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Assessment of *Aspartame* Exposure Due to Consumption of Some Imported Chewing Gums by Microwave Digestion and High Performance Liquid Chromatography Analysis

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ABSTRACT

Aspartame is a widely used artificial sweetener, the long-term safety of which has been controversial ever since it was accepted for human consumption. The main aim of this research is assessment of *aspartame* exposure due to consumption of some imported chewing gums during summer 2015 to Iran by microwave digestion and HPLC analysis. Thirty chewing gums from highly consumed imported ones were collected from retail market in Tehran. Closed vessel microwave digestion was employed for sample preparation using a three phase temperature program. An aliquot of 20 µL of prepared samples was injected into the HPLC column and the aspartame was detected at 254 nm with an on-line detector. Concentration of aspartame in chewing gum samples was between 1.9 and 30.5 µg/g with an average of 11.1 µg/g. In conclusion, despite of existing aspartame in 76.6 percent of samples, however the effective amount of this artificial sweetener is not as high as the levels that international legislations recommended for exposing due to chewing gum consumption.

Keywords: *Aspartame*, Artificial sweeteners, Chewing gum, Microwave digestion.

INTRODUCTION

The chemistry science along with food industry had a positive effect on producing and developing artificial sweeteners that are used as substitutes for the sugar[1]. Artificial sweeteners are widely used in food, beverage, dietary products, confectionery and pharmaceutical industry all over the world[2, 3]. These additives refer to the people who want or need to reduce caloric intake, obesity and tooth decay so, they are recommended for patients with diabetes who are restricted in sugar consumption[1-4]. *Aspartame* is currently considered as safe artificial sweetener and now is the most

popular sweetener in more than 75 countries that may be used separately or in combination with other sweeteners[2]. This sweetener is a dipeptide L-aspartyl-L-phenylalanine methyl ester[3, 5] that its sweetness accidently discovered in 1965 by James M. Schlatter[5]. *Aspartame* is composed of phenylalanine, aspartic acid plus a small amount of methanol[6]. Since phenylalanine can be neurotoxin and can influence on the neurotransmitters synthesis of inhibitory monoamine so, phenylalanine in aspartame can cause neurological side effects. Several clinical studies have shown that excessive intake of aspartame can cause headaches, migraines and memory lose[3]. Despite numerous trials that are done to evaluate the risks of food additives on animals however, the safety of *aspartame* consumption still remains controversial[1]. *Aspartame* is 200 times sweeter than sucrose. Now *aspartame* is used in more than 6000 different products such as non-alcoholic drinks, desserts, icy yogurt, chewable multivitamins, cereals, desktop sweeteners, medicine, etc and millions of people around the world used them[1, 6]. The food and drug administration of America (US-FDA) and the food organization of Europe have set the acceptable daily intake (ADI) of *aspartame* 50 and 40 milligrams per kilogram of body weight per day respectively [4, 6, 7]. The experts of joint committee FAO/WHO in food industry (JECFA) have also set this amount to 40 milligrams per kilogram of body weight[6]. In Iran there are many attractions for consumption of the imported gums, especially for children and teenagers that show the need for the rigorous and periodic safety controls more than ever. According to the dangers due to exposing to the high doses of aspartame and lack of periodic and continuous control for the chewing gums that are often imported, hence it's essential to do proper monitoring and evaluation of aspartame from quantity aspect in this instance. Conventional methods for identifying and measuring the aspartame in food industry and chewing gums have numerous weaknesses particularly in the preparation of samples including the loss of the analyte, being costly, time consuming and lack of the necessary sensitivity. The widespread use of microwave to dissolve samples is gradually replaced by the conventional pressure reactors that particularly are suitable for the determination of organic and inorganic analytes[8, 9]. There were reported some microwave digestion methods which are mainly applied for extraction of minerals from food matrices[10-12]. Microwave extraction is one of the extraction methods based on the warming in a closed vessel containing oxidizing agents. Its principle is that a sample and its appropriate solvent are placed in a container and then pressured and heated by microwaves. After about 5 to 20 minutes extraction is completed and will be allowed to cool. Then the sample mixture is removed and filtered[13, 14]. Extracted solution is analyzed in high performance liquid chromatography as a good way to separate, identify and measure the components of the sample[8, 9]. The main aim of this research is the assessment of aspartame exposure due to the consumption of some imported chewing gums during summer 2015 to Iran by microwave digestion and HPLC analysis.

EXPERIMENTAL

Reagents and chemicals

Aspartame powder with 100% purity was purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, potassium dihydrogen phosphate, methanol, nitric acid and hydrogen peroxide were obtained from Merck, (Darmstadt, Germany).

Equipments and devices

Microwave digestion system (Milestone, Switzerland Co.), high performance liquid chromatography (Younglin, South Korea), Revese-phase ODS chromatography column 250*4.6mm, 5 μm (Teknokroma, Spain) which was set at 25 centigrade degree in a column oven, the micro injection syringe (Hamilton, USA), ultra-pure water system (Younglin Co., Younglin), ultrasonic bath (LIARRE Starsonic 60, Italy) and syringe micro-filter were bought from CNW technologies company, Germany.

Sample collection and preparation

All of the chewing gum samples from highly consumed ten brands with three replicate were randomly collected from different parts of retail markets as the most widely used imported chewing gum brands in Tehran during 2015 summer. All of the samples with appropriate labeling and packaging and without any apparent damage were transferred to the reference food control laboratory and maintained in dry and cool condition until analysis. At first, the moisture content of gum samples was measured

according to the official method[15]. Extracting operations of *aspartame* from gum samples were performed by microwave digestion method in a Milestone microwave system[16]. All of the variables involved in this system such as digestion time, digestion temperature and microwave intensity were examined to extract aspartame from chewing gum samples and to achieve maximum recovery. For this, exactly 0.1 gram of each gum sample was inserted into the sealed Teflon containers then placed in a microwave digestion system and the operations were carried out. These containers were well washed with water and detergents before using and then were placed in the nitric acid solution for 24 hours to be free from any contamination. Five milliliter of concentrated nitric acid and two milliliter of hydrogen peroxide is added to the sample and the Teflon vessel is put under the hood until the reaction is completed

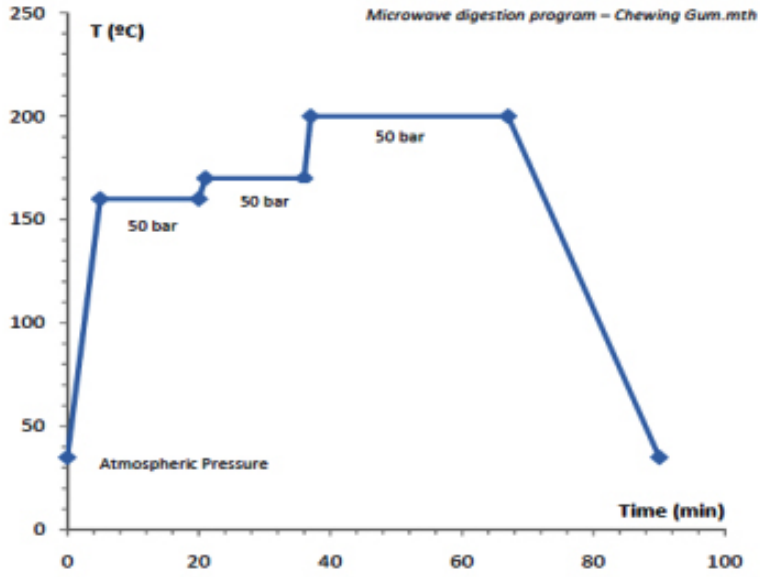

Fig. 1: Microwave digestion program applied for digesting chewing gum samples

Table 1: The results for the moisture content in chewing gum samples

Sample code	Weight of moisture vessel (g)	Weight of crucible with sample(g)		Moisture (w/w%)
		before drying	After drying	
Tr-1	17.5767	17.6787	17.6768	1.96
Viv-1	22.5212	22.6292	22.6270	1.85
Viv-2	20.6008	20.7089	20.7056	2.77
Fv-1	23.9633	24.0654	24.0623	2.94
Fr-1	22.0312	22.1325	22.1300	1.98
Or-1	19.0506	19.1538	19.1506	2.91
Re-1	21.0381	21.1354	21.1322	3.09
Na-1	17.8875	17.9892	17.9871	1.96
Me-1	23.8203	23.9211	23.9201	0.99
On-1	23.8637	23.9669	23.9638	2.91
On-2	32.5384	32.6433	32.6411	1.90
Ex-1	23.7410	23.8402	23.8323	8.08

between the materials. Then the vessels were closed well and placed inside the microwave system.

Figure 1 shows the implementation plan of temperature, pressure and time to digest the chewing gum samples in the microwave digestion system. This figure shows a three phase temperature-pressure program for digestion of gum samples according to the instrument instructions. As can be seen in this diagram, digestion operation is started by pouring the sample and reagents at ambient temperature and atmospheric pressure and reaching to the first step that the temperature is 160 centigrade and the pressure reached to the 50 bar in the vessel. In the second and third steps the temperature of the sample container reached to 170 and 200 centigrade degree respectively in a constant pressure. Then the vessel is cooled and returning to the ambient pressure in the final phase. The total operation of sample digestion was performed in 90 minutes.

At the end of operating program, samples are transferred into 15 ml poly ethylene test tubes and the remaining solution in the vessel was washed with a little distilled water and transferred into the test tube and the obtained solution was diluted up to 10 ml.

Preparation of standard solutions

To prepare standard solutions of *aspartame*, exactly 0.010 g of *aspartame* powder was weighed and after dissolving in a beaker was brought to volume inside a volumetric flask. Then working standard solutions were prepared in a 100 ml volumetric flasks by diluting appropriate amount of stock solution by deionized water.

Table 2: The calibration curve data obtained from injection of aspartame standard solutions in five levels

Concentration of standard solution (mg/L)	Retention time (min)	Area under the curve
0.00	—-	—-
0.01	6.42	8.3550
0.02	6.35	15.7905
0.03	6.42	22.6004
0.04	6.43	30.2175

Sample preparation

To prepare a sample solution, the digested sample was brought to volume inside a volumetric flask and poured in a test tube. For removing any suspended particles in sample, appropriate Wattman syringe filters were used and passed prepared samples through it. Then the prepared sample was sonicated in sonication bath for 30 minutes. Finally 5 micro liters of filtered samples individually and respectively were injected into the HPLC system[17].

RESULTS

Moisture content

Table1 shows the results of mean moisture content for gum samples. As can be seen in this table, sample code of Me-1 with mean moisture of 0.9900 w/w percent has the lowest moisture and sample code of Ex-1 with 8.0808 w/w percent w/w percent accounted for the most amount of the moisture.

***Aspartame* analysis**

The results of the injection of *aspartame* standard solutions to HPLC system is presented in Table2. As can be seen in Figure 2 by injecting the standard solution with a concentration of 4 mg/L, a peak with the retention time of 6.600 minutes appeared in a run time of 10 minutes that is related to the *aspartame*. The chromatograms related to the injection of the *aspartame* standard solutions led to a linear calibration curve with correlation coefficient of 0.9989 (r^2).

It can be seen in Figure 3, the extracted solution of gum sample which have *aspartame* in the list of gum formulations on the sample label. In this HPLC chromatogram there is no peak related to the *aspartame* and it can be seen that no any peak is observed for a certain period of time according to the retention time of standard peak in Figure 2.

In Figure 4, a typical chromatogram related to the injection of extracted solution of gum sample is presented. This analysis is related to the lowest amount of *aspartame* between all of the studied samples. In this chromatogram the observed peak at 6.3033 minutes is related to the *aspartame* with the area under the curve of 13.6327.

In Figure 5, the HPLC chromatogram due to injecting the extractive solution of gum sample with the highest amount of *aspartame* can be seen. This chromatogram shows a peak with area under the curve of 216.0330 in the retention time of 6.4667 minutes.

***Aspartame* in chewing gums as a dry weight**

Comparing the retention time of peaks of the injection of the gum samples with retention time of peaks of the injection of the standard solutions show that 23 out of 30 studied sample contain *aspartame*. Also, although the *aspartame* name

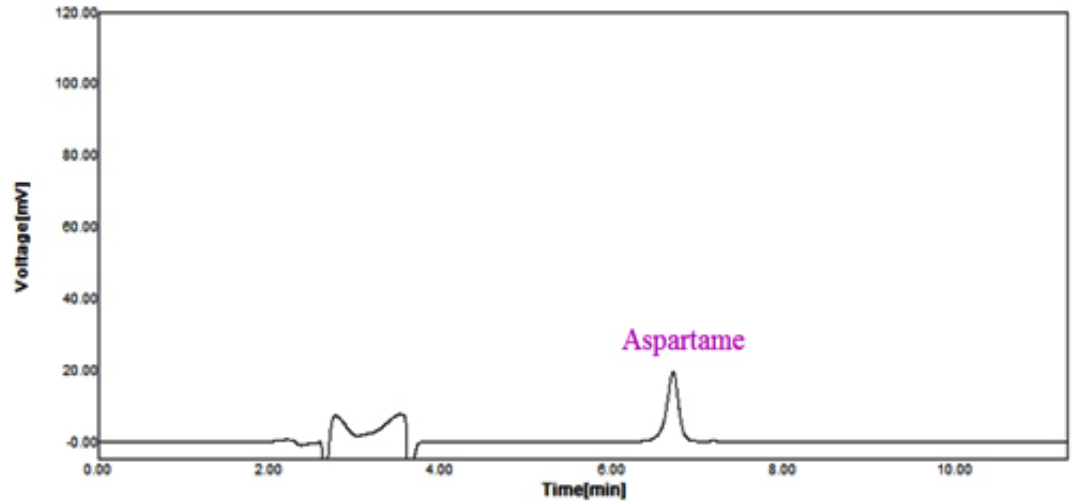

Fig. 2: A typical HPLC Chromatogram for injection of *aspartame* standard solution (Condition: 250*4.6mm, 5µm ODS column, acetonitrile and phosphate buffer (80:20) as mobile phase, 1.0 ml/min flow rate and UV detection in 254 nm)

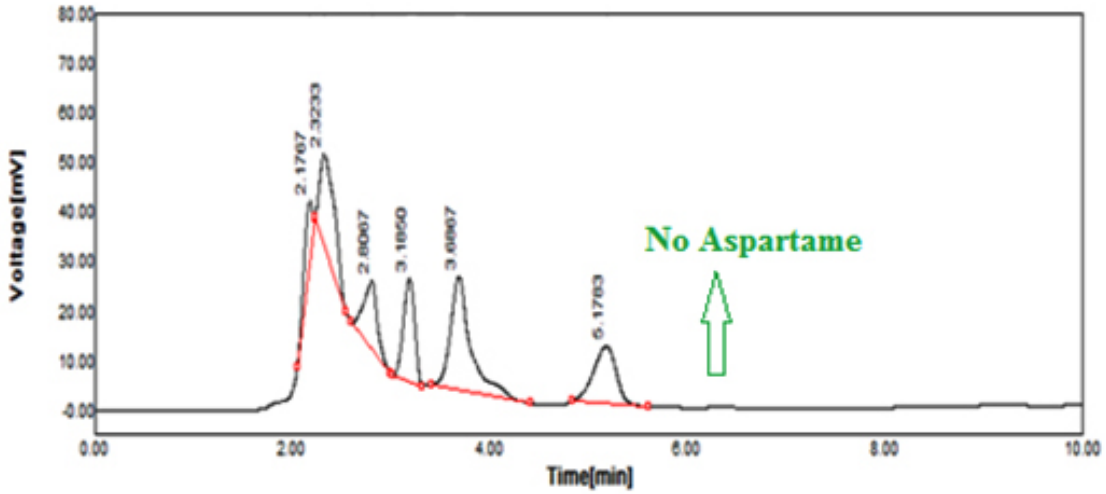

Fig. 3: A typical HPLC Chromatogram for injection of extracted solution of chewing gum sample with no *aspartame* (Condition: 250*4.6mm, 5µm ODS column, acetonitrile and phosphate buffer (80:20) as mobile phase, 1.0 ml/min flow rate and UV detection in 254 nm)

Table 3: The chromatographic results for *aspartame* in chewing gum samples as a dry weight

Sample code	Moisture (w/w%)	*Aspartame* in dry weight (µg/g)	Mean *aspartame* in dry weight (µg/g)	Standard Deviation (µg/g)
Fr-1		24.80		
Fr-2	2.00	29.50	23.80	6.30
Fr-3		17.10		
Viv-1		11.80		
Viv-2	2.30	12.20	11.00	1.70
Viv-3		9.00		
Tr-1		8.60		
Tr-2	2.00	1.90	4.20	3.80
Tr-3		2.20		
Ex-1		7.10		
Ex-2	8.10	6.30	4.50	3.90
Ex-3		N.D*		
Or-1		20.10		
Or-2	2.90	N.D*	15.90	14.30
Or-3		27.70		
Me-1		5.80		
Me-2	1.00	N.D*	3.20	2.90
Me-3		3.70		
Na-1		2.00		
Na-2	2.00	18.50	10.30	8.30
Na-3		10.40		
On-1		30.50		
On-2	2.90	N.D*	15.40	15.30
On-3		15.60		
Fv-1		17.60		
Fv-2	2.90	26.80	14.80	13.60
Fv-3		N.D*		
Re-1		N.D*		
Re-2	3.10	24.80	8.30	14.30
Re-3		N.D*		
	Total mean		11.10	

* N.D. = Not Detected

Table 4: Comparison of mean amount of *aspartame* in chewing gum samples with some permission limits in national and international legislations for human consumption

Sweetener	This research (mg/kg)	FAO/WHO (mg/kg)	FDA (mg/kg)	ISIRI (mg/kg)	EU (mg/kg)	Reference
Aspartame	0.0111	40	50	5500	5500	(18-21)

was listed on the package label of all samples, 7 samples ad no *aspartame* peak wasn't observed during this period. In Table3 the concentration, the humid weight percentage, average and standard deviation of *aspartame* in different gum brands are presented according to their dried weight unit.

Comparing the mean of *aspartame* between sample types

In Figure 6, the average *aspartame* concentration is shown as well as their standard deviation in the studied gum samples. As can be seen in this figure the highest average of *aspartame*

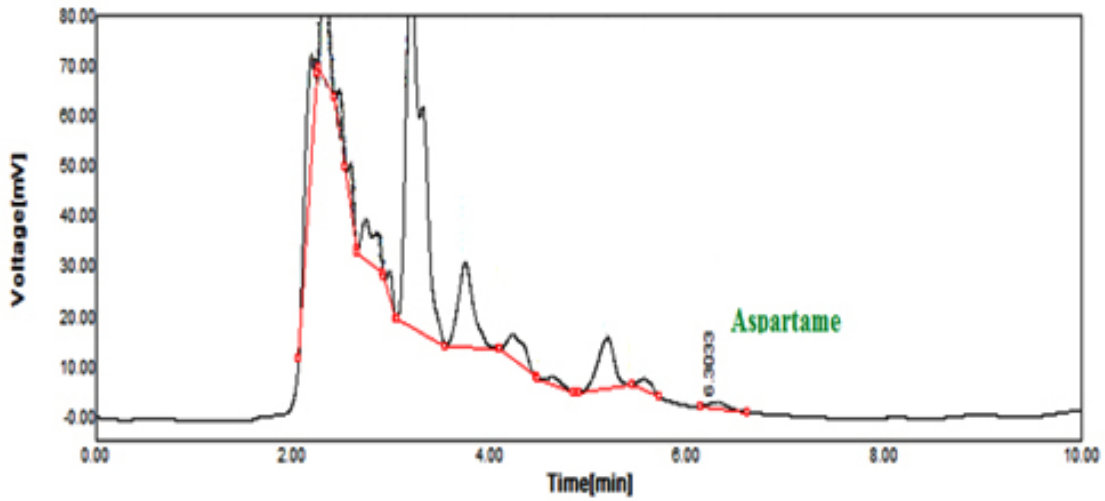

Fig. 4: HPLC Chromatogram for injection an aliquots amount of extracted solution of chewing gum containing the lowest amount of *aspartame* (Condition: 250*4.6mm, 5µm ODS column, acetonitrile and phosphate buffer (80:20) as mobile phase, 1.0 ml/min flow rate and UV detection in 254 nm)

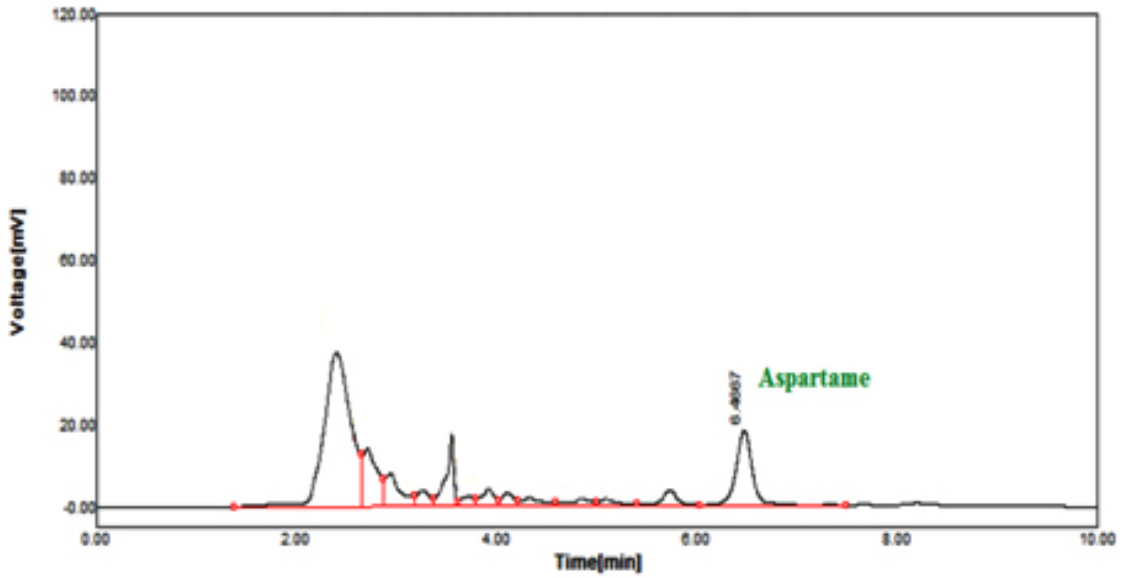

Fig. 5: HPLC Chromatogram for injecting an aliquots amount of extracted solution of chewing gum containing the highest amount of *aspartame* (Condition: 250*4.6mm, 5µm ODS column, acetonitrile and phosphate buffer (80:20) as mobile phase, 1.0 ml/min flow rate and UV detection in 254 nm)

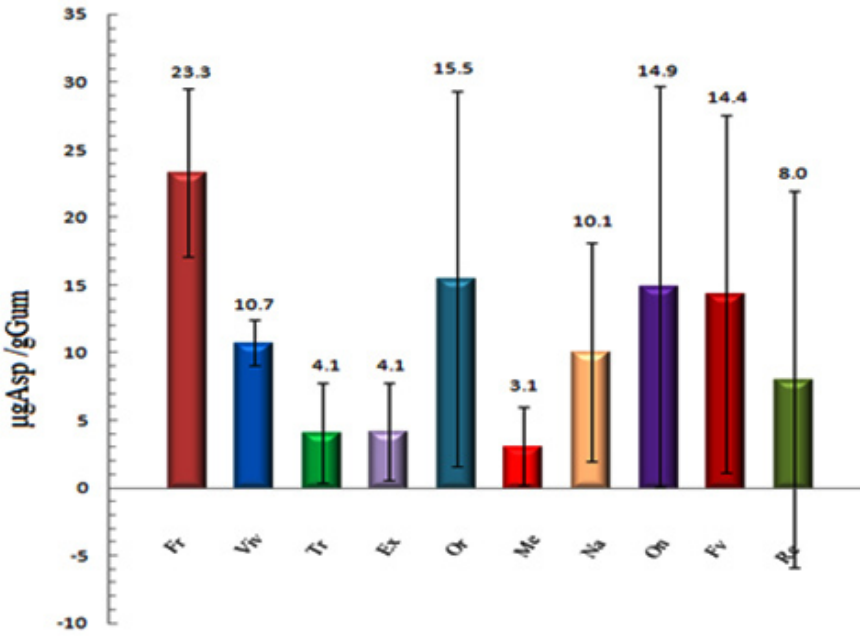

Fig. 6: Variation of *aspartame* mean concentrations in chewing gum samples according µgAsp/gGum

refers to the (Fr) brand and its amount is 23.3 and the least average belongs to (Me) brand that equals to 3.1, also the highest standard deviation belongs to (Re) brand that equals to 13.9 and the least average belongs to (Viv) brand that equals to 1.7.

CONCLUSION

During recent years, the use of imported chewing gums has dramatically increased among teenagers and young adults. In comparison with sucrose, the higher sweetness of *aspartame* cause that people prefer it among gum samples. Nowadays the artificial sweetener is used in more than 6000 different food products and the high consumption of these sweeteners may lead fears in terms of health regulations. Using a new combination method for the extraction of aspartame from chewing gum and its analysis by HPLC method in such a complex matrix following their monitoring, collecting the various samples from imported brands and recording the amount of artificial sweeteners in the form of a coherent set and presentable way to policy makers and health references are new indices of this research. According to the obtained results, some samples contained aspartame under the approved standards in the level of national and international standards and some other had no *aspartame* despite the existence of aspartame in gum formulation listed on the label instance. The aspartame concentration evaluated in the samples and it is revealed that their amounts are comparable with the national regulations and recommended values for daily intake of aspartame. The average amount of *aspartame* in the chewing gum samples revealed that it is compatible with the local criteria in terms of national standards and international FAO/WHO, FDA and EU regulations.

The limits set out in Table 4 show that the obtained mean concentration for *aspartame* in the samples was much less than the specified limits in the national and international levels. While in the investigation that was conducted on 30 samples of gum, a larger amount than the average of 0.0305 mg/kg was also observed, however this amount is much less than the specified limits that in comparison with the limits of daily intake of *aspartame* on the basis of the FAO/WHO and FDA organizations the consumer by using only one gram of chewing gum would

respectively receive 0.07625 and 0.005 percentage of lawful limit of this artificial sweetener use. As can be seen in this table, mean concentration of *aspartame* in chewing gum samples in comparison with lawful limit of FAO/WHO is 0.0227 percent and in comparison with FDA is 0.0222 percent and this amount represents the lower limits of *aspartame* in the sample in comparison with international standards. According to the maximum allowable concentration of *aspartame* and obtained amount of average for the aspartame of gum samples from supply level would conclude that the rate of consumer exposure to the aspartame due to the consumption of imported gum is far less and the percentage is much lower than allowable limit. However according to the obtained concentration in this study and its interval from allowable limit, it can be concluded that the consumption of exported gums doesn't pose a risk to the health of consumer and the concentration of aspartame in comparison with international standards is less than its allowable limit for the consumer. But according to the daily intake of various doses of *aspartame* from other sources containing the artificial sweetener such as non-alcoholic drinks, deserts, icy yogurt, chewable multivitamins, cereals, desktop sweeteners, medicine, etc, to prevent poisoning caused by excessive consumption of these sweeteners caution must be observed.

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