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CHEMICAL ANALYSIS OF SOME COMMERCIALLY SOLD VITAMIN C IN NIGERIA

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ABSTRACT

The chemical analysis of some commercially sold vitamin C tablet in Nigeria was investigated. The results showed that sweeteners were in the range of 9.65 – 10.25%, preservative in the samples were found to contain sodium benzoate, sodium metabisulfite, Sorbic acid and sodium propionate with same concentration (25mg/100g). The dye content were found to contain 1.60mg/ml to 4.78mg/ml. Vitamin C content were observed to contain 120.20 -128.59mg/100g of the sample. Sweeteners, preservative s and artificial dye in this study have been implicated to cause some health abnormalities both in infants and adults. The vitamin C content of the various tablets was of excellent recommendable dietary intake RDI value. The additives from this study should be considered by the pharmaceutical/food industries owing to their risk factor in such products. Since vitamin C is essential in our metabolic function which is more or less important in our daily nutrition, natural sources of vitamin C should be inculcated in our dietary lifestyle.

INTRODUCTION

Vitamin C (ascorbic acid) is an anti-oxidant that is essential for human nutrition. It belongs to the water soluble class of vitamins. Humans are one of the few species who lack the enzymes to convert glucose to vitamin C (Groff *et al.* 1995). The importance of vitamin C was first discovered in 1747 during the 16th Century, numerous sea Voyagers died due to the disease known as scurvy. Vitamin C deficiency can lead to scurvy which is characterized by abnormalities in the bones and teeth. James Lind found out that men suffering from scurvy were cured when given oranges and lemons and he published his findings in the treatise of the scurvy in 1753. These findings were not widely accepted by the rest of the world and scurvy continued to lead to wide spread death throughout the 19th century (Jacob *et al.* 1999). The Vitamin is an important aid in the absorption and conversion of iron to it's storage form. It has been proposed by some researchers to have pharmacological benefits in preventing cancer, infections and common cold. The role of vitamin C in preventing cancer is controversial, but has been studied for cancer of oral cavity, uterus, oesophagus, bladder and pancreas.

In Nigeria, the Vitamin C that is commercially sold has been implicated to low vitamin C content. Also most of the manufacturers have comprised the standard as against National Agency for good and Drugs which includes the presence of banned dye, presence of detrimental sweeteners and the use of deadly preservatives during production. As a result of ill standard practices, most of the prescribed vitamin C to patients has resulted to even more severe illness. These reasons have necessitated this research work. Therefore, the aim of this research is to evaluate the content of vitamin C, determination of preservatives, dye and sweeteners used in some commercially sold Vitamin C tablet in Edo State, Nigeria.

SOURCES OF VITAMIN C

A wide variety of food exists that contains vitamin C. It is widely known by the general public today that the sources of vitamin C are citrus fruits. Fruits with high vitamin C content are not limited to oranges, but also include lemons, peaches, and strawberries, bananas, and grapes fruits. A wide variety of other food also contain sufficient quantities of vitamin C like: cabbage, broccoli, caulis flower, leaf lettuce, tomatoes, potato and beans also have relatively high (7mg/100g to 163mg/100g) vitamin C content (Jacob *et al.* 1999).

ABSORPTION AND BIOAVAILABILITY

Absorption of vitamin C is greater when several individual doses of vitamin C, in quantities less than one gram are taken throughout the day rather than one mega dose (Jacob *et al.* 1999). At the intestine and cells ascorbic acid is oxidized to DHAA, which is more quickly transported across the cell membrane. Vitamin C can be impaired by a number of factors. A

single large dose saturates the enzymes kinetics for Vitamin C leading to excess ascorbic acid in the intestinal lumen, which causes numerous gastro intestinal problems. Furthermore, the bioavailability of synthetic and natural form of the Vitamin differ very little despite the claims made by manufacturers (Groff *et al.* 1995; Jacob *et al.* 1999).

TRANSPORT

Active transport is the main mechanism of vitamin C distribution within the body. Simple diffusion may occur in the mouth and stomach but accounts for only a very small percentage of uptake (Groff *et al* 1995). Sodium independent transport system shuttle vitamin C across the basolateral membrane of the intestinal cells. In the plasma absorbed ascorbic acid and DHAA can either be transported freely or be bound to albumin, ascorbate can also move into the body cells and tissues (Groff *et al.* 1995) as previously mentioned DHAA is the primary form of vitamin C that crosses cellular membranes.

STORAGE

Vitamin is stored throughout body tissues and blood. Ascorbic acid content of blood component, fluid, and tissues varies widely on an individual basis. Tissues concentration exceeds those found in the plasma by three to ten times. Energy driven transport pumps are responsible for the higher concentration of vitamin C versus the plasma. Both tissue and plasma levels of vitamin C is correlated to intake up to 90mg/day (Groff *et al.* 1995). Vitamin C content of cardiac tissue is between 28 and 85ml/100g wet weight, while that in skeletal muscle is approximately 17ml/100g wet weight, (Howald *et al.* 1975). Other tissues with intermediate level of vitamin C include the kidney, brain, liver, lungs and thyroid. The water-soluble properties of vitamin C prevent it from being stored in the adipose tissue of the body.

EXCRETION

The kidney plays a major role in vitamin C excretion and retention. DHAA and ascorbic acid can be reabsorbed by the kidney tubules as long as body pool levels are equal to or less than 1500mg. The average half-life of ascorbic acid is believed to be between 16 and 20 days (Jacob *et al.* 1999). Its half-life is inversely related to intake. The water-soluble properties of vitamin C lead to urinary excretion of the vitamin.

CURRENT DIETARY REFERENCE INTAKE (DRI)

The recommended daily allowance (RDA) has been replaced by a dietary reference intake (DRI) for vitamin C in the year 2000. In 1989 the recommended daily allowance was established at 60mg for adults. This level was believed to be sufficient enough to maintain body pool levels at 1500mg and does not differ from that established in 1980. A

recommended daily allowance for smokers was established because smokers have a higher turnover rate of vitamin C versus non-smoker (Groff *et al.* 1995). The DRI does not differentiate the need between smokers and non-smokers. DRI for vitamin C have been established at 90mg for men and 75mg for women (national Academic of Science et al, 2000).

TOXICITY

The saturable kinetics of vitamin C makes toxicity more likely when multiple large doses (~lg) are consumed throughout a day versus one single dose. A common symptom of unabsorbed vitamin C left in the gastro intestinal tract is osmotic diarrhea (Groff *et al*. 1995). Vitamin C can be transformed in the body to oxalate, which is a common constituent of kidney stones. Doses up to 10g have shown to be associated with higher prevalence of oxalate excretion, but the level does not fall outside of the normal range. As a precaution, people who are prone to kidney stones may want to avoid large doses (10 times the DRI or greater) of the vitamin (Groff *et al.* 1995). People who lack the control to regulate iron uptake should also avoid large doses of the vitamin. As stated earlier vitamin C enhances iron absorption which can lead to toxicity of iron in some people. Furthermore, excess ascorbate in the urine and feaces can falsify laboratory tests such as glucose in urine and fecal occult blood test.

PHYSIOLOGICAL ROLE

Vitamin C has been studied for many years. It participates in numerous biochemical reactions, suggesting that vitamin C is important for everybody process from bone formation to scar tissue repair (Groff *et al*. 1995). The only established role of the vitamin appears to be incurring and preventing scurvy. Vitamin C is the major water-soluble antioxidant within the body. The vitamin readily donates electron to break the chain reaction of lipid peroxidation. The water-soluble properties of vitamin C allow for the quenching of free radicals before they reach the cellular membrane. Tocopherol and glutathione also rely on ascorbic acid for regeneration back to their active isoforms. The relationship between ascorbic acid and glutathione is unique. Vitamin C reduced glutathione back to the active form. Once reduced, glutathione will regenerate vitamin C from its DHAA or oxidized state. A well-known function of ascorbic acid is the role it plays in hydroxylation reactions that are essential for the formation of collagen. Vitamin C is important in collagen formation as it allows for a tight cross-linking of the triple helix, thereby resulting in stabilization of the peptide. Evidence also suggests that ascorbic acid may be involved in collagen gene expression. Carnitine synthesis prefers to use vitamin C as the reducing agent. Carnitine facilitates the beta-oxidation of fat, through its role transporting long chain fatty acids from the cytoplasm into the mitochondrial matrix of cardiac and skeletal muscle. Vitamin C is directly involved in the enzyme activity

of two copper dependent mono-oxygenases which are important in the formation and conversion of iron to its storage form. The protection of neural and endothelial tissue, along with effects on cellular tone can also be attributed to vitamin C. (Groff *et al.* 1995; Jacob *et al.* 1999). Vitamin C has been proposed by some to have pharmacological benefits in preventing cancer, infections, and the common cold. However, these benefits have yet to be reported in the scientific literature. The role of vitamin C in preventing cancer is controversial, but has been studied for cancers of the oral cavity, uterus, oesophagus, bladder, and pancreas. The research is at best equivocal and more studies are needed to further address the role of Vitamin C in preventing cancer.

ANTIOXIDANT

Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. When there are more free radicals (reactive oxygen species ROS) in the human body than antioxidants, the condition is called oxidative stress (Mayne, 2003) and has an impact on cardiovascular disease, hypertension, chronic inflammatory diseases, diabetes (Tak, 2000; Bruch, 2002; Schorah, 1996; Jacques, 1995) as well as on critically ill patients and individuals with severe burns (Mayne, 2003). Individuals experiencing oxidative stress have ascorbate blood levels lower than 45 μmol/L, compared to healthy individuals who range between 61.4-80 μmol/L (Fortherby *et al.* 2000). It is not yet certain whether vitamin C and antioxidants in general prevent oxidative stress-related disease and promote health. Clinical studies regarding the effects of vitamin C supplementation on lipoproteins and cholesterol have found that vitamin C supplementation does not improve disease markers in the blood. (fortherby *et al*. 2000; Simona, *et al*. 2007) vitamin C may contribute to decreased risk of cardiovascular disease and strokes through a small reduction in systolic blood pressure (Mayer-Daris *et al*. 1997) and was also found to both increase ascorbic acid levels and reduce levels of resisting serum, (Bjelakovi *et al*. 2007) another likely determinant of oxidative stress and cardiovascular risk. However, so far there is no consensus that vitamin C intake has an impact on cardiovascular risks in general, and an array of studies found negative results (Satoh and Sakagami, 1997) meta-analysis of a large number of studies on antioxidants including vitamin C and mortality (Muhulhofer *et al*. 2004).

DAILY REQUIREMENTS

The North American dietary reference intake recommends 90 milligrams per day and not more than 2 grams (2,000 milligrams) per day (Milton, 2003). Other related species sharing the same inability to produce Vitamin C and requiring exogenous vitamin C consume 20 to 80 times this reference intake (Knowledge of Health, 2004). There is continuing debate with the scientific community over the best dose schedule (the amount and frequency of intake) of

vitamin C for maintaining optimal health in humans. A balance diet without supplementation usually contains enough vitamin C to prevent scurvy in an average health adult. A 1999 review suggested a dose of 90-100mg vitamin C daily is required to optimally protect against these diseases, in contrast to the lower 45mg daily required to prevent scurvy.

High doses (thousands of milligrams) may result in diarrhea in healthy adults, as a result of the osmotic water-retaining effect of the unabsorbed protein in the gastro intestinal tract (similar to cathartic osmotic laxatives). Proponents of orthomolecular machine claim the onset of diarrhea to be an indication of where the body's true vitamin C requirement lies, though this has not been chemically verified.

CHANCE OF OVERDOSE

Vitamin C is water soluble, with dietary excesses not absorbed and excesses in the blood rapidly excreted in the urine. It exhibits remarkably low toxicity. The LD50 (the does that will kill 50% of a population) in rats is generally accepted to be 11.9g per kilogram of body weight when given by forced gavage (orally). The mechanism of death from such doses (1.2% of body weight, or 0.84 kg for a 70kg human) is unknown, but may be more mechanical than chemical. The LD50 in humans remains unknown, given lack of any accidental or international poisoning death data. However, as with all substances tested in this way, the rat LD50 is taken as a guide to its toxicity in humans.

MATERIALS AND METHODS

SAMPLE PREPARATION

Eight samples were purchased from different patent chemist in Auchi township of Etsako West Local Government Area of Edo state, Nigeria, October 2018. The samples are from A(Emzor), B(Cika), C(Esehi), D(Day by Day), E(Emzor White), F(Nutric C), G(Mekophar) and H(Juhel) vitamin C tablets.

DETERMINATION OF VITAMIN C (ASCORBIC ACID)

PRINCIPLE:

Ascorbic acid reduces oxidation:- Reduction indicator dye, 2,6-diichloroindophenol to colourless solution. At the end point, excess unreduced dye is rose pink in acid solution. The determination of vitamin C was done according to AOAC (2005). Vitamin C (ascorbic acid) is extracted and titration performed in the presence of Metaphosphoric acetic acid solution (HPO_3-CH_3COOH) which helps to maintain proper acidity for reaction and to avoid autoxidation of ascorbic acid at high P^H.

EXTRACTING SOLUTION

Meta-phosphoric acid-acetic acid solution: 15g of meta-phosphoric acid was dissolved in 40ml acetic acid and 200ml distilled water, this was then made up to 500ml with distilled water. The solution was then filtered rapidly through a filter paper whatman No. 1 into a glass-stoppered bottle and stored in the refrigerator until required for usage.

ASCORBIC ACID STANDARD SOLUTION

100mg L-ascorbic acid was accurately weighed, dissolved and volume made up to 100ml immediately with meta-phosphoric acid acetic solution ($HPO^{3-}CH_3COOH$).

INDOPHENOLS STANDARD SOLUTION

50mg 2,6-dichlorophenol Na salt was dissolved in 50ml distilled water to which has been added 42mg $NaHCO_3$ and then made up to 200ml distilled water. The mixture was then filtered through filter paper into amber glass-stoppered bottle, stored in the refrigerator and standardizes every day before usage.

STANDARDIZATION OF DYE

To 2ml of standard ascorbic acid solution was added 5ml of metaphosporic –acetic acid. Titration was then performed rapidly with indophenols dye until light but distinct rosepink persists for more than 5 seconds (the titration should require about 15ml indophenols solution and titration check within 0.1ml.

Procedure

10ml of the sample were immediately extracted with 200ml meta-phosphoric acetic acid solution by blending with warring blender for 3 minutes.

The resulting slurry was then filtered and 50ml of the juice extract was titrated with the dye solution, at the end point the colour changes to rose pink that persist for more than 5 seconds.

Calculation

Mg ascorbic acid/100g = (x –B) x $\frac{F}{E} \times \frac{Y}{F} \times 100$

Where x= Average volume ml for test solution titration

B= Average unit ml for test blank titration

F= mg ascorbic acid equivalent to 1.0ml indophenol standard solution

E= Weight of sample

V= Volume of initial test solution

Y= Volume of test solution titrated.

DETERMINATION OF DYE

The determination of dye was according to the method of Braz (2005).

BROMATED-BROMIDE MIXTURE

A 5mmol^{-1} $KBrO_3$ 50mmol^{-1} KBr solution was prepared by dissolving accurately weighed 418mg of $KBrsO_3$ (sarabhai M chemicals, Baroda, India) and 3g of KBr (qualigens India Ltd. India) in water and diluted to the mark in a 500ml calibrated flask and this solution was used in titrimetric work. For use in spectrophotometric study, a 1000μgml^{-1} $KBrO_3$ solution containing a large excess of KBr in water and diluting to the mark in 100ml calibrated flask this was diluted stephiose to get 10μgml^{-1} and 30μgml^{-1} bromate solutions for use in method B and C respectively.

METHYL ORANGE (50 μgml^{-1})

First, a 500 μgml^{-1} solution was prepared by dissolving 59mg of dye (s.d. fine chem., India, dye content 85%) in water and diluting to the mark in a 100ml calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 μgml^{-1} .

INDIGO CARMINE (200 μgml^{-1})

A 1000 μgml^{-1} solution was first prepared by dissolving 111mg of dye (s.d. fine chem., India, dye content 90%) in water and diluting to the mark in a 100ml calibrated flask and filtered. The stock solution was diluted appropriately to get 200 μgml^{-1} dye solution with water.

SODIUM TRIOSULPHATE

About 8g of chemical (sharabhai M chemicals, Baroda, India) was dissolved in 1L of water and the solution was standardized iodometrically using a pure sample of potassium dichromate.

HYDROCHLORIC ACID (5mol^{-1})

112 ml volume of concentrated acid. (s.d. Finechem, Munbia, India, SP gr 1.18) was diluted to 250ml with water and mixed well.

POTASSIUM IODIDE (10%)

It's prepared by dissolving 25g of chemical (Qualigens Fine chemicals, India) in 250ml of water.

STARCH INDICATOR (1%)

1g of starch paste made in water was slowly poured into 100ml boiling water, boiled for I minute and cooled.

ASTEMIZOLE STANDARD SOLUTION

A 2gml^{-1} standard drug solution was prepared by dissolving 500mg of pharmaceutical grade astermizole (received from UCB India Ltd., Mumbai, India) in 25ml of glacial acetic acid and diluting to the mark with water in a 250ml calibrated flask and was used in titrimetry. This

solution was then diluted with water to get 10 μgml^{-1} and 25 μgml^{-1} solutions for used in method B and method C respectively.

PROCEDURE

VISUAL TITRIMETRY (METHOD A)

A 100ml aliquot of pure drug solution containing 4-16mg of AST was accurately transferred into a 100ml Erlenmeyer flask. 10ml of bromated-bromide solution (5mmol^{-1} w.r.t $KBrO_3$) was transferred to the flask by means of a pipette. The solution was acidified by adding 7ml of 2 mol^{-1} hydrochloric acid. The flask was stoppered, the content mixed well and kept aside for 15 minutes with occasional swirling. The stopper was then washed with 5ml of water and 5ml of 10% potassium iodide solution was added to the flask. The liberated iodine was titrated with 0.03mooll-1 sodium thiosulphate to a starch endpoint. A blank titration was run under identical conditions.

The amount of drug in the measured aliquot was calculated from:

Mg= $\frac{(B-S)M\omega R}{X}$

Where B= volume of thiosulphate consumed in the blank titration in ml

S= volume of thiosulphate of bromated solution in mol^{-1}

$M\omega$= Relative molecular mass of drugs

R= Concentration of bromate reaction with each mole of drug.

SPECTROPHOTOMETRY WITH METHYL ORANGE (METHOD B)

Different aliquot, (0.5 - 4.0ml) of 10 μgml^{-1} AST solution were accurately measured into a series of 10 μl calibrated flasks and the total volume was adjusted to 5ml with water. To each flask was added 1ml each of bromated-bromide solution (10 μgml^{-1} w.r.t $KBrO_3$) and 5mol^{-1} hydrochloric acid. The flasks were stoppered, contents mixed well and let stand for 15minutes with occasional shaking then 1ml of 50 μgml^{-1} methyl orange solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 520nm against a reagent blank after 10minute.

SPECTROPHOTOMETRY WITH INDIGO CARMINE (METHOD C)

Varying aliquots of standard AST solution (0.5-5.0ml, 25 μgml^{-1}) were transferred into a series of 10ml calibrated flasks by means of a micro burette, and the total volume was brought to 5ml by adding water. Accurately measured 1.5ml of bromated-bromide solution (30 μgml^{-1} w.r.t $KBrO_3$) was added to each flask followed by 1ml of 5mol^{-1} hydrochloric acid. The flasks were stoppered, contents mixed and allowed to stand for 15 minutes with occasional shaking then, 1ml of 200 μgml^{-1} indigo carmine solution was added to each flask and diluted to the mark with water. The absorbance was measured at 610nm against a reagent blank after 10 minutes.

In methods B and C, a calibration graph was prepared by plotting absorbance against concentration of drugs and concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the beer's law data. An amount of powdered tablets and syrup equivalent to 100mg of AST was extracted with chloroform (4x10ml), the combined extracts were evaporated on a steam bath and the residue was treated as for preparation of standard drug solution. A convenient aliquot was then subject to analysis by either method.

DETERMINATION OF SWEETENER

The determination of sweetener was according Nahrung (1985).

HPLC system (waters) consisting of a 600E controller pump, a 717 plus auto sampler, 2996 ASCORBIC ACID detector and an inline-degasser. Millinium 32 software for data processing and silica (waters, spherisorb 5 μm silica, 4.6 x 250mm) analytical column was used for separation. The mobile phase consisted of (hexane: ethyl acetate) (60:40, V/V, PH adjusted to 4.5 using 1% acetic acid), with flow rate of 1ml/min. The analysis was carried out at ambient temperature.

STANDARD SOLUTION

Stock solution of aspartame (0.2mg/ml) was prepared by dissolving in water. For drawing calibration curve five different concentrations in linearity range were prepared by diluting stock solution.

SAMPLE PREPARATION

Accurate weight of powdered tablets equivalent to 10mg of drug was transferred quantitatively to 100ml volumetric flask with the aid of 50ml of water. The content of the flask was sonicated for 10 minutes in ultrasonic bath, the flasks were filled up to the mark with water and filtered before derivatization. In a 10ml, screw cap test tube 1ml of each standard solution was added. This was followed by addition of 1ml of 0.2 mol/L sodium bicarbonate ($NaHCO_2$) solution and 1ml of 1% DNFB (a 1ml portion of DNFB was dissolved in 100ml of 1,4-dioxane) these test tubes were then placed on a water bath (60^oC) for 40 minutes. All derivatization reactions were stopped by addition of 0.5ml of 1mol/LHCL. The resulting dinitrophenylanrine (DNP) derivative was extracted with 5ml ethyl acetate and made up to 10ml with mobile phase. All the samples were treated in the same manner as described above for standard. To avoid interference from components soluble in ethyl acetate, all the sample were pre-extracted with 5ml of ethyl acetate before derivatization. Ten (10) μl of each standard sample were injected into the HPLC system.

DETERMINATION OF PRESERVATIVE

PLATE-DIFFUSION ASSAY (THIOMERSAL)

Flat-bottomed petin plates (100mm by 15mm, manufactured specifically for antibiotic assays) were employed. For acceptable results, it is essential that the plates should be placed on a completely level bench or table. To each plate, 21ml of a base agar (BBL antibiotic medium No. 2) 2 were added and allowed to harden for 2 hours; then 4ml of seed agar (BBL antibiotic medium No.1) 2 inoculated with the test organism were layered evenly over the base agar and left for 5-10 minutes. The test organism was maintained on nutrient agar slants (BBL antibiotic medium No.1) by transfer at 14-day intervals. To prepare the test culture, 150ml of the medium were poured into a sterile, cotton-plugged roux bottle and allowed to harden and then dry for 24hours. The medium was then inoculated by spreading evenly over the surface of the growth of a 24-hour slant, which had been washed off with 2.0ml of BBL antibiotic medium No. 3.2. The Roux bottle was incubated at 35^{o}C for 24 hours and the growth was washed off with 80ml of the BBL medium No. 3. Such a suspension may be used for 3-4weeks if it is maintained under refrigeration. To prepare the seed –layer innoculim, 1.0ml of the stock suspension was added to 150ml of seed agar which had been melted and cooled at 52^{o}C. a standard thiomarsal solution was made from reagen-grade crystals and dilutions of 1:2500, 1:5000, 1:10,000 and 1:20000 were prepared, only three consecutive dilution were used for an assay. At the same time, equivalent dilutions of the drug under test were prepared, based on the statement on the label regarding the amount of preservative present. Four plates were used for each dilution; 2 cylinders on each plate were filled to the brim with a given dilution of the standard and the other 2 were filled in a similar manner with the equivalent dilution of the test solution. The plates were covered with porcelain covers glazed only on by one, the outer, side and then incubated at 37^{o}C for 18-24 hours. Subsequently, the zones of inhibition were measured and recorded, the average size of the zone for the different dilutions being determined. Activity regression lines were prepared on semi-logarithm paper and the amount of phenol present in the unknown was determined by direct comparison with the standard.

RESULT

The result of the various experiment carried out are shown below in table 1.

Table 1

Concentration of sweetener (%), preservative (mg/100g), dye (mg/ml) and Vitamin C content (mg/100g) in some commercially sold vitamin C tablets.

S/N	Sweetener type	%	Preservative type	Mg/100g	Vit. C mg/100g	Dye mg/ml
A	Aspartame	10.25	Sodium benzoate	25.0	125.25	1.60
					125.23	4.58
B	Sucralose	9.98	Sodium propionate	25.0	120.55	4.63
					120.57	4.62
C	Saccharin	9.65	Sodium metabisulphite	25.0	128.59	4.55
					128.57	4.52
D	Asesulfame k	9.75	Sorbic acid	25.0	125.55	4.48
					125.56	4.45
E	Aspartame	10.15	Sodium benzoate	25.0	123.33	4.38
					123.34	4.33
F	Aspartame	10.22	Sodium metabisulphite	25.0	120.47	4.28
					120.46	4.26
G	Sucralose	9.88	Sodium benzoate	25.0	122.2	4.77
					122.24	4.78
H	Aspartame	10.05	Sodium propionate	25.0	120.65	4/60
					120.64	4.58

Values are means of triplicate determination (+SEM)

The above result for sweetener shows that sample A has the highest percentage of sweetener while sample C has the least percentage of sweeteners. In preservative all the samples have equal value. While in vitamin C content sample C has the highest mg/100g value while sample F has the least vitamin C content. In Dye sample G has the highest value of dye while sample A has the least value.

DISCUSSION

PRESERVATIVE

The preservative used in the vitamin C tablets from the study carried out are sodium benzoate, (sample A, E and G), Sodium propionate (sample B and H), sorbic acid (sample D) and sodium metabisulfite (sample C and F). They were observed to have the same concentration (25mg/100g). The present of benzoate in food is very safe for most people, though they cause hives, asthma, and or other allergic reaction in sensitive individuals (FDA, 2008). Sodium Benzoate used in Beverages that also contains ascorbic acids, can react together to form small

amount of benzene when present in an acidic solution, a chemical that causes Leukemia and other cancer (FDA, 2008). Some of the food preservatives used to stabilize and protect food can actually harm human when eating in excess, according to the consumer advocacy group, the center for science in the public interest (CSPI). Sodium bicarbonate contribute sodium to food, which can raise a person blood pressure when eating too often. People on low sodium diet for hypertension may experience a worsening of their condition if they consume food with sodium bicarbonate (USDA, 2011). The two food preservative that can cause allergies are sodium bisulfite and sodium metabisulfite in sample C and G. According to (WHO, 2008), the consumption of preservatives may have harmful effect on the pancreas. Elimination of the intake of hazardous preservatives and chemical food additives help to reduce the risk of developing pancreatitis (an inflammation of the pancreas) (FDA, 2008). It also helps to reduce symptoms associated with pancreatitis such as nausea, vomiting, fever, pain, and abnormal tenderness. Among all, sorbic acid observed in sample D is generally regarded as safe (GRAS) (FDA, 2008).

SWEETNER

The sweetner identified in the study are aspartame (sample A, E, F and H), saccharin (sample C), Sucralose (sample B, G) and asesulfume K (sample D). From the result it was shown that Aspartame have the highest percentage of sweetner. Excess consumption of aspartame might cause cancer or neurological problems such as dizziness or hallucination (Food and Drugs Agency, 2008). Large dosage of Asesulfume K can affect the thyroid gland of human and animals and small amount of it is not harmful to the body (World Health Organisation, 2007). Asesulfume K and Saccharin is about 200 and 350 times sweeter than sugar respectively (United State Department of Agriculture, 2011). From this study, Saccharin was shown to have the least percentage of sweetner. This sweetner is usually found in diet, soft drinks and excess of it can cause cancer of the urinary bladder, uterus, ovaries, blood vessels, skin and other organs and it increase the potency of other cancer causing chemicals (FDA, 2011). Sucralose is an artificial sweetner found in baked food, Ice cream, frozen desert and other food product. Sucralose is safer than Saccharin, asesulfume K and cyclamate (FDA, 20111). The centre for science in the public interest (CSPI) have indicated in a research conducted that sucralose causes premature shrinkage of the thymus gland (part of the immune system). Although other studies carried out have implicated sucralose to be safe (FDA, 2011).

DYE

The presence of Dye (artificial dye) was determined during this study. The highest concentration of Dye was found in sample G while the least concentration was obtained in sample A. the presence of artificial dye in drugs and food products have been implicated to the cause of Cancer (FDA, 2008). The Dye in all the samples were obtained to have almost the same range of concentration (mg/ml). The presence of Dye in food causes liver Cancer, possible carcinogen (USDA, 1956). High level of Dye damage the adrenal cortex of dog and internal organs (FDA, 2008).

VITAMIN C

According to WHO (2007), NAFDAC (2009) and FDA (2008) vitamin C is a good natural antioxidant in the body. Fruit and vegetable are the best sources of vitamin C (USDA, *et al.* 2011). Citrus fruits, tomatoes and tomatoes juice and potatoes and are major contributors of vitamin C to the American diet (Institute of medicine, 2000). The average daily recommended allowance sufficient to meet the nutrient requirement involves (97 -98%) healthy individuals. From this study the vitamin C content investigated are sufficient (120.64 – 125.56 mg/100g) to meet Recommended Dietary Intake (RDI). Excess accumulation of vitamin C causes mist common complaints such as diarrhea, nausea, abdominal cramp and other gastrointestinal disturbance due to osmotic effect of unabsorbed vitamin C in GIT (Jacob *et al.* 2002; Institute of Medicine, 2000). Due to its function as antioxidant and its role in immune function, vitamin C has promoted as a means to help prevent and/or treat numerous health conditions. Vitamin C has low toxicity and is not believed to cause serious adverse effects at high intake (Institute of Medicine, 2000). Therefore the vitamin C contents of the various tablets investigated are found to be rich in vitamin C to meet normal metabolic functions.

CONCLUSION

The relevance of vitamin C cannot be over emphasized. The vitamin C tablet investigated resulted to an excellent recommendable dietary intake RDI value for all samples. The level of preservative, sweetener and dye in the tablet may pose a risk factor for infant and human health. Further research should be considered on the types of dye used in the vitamin C tablets so as to evaluate the hazardous effect of the artificial dyes, and possibly quantify them.

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