

DETERMINATION AND COMPARISON OF ASPARTAME LEVELS IN SOME SELECTED SOFT DRINKS CONSUMED IN MUBI, ADAMAWA STATE, NIGERIA

ABSTRACT

Aspartame (ASP) is an Artificial Sweetener used to substitute sugar which was said to cause some health disorders. It is about 200 times sweeter than sugar, with almost the same calorie value of 17KJ per gram; a smaller quantity then, provides the same sweetness as sugar, consequently providing lesser calorie and thereby reducing the health disorders associated with sugar intake. However, some health disorders have been said to associate with high intake of aspartame, and especially PKUs who must completely avoid sources of Phenylalanine in their diet. This research has therefore investigated the presence and concentrations of Aspartame in five soft drink samples consumed in Mubi, Adamawa State. Ninhydrin test was used for the Qualitative analysis and High Performance Liquid Chromatography (HPLC) was used for the Quantitative analysis and the results were found to be $438.45 \pm 0.03 \text{ mg/L}$ in Zero Coke (ZRC), $365.81 \pm 0.02 \text{ mg/L}$ in Pepsi Lite (PSL), $525.36 \pm 0.02 \text{ mg/L}$ in Origin Zero (ORZ), $337.06 \pm 0.01 \text{ mg/L}$ in Sans Cream Soda (SNW) and $306.13 \pm 0.02 \text{ mg/L}$ in Malta Guinness Herbs Lite (MHL); none of these violated the standard of 3000 mg/L set by NAFDAC. PKUs, therefore, is to avoid these drinks and other consumers should not exceed the ADI recommendations.

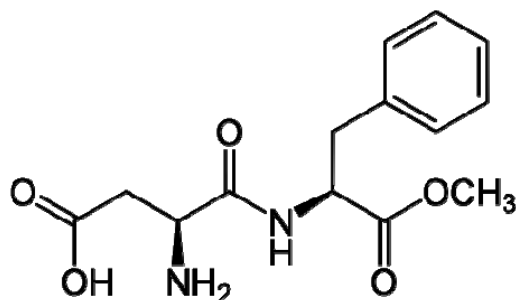
Key Words: Aspartame, soft drink, phenylketonuria, Mubi

1. INTRODUCTION

Food additives are substances added with or without consent to foods; these, as a result, may be expected to directly or indirectly become components or goes to affect the characteristics of any food. These substances may be used in production; processing, treatment or any phase of food utilization such as packaging, transportation or storage [1,2] to improve and sustain some desirable qualities of the food such as colour, taste, flavour etc. Additives are used in foods to maintain product consistency, to improve or maintain nutritional value, to maintain palatability and wholesomeness, to provide leavening or control acidity/alkalinity and to enhance flavour or impart desired colour [3,4,5]. Soft drinks typically contain carbonated water, a sweetener, and a natural or artificial flavouring. The sweetener may be sugar, high-fructose corn syrup, fruit juice, sugar substitutes (in the case of diet drinks), or some combination of these. All additives in foods are regulated, evaluated and approved for consumption by food regulating agencies such as FDA, EU – SCF, JECFA, NAFDAC and EFSA in different countries.

Aspartame, (also called NutraSweet, Equal, E951, and Equal – measure) a low calorie artificial sweetener like other protein substances has a caloric value of 17 KJ per gram. It has the chemical name, 3-Amino-N-(alpha-carbomethoxy-phenethyl)-succinamic acid or N-L-alphaaspartyl-L-phenylalanine-1-methyl ester, with the chemical formula, $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$ and its C.A.S number is 22839-47-0, density; 1.347 g/cm^3 molecular weight; $294.34 \text{ g/mol}^{-1}$. It is obtained by synthesis from two amino acids, L-phenylalanine and L-aspartic acid, which are also components of proteins in our body and in food. Approximately 50% of the Aspartame

43 molecule is phenylalanine, 40% is aspartic acid, and 10% is methanol; it was first approved
44 for use by FDA for dry goods in 1981 and carbonated beverages in 1983 [6]. The
45 phenylalanine in Aspartame is slightly modified by adding a methyl group which gives
46 Aspartame its sweet taste. Its structural formula can be seen in the figure below;



47
48 **Fig. 1. Aspartame structural formula**
49

50 Aspartame is a white crystalline powder and is about 200 times as sweet as sucrose [7], it is
51 widely used in food products including beverages, yoghurts, breakfast cereals and
52 confectionary products, and in pharmaceutical industries [7,8,9]; although it was said that,
53 high intake of Aspartame leads to various side effects such as Night vision, Headache,
54 Dizziness, Alzheimer disease, Seizures, Insomnia, Mental depression, Anxiety, Feeling
55 aggressive among others.

56 Aspartame is fully metabolized in our gut to aspartic acid and phenylalanine, which are
57 absorbed into the body; the methyl group modified from phenylalanine is then released to
58 form methanol which also gets absorbed, and most of it used to produce energy. All the
59 scientific studies to date in animals and human volunteers have shown that the breakdown of
60 Aspartame in the gut is very rapid and complete; no Aspartame has ever been found in the
61 blood or any organ after ingestion. Any effect reported to occur in the body following
62 ingestion of Aspartame will be caused by one or more of the three constituents; aspartic acid,
63 phenylalanine or methanol [10].

64 Phenylalanine is an essential amino acid, though cannot be biosynthesized; it is essential
65 because it can be converted into tyrosine in the body, this act as a precursor for a variety of
66 neurotransmitters like dopamine, noradrenalin (norepinephrine) and adrenalin (epinephrine)
67 and for such substances as thyroid hormone and melanin [11]. Phenylalanine and its
68 derivatives accumulate after ingestion of Aspartame in the blood, tissues and urine; this is
69 dangerous to individuals who have the disorder called, phenylketonuria; an inborn disorder
70 caused by lack of Phenylalanine Hydroxylase Enzyme, where phenylalanine cannot be
71 metabolized to tyrosine [12]. Consequently, such accumulation result in mental retardation,
72 growth retardation and hosts of other health disorders (Carol C., 2015).

73 To control these effects, the Joint FAO/WHO Expert Committee on Food Additives
74 (JECFA), the EU Scientific Committee for Food (SCF) and the European Food Safety
75 Authority (EFSA) have evaluated and established an acceptable daily intake (ADI) for

Aspartame as 40mg/kg body weight/day and that all foods containing Aspartame should indicate a warning on their label that it “Contains Aspartame, a source of Phenylalanine” [13] so as to help Phenylketonurics avoid such substances. In Nigeria, the NAFDAC approved concentration of Aspartame in soft drinks is 3000mg/L.

The evaluation of the presence and amounts of Aspartame in soft drinks consumed in Mubi, Adamawa State and awareness about such has, therefore, become necessary, as many of the population consumes Aspartame drinks; which may include even ignorant Phenylketonurics, also there is tendency that some industries may have used Aspartame above the accepted level or may not have even indicated it on their label that Aspartame is contained.

2. MATERIALS AND METHODS

2.1 Study Area

Mubi metropolis is a geo-political area comprising of two local government areas; Mubi North and Mubi South. The metropolis is located between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12' and 13° 19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307 Km² and support a total population 260,009 people (National Population Census 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and the Cameroon Republic in the East.

Varieties of Business activities take place in the area to meet the demand of the population. Though no industry is located in this area, many products from industries in other parts of the country are brought for consumption. The large market; located in the heart of the town, and other large and small-scale retail businesses and shop owners have been able to accommodate the different variety of products, especially soft drinks coming into the town, and provides them readily to the consuming population.

2.2 Samples and sampling

Five samples were collected randomly from shops in Mubi Main Market for the analysis; ZRC (Zero Coke, Coke Zero Sugar) produced by the Coca-Cola company, PSL (Pepsi Lite, Diet Pepsi) produced by PepsiCo company, ORZ (Origin Zero) produced by Guinness Nigeria Plc., SNW (Sans Cream Soda) produced by PharmaDeko Plc. and MHL (Malta Guinness Herbs Lite) produced by Guinness Nigeria Plc. Bottle (PET, Polyethylene Terephthalate) containers of ZRC, PSL, SNW and can containers of ORZ and MHL were used. The expiry date of the samples was cross-checked and those not expired were used.

2.3 Analysis

The presence (Qualitative analysis) of Aspartame in the samples collected was determined by Ninhydrin test; as was adopted by Rituet *al.*, (2015 [14]) and the amount (Quantitative analysis) of Aspartame was determined using HPLC.

2.3.1 Ninhydrin test (Qualitative analysis)

Ninhydrin Solution: 1.0g of Ninhydrin was dissolved in 50ml of 95% ethanol and 10ml of glacial acetic acid. Acetonitrile Phosphate Buffer: 10ml of acetonitrile was added to 90ml of 6.8g/Sol. of potassium dihydrogen phosphate and adjusted to pH 3.7 with phosphoric acid. 1.0ml of each sample was dissolved in water, and 0.5ml acetonitrile phosphate buffer (3.7pH) added which was kept for five minutes, after which 1.0ml Ninhydrin solution was added. This was warmed for seven minutes; this was done in turn for all the samples, and each of the samples gave a pink colour.

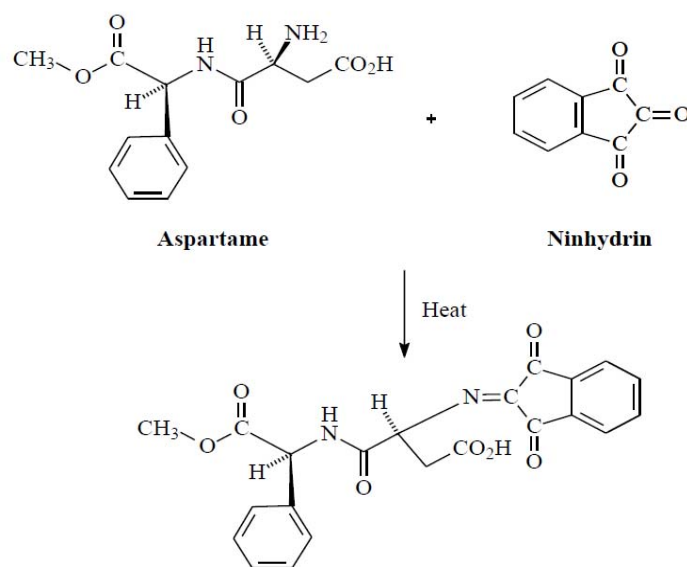


Fig. 2. The chemical reaction of Ninhydrin test

2.3.2 HPLC (Quantitative) Analysis

HPLC with UV detector; 220nm, integrator and C18 column, sampler and sampling pump cables of 1 to 3 L/min with flexible connecting tubes, syringes and syringe filters, vials, glass, scintillation, 20mL with PTFE-lined caps, pipettes of various sizes, tweezers, ultrasonic water bath, pH meter were the equipment used and Aspartame,TM 96% pure, deionized water, acetonitrile; distilled in glass, methanol; distilled in glass, 1-Heptanesulfonic acid sodium salt, monobasic potassium phosphate, phosphoric; acid reagent grade. Eluent: 2.062g of 1-Heptanesulfonic acid sodium salt was added to 1.0L deionized water and adjusted to pH 3.0 with dilute phosphoric acid. Calibration stock solution (5mg/mL): 0.05g Aspartame was diluted with 10mL of Eluent; these were the reagents used.

Six working standards were used for calibration; known amounts of calibration stock solutions were added to eluent using serial dilution to obtain a concentration in the ranges of 1 to 400mg/mL and analyzed with samples and blanks. A calibration graph (peak area vs. mass of Aspartame, mg per sample) was prepared. Sample aliquot was injected and the peak areas measured.

Conditions: 0.35ml/min Flow rate, 5min post time and 40°C in column compartment.

3. RESULTS AND DISCUSSION

The results obtained from Ninhydrin test (Qualitative analysis) indicated that all the samples contain Aspartame, as they all gave the “pink colour” after the test.

The results from HPLC Analysis (Quantitative analysis) are on table 1.

Table 1. Concentration (mg/L) of Aspartame in soft drinks samples with Mean $\pm$ SD

Samples	Tests			Mean $\pm$ SD
	First	Second	Third	
ZRC	438.45	438.47	438.42	438.45 $\pm$ 0.03
PSL	365.81	365.83	365.80	365.81 $\pm$ 0.02
ORZ	525.34	525.36	525.37	525.36 $\pm$ 0.02
SNW	337.05	337.07	337.05	337.06 $\pm$ 0.01
MHL	306.11	306.13	306.14	306.13 $\pm$ 0.02

Concentrations of Aspartame ranged from 306.11mg/L – 525.34mg/L in the samples analyzed; this is also shown as: ORZ > ZRC > PSL > SNW > MHL (Decreasing magnitude of Aspartame concentration in the samples)

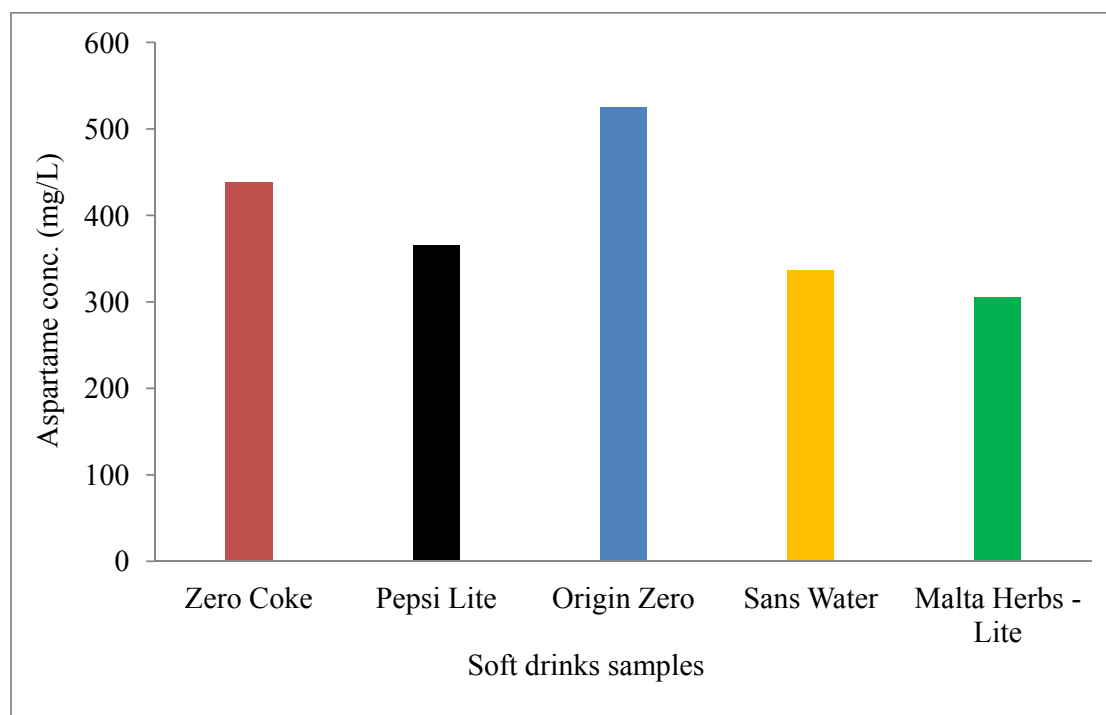


Fig. 3. The concentration of Aspartame in Soft drinks sold in Mubi

153 Ninhydrin (indane-1,2,3-trione) test has been used to test for the presence of Aspartame
 154 which contains the amino acids Phenylalanine and Aspartic acid; this is a sensitive method
 155 used for low amount sugar detection, up to 7.5 microgram. A positive test is indicated by the
 156 formation of a yellow coloured complex (figure 2). Thus a negative test with the absence of
 157 the yellow colour indicates the absence of Aspartame. From the Ninhydrin test carried out on
 158 the samples; the yellow colour was observed in all the samples analyzed, thereby confirming
 159 the presence of Aspartame in the soft drinks. [15] analyzed Aspartame contents of four Soft
 160 drink samples in Thailand, in the research work aimed at evaluating the identification and
 161 determination methods of Aspartame. [14] also analyzed the Aspartame content of ten Soft
 162 drink samples in India; Ninhydrin test was used to confirm Aspartame in the soft drinks, and
 163 all their samples also indicated a Positive result. This then implies that ZRC, PSL, ORZ,
 164 SNW and MHL actually contains Aspartame Sweetener as indicated on their labels; this is in
 165 accordance with the law set by FDA, NAFDAC, EU – SCF etc., that all food substances
 166 containing Aspartame should indicate "Contains Aspartame a source of Phenylalanine", as it
 167 will help Phenylketonurics to avoid such food substances in their diets.

168 The HPLC analysis results are represented on Table 1 for ZRC ($438.45 \pm 0.03\text{mg/L}$), PSL
 169 ($365.81 \pm 0.02\text{mg/L}$), ORZ ($525.36 \pm 0.02\text{mg/L}$), SNW ($337.06 \pm 0.01\text{mg/L}$) and MHL
 170 ($306.13 \pm 0.02\text{mg/L}$), the results indicate that all the samples contain Aspartame amounts not
 171 more than the NAFDAC recommendation of 3000mg/L . The analysis shows that ORZ has
 172 the highest concentration with $525.36 \pm 0.02\text{mg/L}$ and MHL has the least concentration with
 173 $306.13 \pm 0.02\text{mg/L}$; this is in close agreement with the results obtained by [16] where the
 174 mean values for the concentrations of Aspartame for samples A, B, C, D, E and F brands in
 175 Ankara Turkey, were found as $156.81 \pm 7.29\text{mg/L}$, $208.67 \pm 8.97\text{mg/L}$, $236.58 \pm 17.91\text{mg/L}$,
 176 $299.54 \pm 26.1 \text{ mg/L}$, $202.39 \pm 8.08\text{mg/L}$ and $223.28 \pm 14.08\text{mg/L}$, the data obtained from the
 177 analysis revealed that mean levels of Aspartame were found within the Turkish Food Codex
 178 (TFC) of 600mg/L in all the samples, however, some samples were not found appropriate
 179 according to the label information. The difference between the mean levels of Aspartame in
 180 samples of brands (A, B, C, D, E, F) and TFC values (600 mg/L) were statistically significant
 181 ($p < 0.001$). Also, the difference between the mean levels of two different brands (A and B) of
 182 the same firm was statistically significant ($p < 0.001$). However, the difference between the
 183 mean levels of two different brands (C and D) of the same firm were statistically insignificant
 184 ($p > 0.05$). From the results of the analysis carried out in this research, it can also be seen then
 185 that, all samples were within the NAFDAC standard of 3000mg/L in Nigeria.

186 [17] analyzed fourteen energy drink samples in Zaria, Nigeria for their physicochemical
 187 properties (pH, turbidity, conductivity and total dissolved solids), trace and heavy metals,
 188 Aspartame, sugar and caffeine; concentrations of Aspartame was found ranging from
 189 6.51mg/L – 1491.19mg/L which were also below the NAFDAC standard of 3000mg/L as
 190 were the results of this research. Although there is wide difference between the least and
 191 highest concentrations with those of this analysis; this could be due to the fact that NAFDAC
 192 has only set a standard for the maximum amount (3000mg/L) of Aspartame to be used. Thus
 193 any amount can be used so long as it has not exceeded the set standard.

[18] also found Aspartame concentrations ranging from 283.84ppm – 956.82ppm in ten brands of energy drinks; all these were also not more than the NAFDAC standard. The results from all these findings indicate that most Soft drinks industries comply with the standards set by the monitoring agencies.

From the **analysis carried out**, it can also be seen that ORZ and MHL produced by the same company; Guinness Nigeria Plc., has the highest (525.36mg/L) and least (306.13mg/L) concentration of Aspartame respectively, this implies that the company did not use the same concentration of Aspartame in these drinks which could mean that, the concentration of Aspartame in all their products are not consistent; this, of course, does not violate any set law. But, the company can do well to indicate the amounts of Aspartame contained in each of their products. Also considering that the highest and least level of Aspartame was contained in the soft drinks samples with can containers, while the intermediate levels were contained in samples with bottled containers; this should mean that the storage medium does not necessarily determine the Aspartame contents of soft drinks.

With regards to the ADI of 40mg/kg body weight established by the FDA, EU – SCF and other food control agencies; the results **from this analysis suggest that** an individual with 20kg body weight, for instance, can take 800mg of Aspartame daily. Such individual can therefore; take about seven bottles of 250ml (0.25L) ZRC which should contain 109.61mg of Aspartame each, or about Eight bottles of 250ml (0.25L) PSL which should contain 91.45mg of Aspartame each, or about Six bottles of 250ml (0.25L) ORZ which should contain 131.34mg of Aspartame each, or Nine bottles of 250ml (0.25L) SNW which should contain 84.27mg of Aspartame each, or Ten bottles of 250ml (0.25L) MHL which should contain 76.53mg of Aspartame each, daily; consumption of these drinks above this rate daily may subject such individual to the health disorders said to be associated with high intake of Aspartame. Also since Approximately 50% of the Aspartame molecule is phenylalanine, a Phenylketonuric(PKU) with 20kg body weight for instance takes in 54.81mg Phenylalanine per 250ml (0.25L) ZRC bottle, 45.73mg Phenylalanine per 250ml (0.25L) PSL bottle, 65.67mg Phenylalanine per 250ml (0.25L) ORZ bottle, 42.14mg Phenylalanine per 250ml (0.25L) SNW bottle and 38.27mg Phenylalanine per 250ml (0.25L) MHL bottle. From above it can be seen that only minute amounts of Phenylalanine are consumed in the samples, but because PKU actually arises due to accumulation of Phenylalanine in the body of Phenylketonurics (PKUs); its effect will manifest sooner or later with continuous consumption of these drinks, it is therefore rather recommended that PKUs should entirely not reduce, the intake of these drinks.

4. CONCLUSION

This research reveals that the amount of Aspartame sweetener in ZRC, PSL, ORZ, SNW and MHL are within the approved level in compliance with the NAFDAC standard, although the concentrations were not indicated on the labels. This research also confirms the presence of Aspartame in these samples as indicated on their label which is also by the requirement set by the food control agencies.

From the results obtained, all Phenylketonurics should, therefore, avoid these drinks as they contain Aspartame a source of Phenylalanine which their body system cannot convert to Tyrosine due to the absence of Phenylalanine Hydroxylase Enzyme, and also other consumers should adhere to the recommended amounts of these soft drinks in their daily intake in order to avoid the health disorders associated with high intake of Aspartame.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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