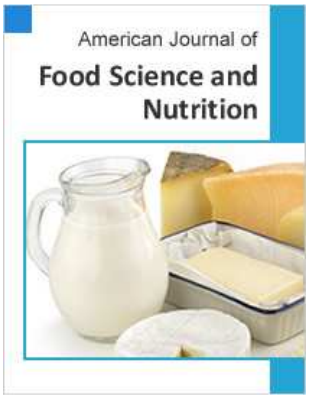
American Journal of Food Science and Nutrition
2015; 2(5): 82-88
Published online September 20, 2015 (http://www.aascit.org/journal/ajfsn)
ISSN: 2375-3935

American Association for Science and Technology



Keywords
Quality Control,
Food Analysis,
Aspartame,
Diet and Low Calories
Beverages

Received: August 29, 2015
Revised: September 8, 2015
Accepted: September 9, 2015

Study of Thermal Degradation of Aspartame in *Coca-Cola Zero* and *Pepsi Light* Markted in Libya

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Citation
Mohamed N. El Attug, Amal A. Ammar, Nahla M. Almosrati, Lyala M. Ben Yousef, Abdurrahman A. El Maremy. Study of Thermal Degradation of Aspartame in *Coca-Cola Zero* and *Pepsi Light* Markted in Libya. *American Journal of Food Science and Nutrition*. Vol. 2, No. 5, 2015, pp. 82-88.

Abstract
Aspartame (Asp) has been used as sweetener in food products, including carbonated soft drinks, powdered soft drinks and some pharmaceuticals such as vitamins and sugar-free cough drops. Since Asp could be degraded under certain conditions such as moisture, temperature and pH into methanol, diketopiperazine, aspartyl phenylalanine, phenylalanine and aspartate. In most stores, the soft drinks which contain aspartame has been placed in front of stores exposure to sun and high temperature, which leads to increase instability of aspartame and to produce a toxic degradation products. The objective of this project was to evaluate the stability of aspartame in commercially beverages that manufactured in different countries by different companies like *Pepsi Light* and *Coca Cola Zero* which are available in Libyan market. The effect of temperature on aspartame stability in these beverages was studied. This study was also conducted to determine % lost (*degradation*) of aspartame for both *Pepsi Light* (4.6 % & 13.2 %) and *Coca Cola Zero* (5.0 % & 11.7 %) after exposed to the January's and February's sun temperature for 2 and 4 weeks, respectively. The storage and display temperature appeared to be the important factor for instability of Aspartame. In our project (*Coca Cola Zero* and *Pepsi Light*) have been chosen as diet and low calories beverages containing Asp, the remaining aspartame in the analyzed samples was determined after exposed to sun temperature for 2 and 4 weeks.

1. Introduction

Aspartame (Asp) 1-methyl N-*L*-a-aspartyl-*L*-phenylalanine, is an artificial sweetener consisting aspartic acid, phenylalanine and methanol [1]. It is a dipeptide consisting of two amino acids in an ester bond with methanol, Aspartic acid is at the N-terminus and phenylalanine is at the C-terminus where it bonds with methanol. It is produced from the methyl ester of two amino acids, L-aspartic and *L*-phenylalanine [2].

There are two forms of aspartame, an *alpha* and *beta* form and only *alpha* form is sweet. Asp has four different configurations when it is synthesized, because aspartame has two chiral carbons, only one of the four configurations fits into the receptor site which contributes to the sweet sensation, two of the other configurations are tasteless and the other is bitter[3].

Chemistry and classification

Aspartame's chemical name is *alpha L-aspartyl-L-phenylalanine-1-methyl ester*, with a molecular weight of 294.3, a density of 1.3 g/cm^3 [4] and a molecular formula is $C_{14}H_{18}N_2O_5$ as shown in Figure. 1.

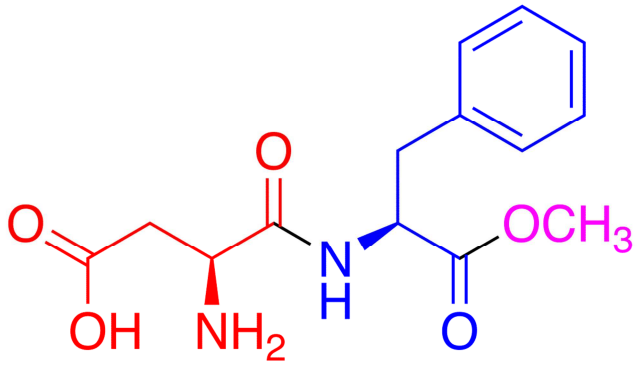

Figure 1. *Chemical Structure of Aspartame.*

Asp has long been used as a substitute of sugar in low-calorie meals, soft drinks and frozen desserts [5, 6]. Asp is 180 times higher sweetness than sucrose [7], Asp is widely used in foods and beverages because its taste is very similar to that of table sugar [8]. Asp is widely used in food products including beverages, breakfast cereals, yoghurts, and confectionary products, and pharmaceutical industries [1, 9-11].

Asp is very stable in dry form, but it has poor stability at various pH and temperatures, and therefore has different degrees of degradation, which can produce aspartic acid, phenylalanine, and diketopiperazine (DKP) [12]. These substances, especially DKP, may be harmful to the metabolic processes in the human body [13]. DKP is one of breakdown products of aspartame, which is a carcinogenic in the *Centre Nervous System* (CNS). DKP contributes to the formation of tumors in the CNS such as gliomas, medulloblastomas and meningioma [14].

Asp may generate methanol by hydrolysis under strongly acidic or alkaline conditions, under more severe conditions the peptide bonds are also hydrolyzed resulting free amino acids [15]. ASP is rapidly metabolized after oral administrated to aspartic acid, phenylalanine and methanol [16].

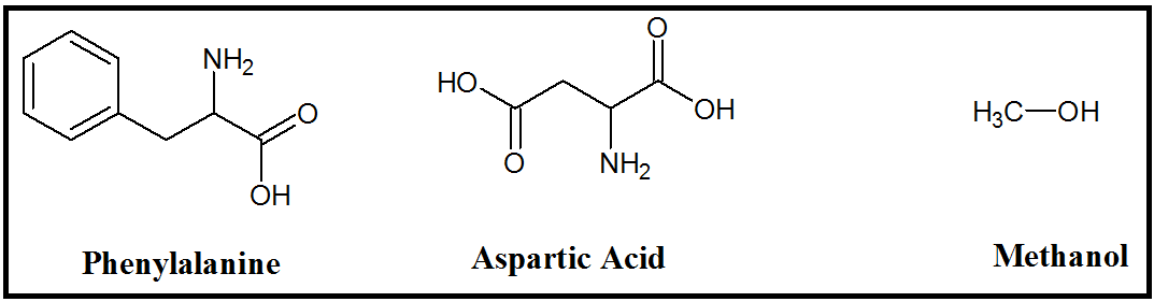

Figure 2. *Metabolites of aspartame.*

Aspartame must be avoided by people with the genetic condition phenyl keton urea (PKU) and liver diseases [17]. So any food containing the additive must bear on its label the following statement: "*Phenyl keton urics contains phenylalanine*". In addition, when aspartame is used as a sugar substitute for table use, the label must bear instructions not to use it in cooking or baking, due to the aspartame breaks down and resulting in a loss of sweetness [18]. Methanol is a toxicant that causes systemic toxicity, after consumption of aspartame, methanol is converted to formaldehyde and then to formic acid [11]. Initially, methanol, which is weaker than ethanol, affects the depression of the central nervous system, this is the latency period after which blurred vision and even blindness occur under the influence of the transformation of methanol to formaldehyde in the retina. As a result of the accumulation of formate, metabolic acidosis occurs and this may eventually cause CNS depression, coma and death from respiratory system paralysis. Methanol poisoning can occur after consumption of chilled beverages containing aspartame after physical exertion or after losing a large amount of fluid under the influence of high air temperature [19, 20].

Aspartic acid and phenylalanine might cross the blood brain-barrier and causes memory loss because deterioration in the neurons of the brain [21].

The aim of this project was to determine the degradation products and the amount of aspartame remaining in the diet Beverages (*Coca Cola Zero* and *Pepsi Light*) post exposed to sun temperature for two and four weeks in mid of January to mid of February of year **2015** on building roof as simulating of shop display of their products in front of shops exposed direct to sun light and temperature.

2. Material & Methods

2.1. Reagents and Solutions

All chemicals used were of analytical grade and used without further purification, Methanol for LC grade was obtained from Fluka analytical company (Germany), Aspartame, Sodium Benzoate reference standards were obtained from Supelco. Analytical company (USA), Acesulfame K was obtained from Fluka analytical company

(Germany) and Caffeine was obtained from Sigma Aldrich (China), *Pepsi Light* from Africa Company for manufacturing and filling of Beverages (Tripoli, Libya) and *Coca Cola Zero* from Tunisian Beverages Manufacturing Company (Street Earth, Center Urbain Nord) were bought from local market.

All solutions were prepared by using ultrapure MilliQ-water (Millipore, Milford, MA, USA) and were filtered with a 0.2 µm membrane filter syringe (Dassel, Germany). All samples were weighed using Sartorius CP64A analytical balance.

2.2. Methods

2.2.1. A-stock Solution of Stranded Preparation

A stock solution of mixture of Aspartame, Acesulfame K, Caffeine and Sodium benzoate CRS was prepared by dissolving a 50 mg each in Methanol and complete to 100 mL of volumetric with LC grade Methanol, the mixture was mixed well by ultrasonic for 15 min then rest for 3-4 min.at 25°C

2.2.2. Stranded Solution Preparation for Calibration Curve

Prepare 5 standards for calibration and calculation of aspartame were prepared by taking 1 mL, 2 mL, 4 mL, 6 mL, 8 mL and 10 mL of ($0.50\ mg.mL^{-1}$) of mixture solution in 50 mL volumetric flask with distilled water.

2.2.3. Preparation of Mobile Phase

i. Preparation of Buffer [22]

Weigh and dissolve a 2.72 mg from KH_2PO_4 (0.02 M) in 1000 mL distilled water, adjust it to pH 2.5 by adding few drops of diluted Ortho Phosphoric Acid (OPA).

2.2.4. Sample Preparation

Sample was prepared by taking 25 mL from sample (*Pepsi Light*, *Coca Cola Zero*) after degassed by ultrasonic for 15 min., then complete volume to 50 mL by Millipore water, mixed well by ultrasonic for few min., fill samples into vials after filtered with 0.45 µm filter device connected to syringe, then run in the HPLC.

2.3. Samples

Table (1). *Samples information and coding.*

Sample	Pepsi light	Coca Cola Zero
1	Control	Control
2	2 weeks	2 weeks
3	4 weeks	4 weeks

2.4. Equipments

2.4.1. Liquid Chromatographic System and Conditions

The assay of Asp performed on Perkin Elemr (Series 200 full Auto sampler system). System consisting of a LC a quaternary pump, an auto injector UV-VIS detector. The column temperature was maintained by using oven, data acquisition was supported, by (PerkinElmer Life and Analytical Sciences, 710 Bridgeport Avenue, Shelton, USA). The stationary phase used for the separation of Asp was C18 (250 x 4.6 mm ID, 5 µm) (SUPLECO Analytical, Bellefonte, USA). , the mobile phase used for analysis of aspartame was delivered with Flow Rate 1.0 $mL.min^{-1}$.

The amount of sample will be injected 10 µL, The Colum oven temperature was maintained at 25ºC, the UV detector wave length 254 nm.

2.4.2. Assay of Aspartame

Different samples were tested during the assay by LC method according to in house validated method, under the condition of the chromatography system as shown in Table (2).

Table (2). *Chromatographic Conditions Assay of Aspartame content [22].*

Mobile phase	Buffer :Methanol: ACN 65:20:15
Elution	Isocratic: 100%
Column Temp	25 °C
Flow rate	1 $mL.min^{-1}$
Detection (UV)	254 nm
Injection vol.	10 µL

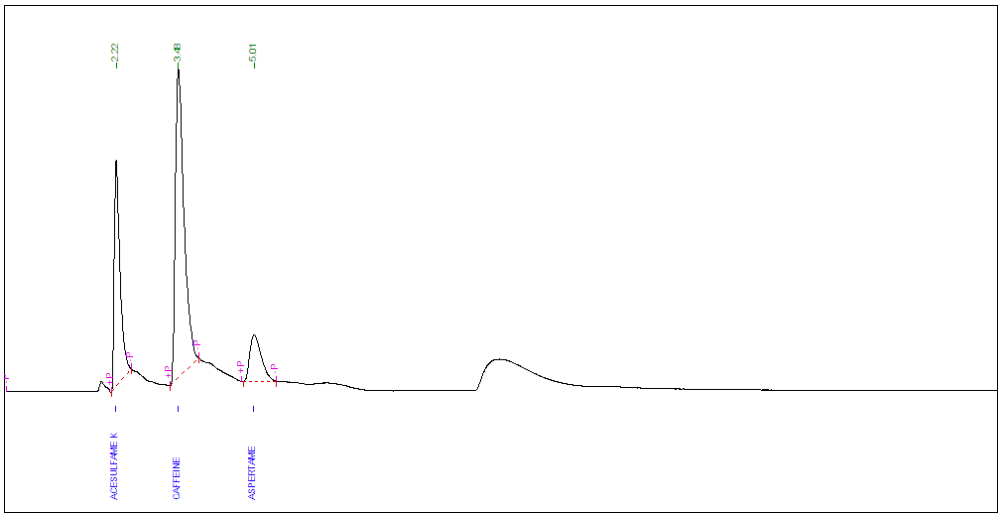

Figure 3. *Shows typical chromatogram of Caffeine, Aspartame and Acesulfame K (CRS).*

In this test amounts of aspartame in the different samples are determined. A three cans of each sample of (*Pepsi Light* and *Coca Cola Zero*), which were exposured to sun temperature on the house roof to simulate the display of products in front of the markets on the streets for 2 and 4 weeks plus control sample kept in ambient temperature in cupboard a way from light.

It is also to measure the amount of the aspartame left and its degradations if it is possible. Figure 4 and 5 show typical chromatograms overlay of different samples for each product. More variation could lead to bad effectiveness (loss of sweetness taste) and degredants, which lead to toxicity effect to the consumer.

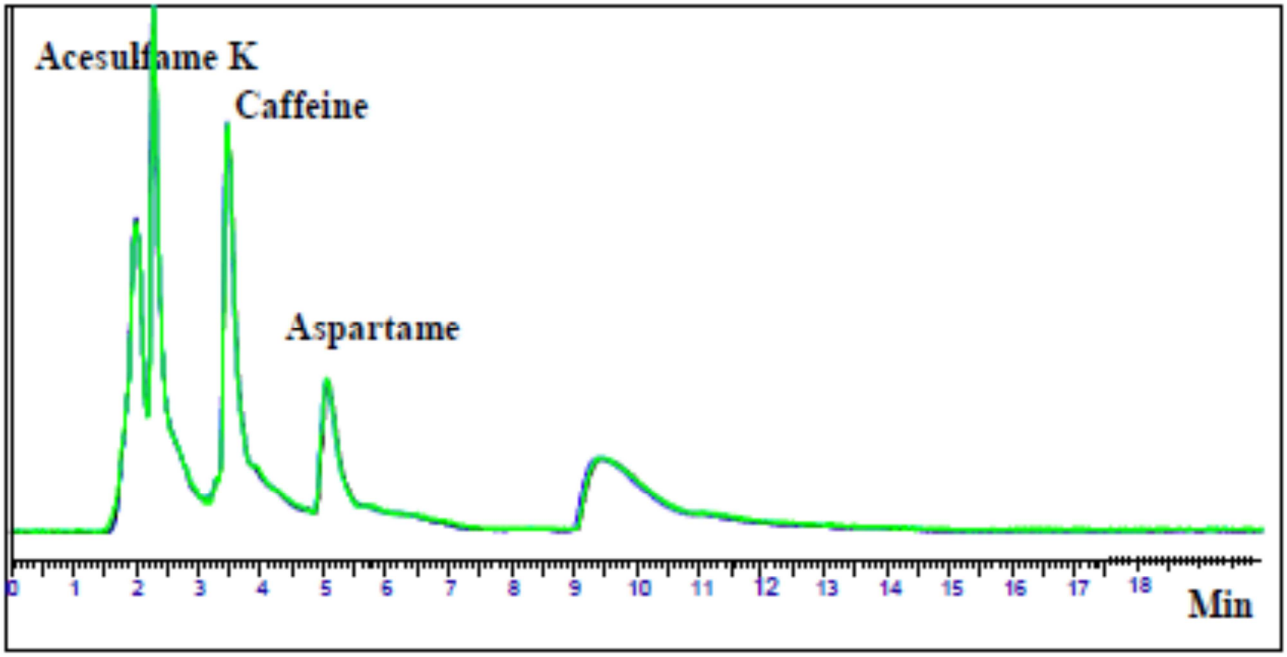

Figure 4. *Shows typical overlay chromatograms of Coca Cola Zero samples (Control, 2 and 4 weeks exposure to sun temperature).*

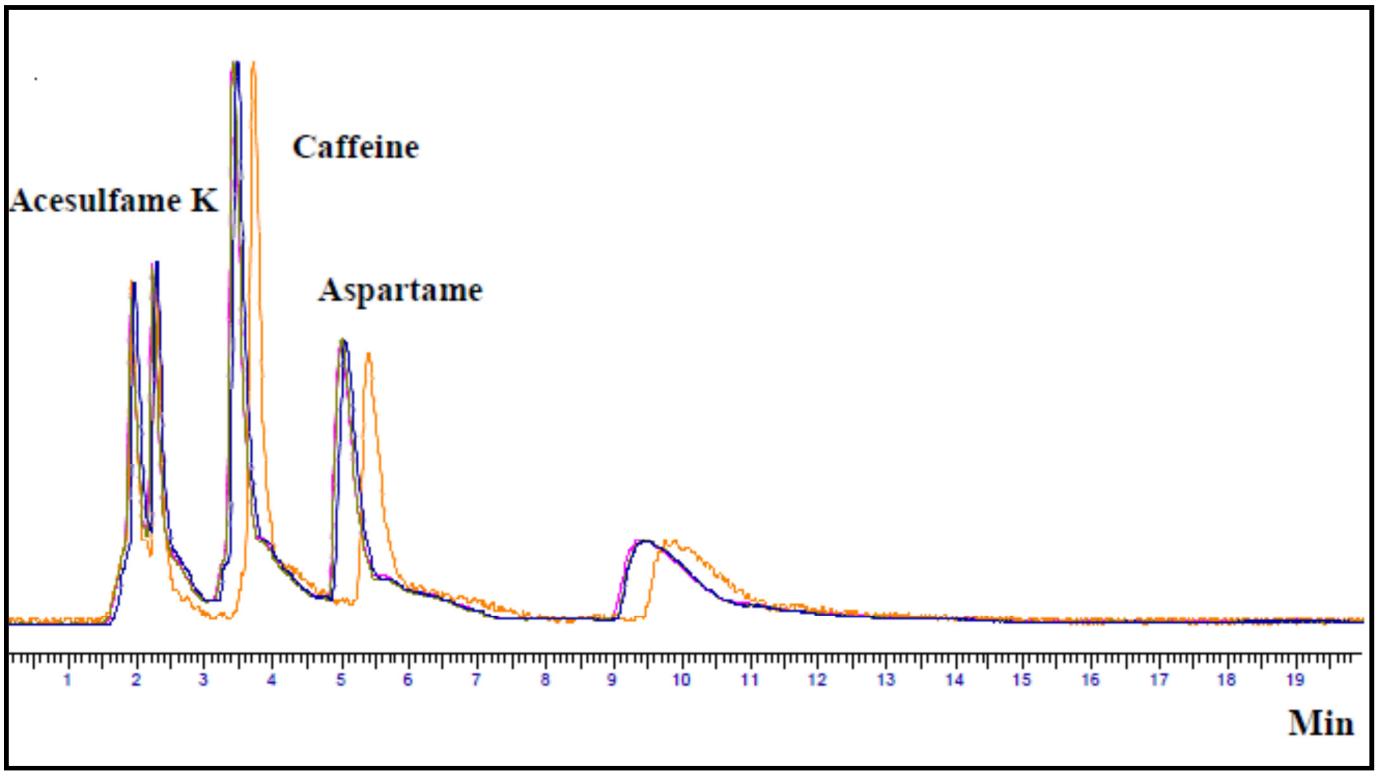

Figure 5. *Shows typical overlay chromatograms of Pepsi Light samples (Control, 2 and 4 weeks exposure to sun temperature).*

3. Results & Discussion

3.1. Results

The quality control tests results for the different samples of both products (*Pepsi Light* and *Coca Cola Zero*) are summarized in Tables (3). For the assay of aspartame in control samples since the average values found were 453 and 240 mg.L^{-1} for *Pepsi Light* and *Coca Cola Zero*, respectively.

3.1.1. Linearity for Aspartame

The separation was obtained in less than 8 min with good linearity (R^2 = 0.9994) for Asp, with 6 concentration points injected in triplicate for concentrations from 10 to 100 mg.L^{-1}. The following regression equation was obtained: $y = 41.583x + 92.65$, S_{yx} = 0.13, where y: peak area, x: concentration (mg.L^{-1}), S_{yx}: standard error of estimate. The intercept was found to be not statistically different from zero as shown in Figure 6.

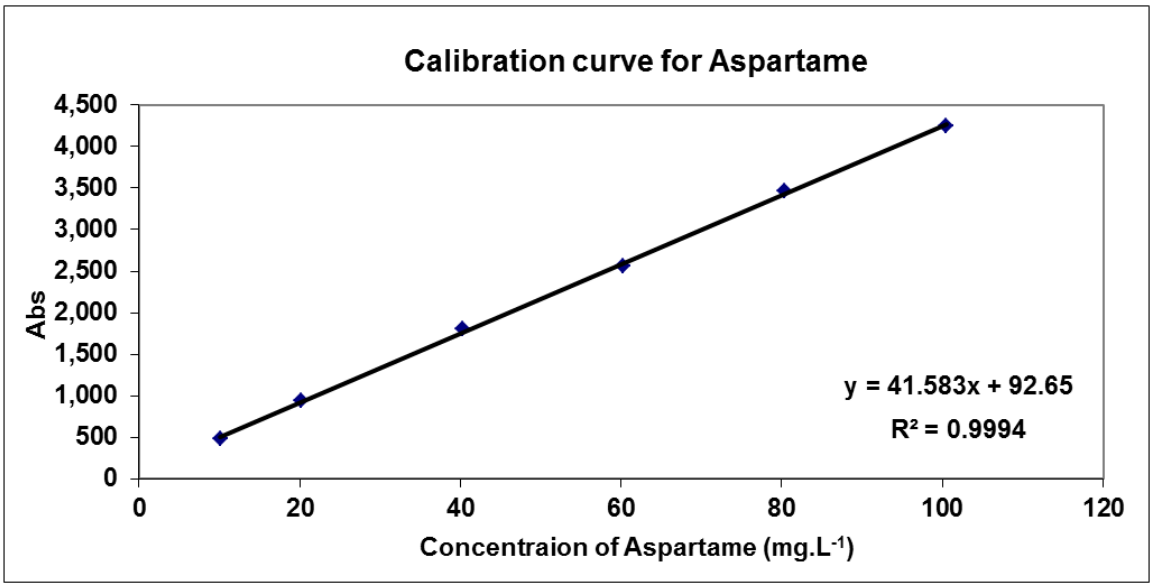

Figure 6. *Linearity (Calibration curve) for Aspartame CRS.*

3.1.2. Determination of Aspartame Content

Table (3). *The amount Aspartame content in ppm (mg.L^{-1}) and % reduction.*

Product Name	*Pepsi Light*	% Reduction	*Coca Cola Zero*	% Reduction
1 Control	453 ± (0.30)	--	240 ± (0.26)	--
2 (2 weeks)	432 ± (0.72)	4.6 %	228 ± (0.42)	5.0 %
3 (4 weeks)	393 ± (0.45)	13.2 %	212 ± (0.84)	11.7 %

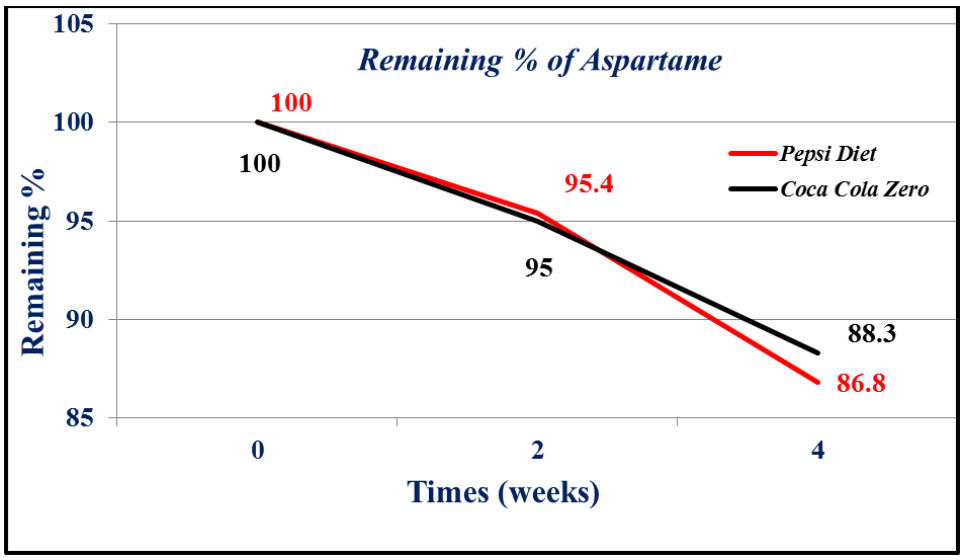

Figure 7. *Percentage (%) Remaining of Aspartame in Pepsi Light and Coca Cola Zero samples.*

3.2. Discussion

The results presented in Table (3), indicate that all the analyzed samples by official method. Since it is common and expected for the aspartame degradation to methanol, phenylalanine and piprazine derivatives as a function of exposure to heat or by increasing the temperature by sun temperature on the building roof as simulating of in front shop display.

The results of the assays presented in Table (3) showed that the average content of Asp for the analyzed samples those exposed to sun temperature ranged from 393 to 432 $mg.L^{-1}$ in *Pepsi Light* Product, while its degradation (lost % percentage) were 4.6 to 13.2 % for 2 and 4 weeks, respectively from the initial amount of aspartame 453 $mg.L^{-1}$, and 212 to 228 $mg.L^{-1}$ in *Coca Cola Zero* Product, the degradation (lost % percentage) were 5.0 to 11.7 % for 2 and 4 weeks, respectively from the initial amount of aspartame 240 $mg.L^{-1}$, when both products exposured to sun heating for 2 and 4 weeks, , respectively.

4. Conclusion

The study on degradation of Asp in *Pepsi Light* and *Coca Cola Zero* at sun temperatures was carried out easily using the official method to determine the (%) Remaining of Aspartame.

Therefore as a conclusions all tested samples of both products of diet beverages (*Pepsi Light* and *Coca Cola* Zero) from different sources, were affected by increasing the temperature.

This project has been carried out to conduct a comparison of various beverages which use aspartame as sweeteners for diet and low calories beverages. By looking to the analytical results that are obtained in the evaluation of the effect of temperature as misconduct of shop keepers when they display their products in direct sunlight and temperature it has been found that *Pepsi Light* drinks had the highest percentage of degradation which was 13.2% from the initial concentration of aspartame 453 $mg.L^{-1}$. *Pepsi Light* drinks also had the highest mean level for the aspartame 453 $mg.L^{-1}$ and the *Coca Cola Zero* had 240 $mg.L^{-1}$.

Acknowledgements

The authors are thankful to Department of Food Control at Centre for Food and Drug Control (CFDC), Gurgi, Tripoli-Libya for providing raw materials, reagents as well as analysis facilities and also grateful for their Staff for support and cooperation.

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