). The biogenic amine identification is important because of their potential use as freshness indicators and their toxicity (Baston O., Barna O., 2010). As Naila A. *et al* (2010) mentioned in their study, the existing method for controlling biogenic amines in food is refrigeration.

[1] "Ştefan cel Mare" University, Faculty of Food Engineering, Suceava

Table 1

Biogenic amine levels in food for which no adverse health effects (NOAEL) were observed

Biogenic amine	Concentration (mg/kg)	Reference
Putrescine	600	Rauscher-Gabernig E. *et al* (2012)
Cadaverine	510	
Spermidine	300	Bobba M. *et al* (2015)
Spermine	50	
Tryptamine	-	-
Phenylethylamine	120	Bobba M. *et al* (2015)
Histamine	200	EFSA (2011)
Tyramine	600	
Agmatine	-	-

Although certain bacteria can develop at low temperature biogenic amines may be formed. Consequently refrigeration exclusively cannot ensure the biogenic amines absence.

The purpose of the present study is to evaluate the influence of refrigeration process on four comercial sauces quality by identifying the development of biogenic amines.

MATERIAL AND METHOD

Samples

Samples (n = 4) of sauce were purchased at supermarkets in Suceava, Romania, in April, 2016. From the different brands available in the market the sauces included in this study were choose based on the statistics made according to the sales achieved in the near period. The sauces were American Cesar dressing (C), salad dressing with yoghurt and willed garlic (U), dressing vinaigrette (V) and salad dressing with aromatic herbs (D).

The products, according to the labels contain water, vegetal oils, vinegar, refined salt, sugar, egg yolk, and xantan. American Cesar dressing also listed butter and hard cheese as ingredients on the label. Salad dressing with yoghurt and willed garlic contains milk proteins and yoghurt aroma. Salad dressing with aromatic herbs contains yoghurt and some condiments.

For the proposed determinations the samples were taken immediately after opening the containers, then the sauces were kept at refrigerated temperature.The samples stored under refrigeration conditions were analysed after 7 and 14 days of storage.

Reagents and solvents

The reagents used were purchased from Sigma-Aldrich: standards solution of biogenic amines with 95-99% purity (putrescine (Put), cadaverine (Cad), spermidine (Spd), spermine (Spm), tryptamine (Try), 2-phenylethylamine (Phe), histamine (His), tyramine (Tyr) and agmatine (Agm)), perchloric acid, sodium hydroxide, benzoyl chloride, diethyl ether and acetonitrile.

Sauces sample preparation:

Over the estabilished amount of the sauce sample, weighed at analytical balance, perchloric acid (0.4 M) was added. The sample was homogenized and centrifuged at 4000 rpm. The supernatant obtained was diluted with perchloric acid (0.4 M). 2 ml of extract were taken NaOH (2 M) and benzoyl chloride was added. The obtained solution was heated to 30°C and NaCl saturated solution was added. The resulting sample was extracted with diethyl ether and the extract was taken to dryness using the water bath.

The residue obtained was dissolved in acetonitrile and before being placed in vials was passed through a filter was filtered through an filter of 0.22 µm pore size.

Biogenic amines determination

Biogenic amines were determined using Shimadzu HPLC detector's SPD M20 Diode Array A (254 nm) from Faculty of Food Engineering (Stefan cel Mare University of Suceava) laboratory.

In order to estabilish the biogenic amines concentration (mg BA/g product) in the analized samples equation (1) was used:

$$C = C_0 x A/As \quad (1)$$

where: C is the determined biogenic amine concentration, C_0 is the standard concentration of biogenic amine A is the biogenic amine area from the sample and As is the standard area for the biogenic amine.

RESULTS AND DISCUTIONS

The biogenic amine contents in four samples of sauce used for salad enrichment were determined using HPLC. From the fresh sampled opened after purchase, just two were identified

with traces of biogenic amines, namely the C and D sauces (*table 2*). Of the nine biogenic amines under study, six of them were identified in the samples collected on 7 days ~~seven~~ after the opening and stored in refrigeration conditions: putrescine (1%), spermidine (1%), spermine (1%), tryamine (4%), phenylethylamine (72%) and histamine (21%) (*table 3*). The total content for the identified amine in each sauce was 150.45 mg/g with a range from 2.55 mg/g to 112.75 mg/g (*figure 1*). The samples analyzed after 14 days of storage showed that the C, D and U sauces contain one type of biogenic amine namely: spermidine (0.61 mg/g), putrescine (0.22 mg/g) and phenylethylamine (861.67 mg/g), respectively.

According to FAO&WHO (2012) refrigeration storage at 4°C will prevent the growth of histamine-producing bacteria. The study conducted by Guidi L.R., Gloria M.B.A. (2012) found in fresh soy sauce the following biogenic amine: tyramine, putrescine, histamine, phenylethylamine and cadaverine. No researches were reported related to refrigeration and biogenic amines development in sauces.

Table 2

Identified biogenic amine in the analized samples

Sample	Biogenic amine		
	Fresh open	After 7 days	After 14 Days
C	Put	Phe	Spd
D	Put	Phe	Put
U	-	Put, Spd, Spm, Try, Phe, His	Phe
V	-	Try, Phe, His, Tyr, Agm	-

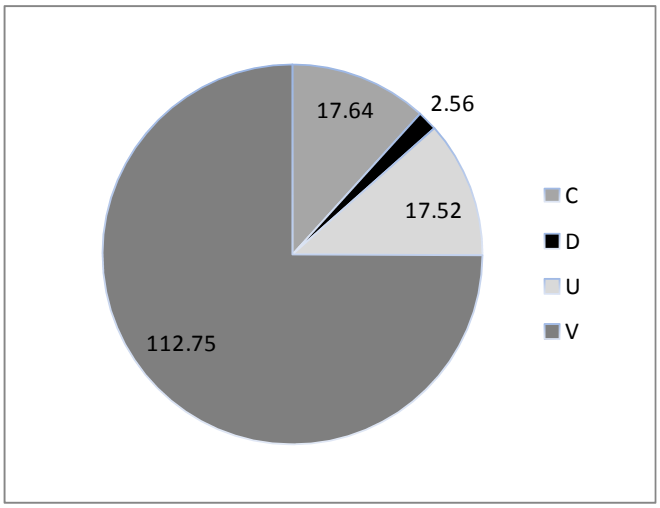

Figura 1 - **Total biogenic amine identified after 7 days of refrigeration**

Table 3

Percentage (%) of Biogenic Amine Identified After 7 Days of Refrigeration in the analyzed sauces

	C	D	U	D
Put	-		0.50	-
Cad	-		-	-
Spd	-		0.92	-
Spm	-		0.35	-
Try	-		3.78	0.92
Phe	100	100	72.81	46.68
His	-		21.64	48.45
Tyr	-		-	0.20
Agm	-		-	3.75

CONCLUSIONS

Refrigeration helps the flavor and quality characteristics to remain at their peak for a longer period. The biogenic amine of four commercial sauces were taken immediately after opening the containers, then the sauces were kept at refrigerated temperature and sample for analysis were carrying in day seven and fourteen.

In the case of the fresh open sauces putrescine was identified in C and D sauces. After seven days the higher amount of biogenic amine was determined in V sauce (112.75 mg/g), followed by C and U (17 mg/g) and D (2.55 mg/g).

In the fourteen day the C, D and U sauces were found to contain one type of biogenic amine namely: spermidine (0.61 mg/g), putrescine (0.22 mg/g) and phenylethylamine (861.67 mg/g).

Sauces refrigerated storage can prevent the growth of biogenic amine producing bacteria in the case of three sauces and inhibited the development of the existing ones.

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