Journal of Advances in Microbiology

13(4): 1-8, 2018; Article no.JAMB.45704
ISSN: 2456-7116

Evaluation of the Fate of Saccharin during Storage of Sobo Drink

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Authors' contributions

This work was carried out in collaboration between all authors. Author KBA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AAF and ADI managed the analyses of the study. Author AUR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/45704
Editor(s):
(1) Dr. Luciana Furlaneto-Maia, Technological Federal University of Paraná, Brazil.
(2) Dr. Muhsin Jamal, Assistant Professor, Department of Microbiology, Abdul Wali Khan University, Garden Campus, Pakistan.
Reviewers:
(1) Dennis, Amaechi, Veritas University, Nigeria.
(2) Takeshi Nagai, Graduate School of Yamagata University, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/27901

Original Research Article

Received 24 September 2018
Accepted 05 December 2018
Published 21 December 2018

ABSTRACT

Artificial sweeteners such as saccharin were used in place of sugar in the production of sobo drink. Sobo (*Hibiscus sabdariffa*) is a local drink produced by boiling the Sobo (*Hibiscus sabdariffa*), sieving out the calyces and addition of artificial sweetener such as saccharin Upon ingestion of the sobo drink, saccharin goes through the human digestive system where it is neither absorbed nor metabolised; it is excreted unmodified via the kidney. Samples were collected from Kasuwar Daji market in sokoto. The drinks were stored at room temperature and monitored for 21 days. The aim of this study is to evaluate the fate of saccharin during storage of sobo drink using microbial load and spectrophotometer. Bacteria load and type was determined using standard bacteriological analysis. The concentration of saccharin in the spoilt sobo drink was determined using spectrophotometer. GC-MS analysis was used for the identification and quantification of volatile compounds. The degradation process was found to mostly occur on the third day and the twelfth

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day of the storage, the degradation rate was found to be higher in lower concentrations of saccharin and lower in higher concentration of saccharin. The bacteria species isolated from the sobo drink, includes *Bacillus subtilis, Bacillus pumilis, Bacillus azotomonas, Micrococcus varians, Aeromonas hydrophila, Enterobacter aeromonas, Lactobacillus acidophilus*. This research shown that prolonged storage results in the predominance of common spoilage bacteria and coliform bacteria in the sobo drink and were found to be capable of degrading saccharin which results in the formation of 2-sulfano benzamide as the degradation product.

Keywords: Artificial sweeteners; Sobo; biodegradation; Bacillus spp; Micrococcus spp.

1. INTRODUCTION

Artificial sweeteners are substances that are used in place of sweeteners like sugar (sucrose) or sugar alcohols [1]. They may also be called sugar substitutes, non nutritive sweeteners (NNS), and non caloric sweeteners [1]. Sugar substitutes can help people trying to lose weight. They provide sweetness to foods and drinks without adding extra calories. Using artificial sweeteners in place of sugar can also help prevent dental decay and aid in blood sugar control in people with diabetes, all artificial sweeteners are chemically processed, usually added to food during preparation and can also be added during eating. Most diet or low-calorie food products sold at the store are made using artificial sweeteners [1]. Saccharin has no food energy and no nutritional value. It is safe to consume for individuals with diabetes. In the sobo drink, saccharin goes through the human digestive system where it is neither absorbed nor metabolised; it is excreted unmodified via the kidney unchanged in the urine, it is not metabolised [2] and the unabsorbed portion is excreted with faeces [3]. Saccharin is very stable, its solutions buffered at pH ranging from 3.3 to 8.0 were essentially unchanged.

Saccharin occurs in groundwater due to old landfills, application of fertilisers in agriculture, degradation of sulfonylurea herbicides, irrigation, soil water management, use of sludge as a fertiliser and leaks in the ducts. Saccharin and its salts are found in municipal wastewater and sewage [3]. In the 1970s, studies performed on laboratory rats found an association between consumption of high doses of saccharin and the development of bladder cancer [4]. However, further study determined that this effect was due to a mechanism that is not relevant to humans. Epidemiological studies have shown no evidence that saccharin is associated with bladder cancer in humans [4]. The International Agency for Research on Cancer (IARC) originally classified saccharin in Group 2B("possibly carcinogenic to humans") based on the rat studies, but downgraded it to Group 3 ("not classifiable as to the carcinogenicity to humans") upon review of the subsequent research [5]. Saccharin is an artificial sweetener that has been used for over a century to sweeten food and beverages without adding calories or carbohydrate. It is found in food such as soft drinks, baked goods, chewing gum, canned fruits, salad dressing, cosmetic products and pharmaceuticals. Upon ingestion, saccharin goes through the human digestive system where it is neither absorbed nor metabolised; it is excreted unmodified via the kidney. On this basis that saccharin is not metabolised, the Food and Drug Administration of the USA considers it safe [6]. Most of the commercial local drinks produced are made using one or more artificial sweeteners such as Saccharin. These drinks when consumed and excreted end up in the environment. Little is known on the activity of microbes and chemical fate of saccharin during storage of Sobo drink.

This study focuses on knowing the fate of Saccharin in the Sobo drinks during storage for 21 days. Although artificial sweeteners are not classified as pollutants, there is increasing concern that continued discharge by urination and accumulation of these sweeteners may have huge environmental effect in the long run. For this reason, and the fact that little is known about the occurrence and fate of this saccharin in Sokoto, it is necessary to provide this information to fill in the knowledge gap. The aim of this study is to evaluate the fate of saccharin during storage of sobo drink using microbial load and spectrophotometer.

2. MATERIALS AND METHODS

The sobo drink was prepared by rinsing the calyces and sample was taken from the rinsing water and cultured on a nutrient agar medium. After decanting the rinsing water, clean water was added and the mixture was boiled for 15 minutes at 100ºC. After boiling the mixture was

allowed to cool and then the Calyx was sieved out of the drink. The drink was divided into three (3) sets, first set of the drink was prepared without sugar or saccharin which serves as the control, In the second set of the drink 2.0g sugar was added to the drink and In the third set, saccharin was added with five different concentrations, that is; 0.25, 0.5, 1.0, 1.5 and 2.0 g/L of Sobo drink.

2.1 Storage and Monitoring the Fate of the Drinks

The (sets 1-3) of the sobo drinks was stored and monitored for 21days. The Sobo drinks was kept at room temperature and was monitored until evidence of spoilage is manifested either by off odour / taste.

2.2 Determination of Bacterial Load and Population

The determination of bacterial load and population started from the production i.e day 0 to the 21st day. The drink was serially diluted in three dilution factors and the last dilution factor was inoculated on nutrient agar. The plates were incubated for 24 hours at 26ºC. Following incubation, the colonies were selected and propagated on the same media until pure cultures were obtained and use for further studies. Characterisation of isolated microflora employed cultural and microscopic examination as well as conventional physiological and biochemical tests such as patterns of sugar fermentation, assimilation of sugars, and production of certain enzymes. At intervals, colonies were randomly picked from incubated plates, purified by repeated sub-culturing before being examined microscopically for Gram reaction [7], cell morphology (using 24 h old cultures), motility, pigmentation and sporulation [8]. Biochemical analysis included catalase and oxidase activities, nitrate reduction, patterns of sugar utilisation as well as urea and starch hydrolysis [9].

2.3 Spectrophotometric Determination of Saccharin Degradation in the Sobo Drink

The rate of saccharin degradation in the sobo drink was determined using spectrophotometer [10]. The method is based on bromination of saccharin to form N-bromo derivative, which on reaction with potassium iodide liberates iodine, imparting yellow colour to the solution. On addition of surfactant cetyl trimethyl ammonium bromide, the intensity of yellow colour increases; it is then extracted in isoamyl alcohol. The absorption maximum was observed at 400 nm, and Beer's law was found to obey over the range 1.5-15 µg of saccharin per 50 ml final solution (0.03-0.3 ppm). To an aliquot of working standard containing 1.5-15 µg of saccharin, 0.5 ml of bromine water was added, and the mixture was shaken gently for 2 min. The excess of bromine was removed by drop-wise addition of formic acid, after which 1 ml of potassium iodide was added. The yellow solution obtained was shaken gently for a few seconds, and 1 ml of cetyl trimethyl ammonium bromide was added and shaken well. The solution was made up to 50 ml mark and transferred to a 100 ml separating funnel. The yellow colour was extracted in 2 × 3 ml of isoamyl alcohol, and the absorption was measured at 400 nm against reagent blank.

2.4 GC-MS Analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with flame ionisation detector (FID). The derivitasation was performed using 0.5ml of TRI-SIL reagent (Thermo-scientific,UK) and the injection was conducted in split less mode at 250°C for 3 minutes by using an inlet of 0.75 mm to minimise peak broadening. Chromatography separations were performed by using DB-WAX analytical column 30 m 0.25 mm, 0.25 mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/min. The oven temperature was programmed at 60°C for 5 min, followed by an increase (held for 5 minutes), and finally at 10°C/min to 280°C (held for 10 min). The temperature of the FID was set to 250°C. MS operating conditions (electron impact ionisation mode) were an ion source temperature of 200°C, ionisation voltage of 70 eV and mass scan range of m/z 23- 450 at 2.76 scans/s [11].

3 RESULTS AND DISCUSSION

3.1 Determination of Bacterial Load and Population

The result of bacterial colony count showed that sobo drink containing 0.25g of saccharin has the highest colony count while sobo drink containing 2.0g of saccharin has the lowest colony count (Fig. 1).

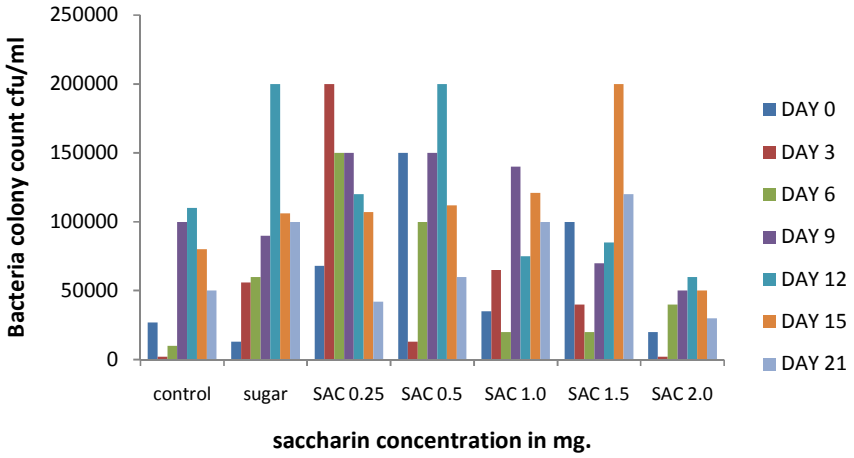

Fig. 1. Bacteria colony count (cfu/ml) during storage of sobo drink from day 0 to day 21 at different concentrations of saccharin

Key: SAC 0.25: 0.25g of Saccharin, SAC 0.5:0.5g of Saccharin, SAC 1.0:1.0g of Saccharin, SAC 1.5:1.5g of Saccharin, SAC 2.0:2.0g of Saccharin

Bacteria isolates were identified on the basis of the results obtained from biochemical characterisation complemented with the API identification kits (API System, France). The results were analysed using Bergey's manual of systematic bacteriology [12].Bacteria such as *Bacillus subtilis, Bacillus pumilis, Bacillus azotomonas, Micrococcus varians, Aeromonas hydrophila, Enterobacter aeromonas, Lactobacillus acidophilus* were isolated from the sobo drink. *Bacillus subtilis* was found in both fresh and spoilt sobo drink, which correspond with the work of [13] who also isolated *Bacillus subtilis* from both fresh and two weeks old prepared sobo drink. *Lactobacillus acidophilus* was isolated from the spoilt sobo drink which is also in line with the study of [13] who also isolated *Lactobacillus spp* from both dried *Hibiscus* calyces, fresh and also two weeks old sobo drink. From the results obtained, it shows that most of the bacterial isolates were coliforms. Most coliform bacteria have been implicated in food poisoning outbreak of some products [14]. Bacillus species are spore formers whose spores could survive high temperatures of processing. Rhodes and Fletcher [15] found that *Bacillus* and *Lactobacillus* species were readily found in foods of low acid content like juices and beverages where they produce organic acids. The presence of microbes (coliform bacteria) in the sobo drink is probably related to the sources or quality of water used for the processing. However, the current research disagree with the previous researches of Omemu [13] and [14] that failed to isolate *Micrococcus varians, Aeromonas hydrophila, Enterobacter aeromonas* in their researches, probably due to difference in period of storage.

3.2 Spectrophotometric Determination of Saccharin Degradation

Saccharin was found to be degraded at different day's interval during the period of storage. The degradation process was found to mostly occur on the twelfth day and the third day of the storage. Sobo drink containing 0.25g concentration of saccharin had 50% degradation rate which occurred on the twelfth day , 0.5g concentration 64.5% occurred on the third day and the rest occurring all on the twelfth day, 1.0g concentration 19.3% , 1.5g concentration 8.1% and 2.0g concentration of saccharin had the degradation rate of 3.1% (Fig. 2).

The use of GC-MS in monitoring the degradation of organic molecules such as saccharin is a widely accepted technique in analytical chemistry. 2-sulfonobenzamide was detected in sobo drink with lower concentration of saccharin such as 0.25 and 0.5g but not in sobo drink with higher concentration such as 1.0g and 1.5g of saccharin. This is because organisms such as bacteria present in the sobo drink might have utilised the available saccharin found in sobo drink with lower concentration of saccharin. *Sphingomona xenophaga* SKN was able to utilise saccharin as its carbon source and energy [16]. The compound can also be utilised as a source of sulfur by many bacteria [17]. Several

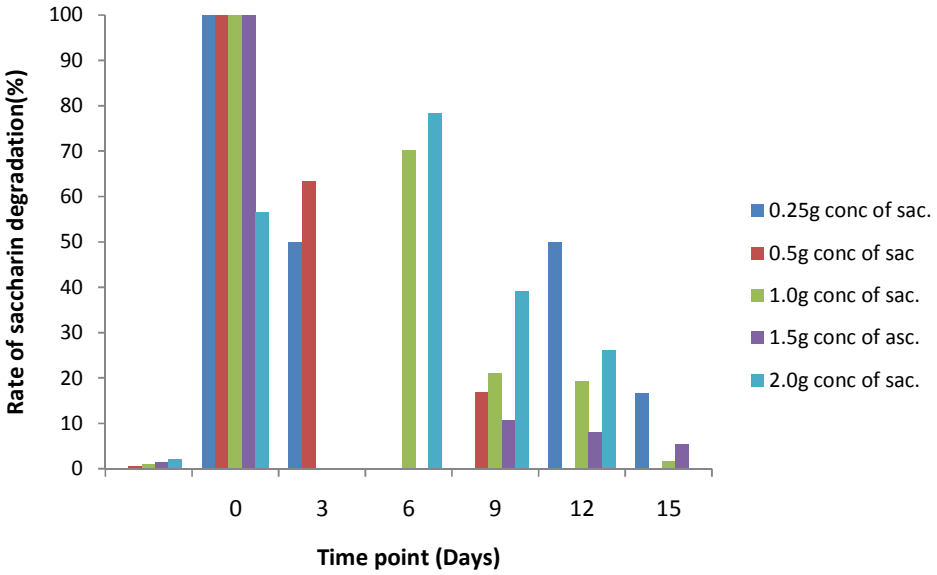

Fig. 2. Rate of saccharin degradation in sobo drink containing varying concentrations of saccharin at different day interval.

Key: sac=saccharin, Conc=concentration

bacteria able to assimilate sulfur from saccharin have been isolated from soil, suggesting that saccharin will tend to be attacked in soils without sulfate fertilisation [18].

The result shows that saccharin has been utilised in the sobo drink, and also higher concentration of saccharin can inhibit bacteria growth. 2-sulfonobenzamide is a saccharin degradation product that is used to produce drug like sulfabenzamide that is use as an antibacterial, which is often use in conjunction with sulfathiazole and sulfacetamide as a topical intra vaginal antibacterial preparation. Studies in rats have shown that long-term administration of sulfonamide may cause thyroid malignancy. However, rats appear to be especially susceptible to the goitrogenic effects of sulfonamide. 1-(+)-Ascorbic acid, 2, 6-dihexadecanoate was also detected in abundance. Ascorbic acid have been efficiently determined using a different method from hibiscus sabdariffa. Ascorbic acid commonly known as vitamin C is a water soluble vitamin that have wide range of biochemical role in the body owing to its anti oxidant and therapeutic properties. It is a vital, ubiquitous substance in the life process which can either be synthesised by living organisms, get it from exogenous sources or perish due to scurvy a result of uncorrected hypoascorbemia [19]. Studies [20] have shown that the Chemotherapeutic action of vitamin C is similar to those of sulfonamide compounds or mycelial antibiotics in acute infections as toxins such as those in snake bites, spider bites and insect stings are neutralised. It is germane in the formation and maintenance of collagen, absorption of iron from the intestine, metabolism of amino acids and prevention of tissue damage [21] in the treatment of diseases such as common cold, anaemia, scurvy, infertility and haemorrhage [19], maintenance of biochemical homeostasis under stress and in the treatment of intraocular pressure in the glaucomatous eye, diphtheria, streptococcus and staphylococcus infections [19]. Cyclotrisiloxane hexamethyl is another volatile compound detected which has not been classified as harmful by ingestion. However, it can cause eye, skin. and respiratory irritation and damage in some persons. Benzene acetic also known as Phenylacetic acid (PAA) (conjugate base phenylacetate), also known by various synonyms, is an organic compound containing a phenyl functional group and a carboxylic acid functional group. It is a white solid with a disagreeable odour. Endogeneously, it is a catabolite of phenylalanine. Phenylacetic acid has been found to be an active auxin (a type of plant hormone) found predominantly in fruits. However, its effect is much weaker than the effect of the basic auxin molecule indole-3-acetic acid. In addition, the molecule is naturally produced by the metapleural gland of most ant

species and used as an antimicrobial. It is also the oxidation product of phenethylamine in humans following metabolism by monoamine oxidase and subsequent metabolism of the intermediate product, phenylacetaldehyde, by the aldehyde dehydrogenase enzyme; these enzymes are also found in many other organisms. Tetradecanoic acid was also among the volatile compounds found; it has an antimicrobial, antispasmodic and anti-inflammatory effect [22].

Table 1. GC-MS analysis of the volatile organic compound profile of saccharin containing sobo drink stored at room temperature

Volatile organic compounds	Peak area (%)					
	RT^{-1} (min)	C	0.25	0.5	1.0	1.5
2 (3h)-Firanone	3.22	-	-	-	-	0.31
Methanamine N,methoxy	3.49	-	-	-	-	0.40
Urea, N-butyl-N(3,4-dichlorophenyl)	3.73	-	-	-	-	0.16
Propane nitrile 3,3-iminobis	3.74	-	-	-	0.29	-
4-Hexen-3-one,5 methyl	4.1	-	-	-	0.45	-
3,3-diethoxy-1-propyne	4.52	-	-	-	0.26	-
Butanedioc acid	5.07	-	0.17	-	-	-
Napthalene	5.36	-	0.13	-	-	-
3, cyclohexene 1-methanol alpha phara 4-trimethyl	5.36	-	-	-	0.21	-
2-oxopentanedioc acid	5.50	-	-	-	0.09	-
2-sulfano benzamide	5.42	-	-	12.55	-	-
2-sec-Butyl cyclohexane	5.48	-	-	3.00	-	-
3-Hexen-2-one	5.78	-	-	0.06	-	-
2-Penetanol, 2,5 dimethyl	5.92	-	-	-	0.52	-
4-isothio carboxamide	6.01	0.59	-	-	-	-
Phenol-2-methyl-5-(1-methyl)	6.23	-	-	-	0.10	-
2,4-decadienal	6.28	-	-	057	-	-
Cyclo hexane,dodeca methyl	6.36	-	-	1.18	-	-
Cyclo hexen-3-bromo	6.48	-	-	-	0.90	-
Silane trimethyl-2-(phenylethyl)	6.49	0.26	-	-	-	-
Bicyclo(2.1.0)pentane-1-dimethyl	6.49	-	-	-	0.05	-
Octane-2,7-dimethyl	6.63	-	-	1.47	-	-
Benzen acetic acid	6.68	0.38	-	-	-	0.43
1-Hexene-3,5,5-trimethyl	6.76	-	-	1.44	-	-
Ethyl hydrogen fumarata	6.69	-	-	-	0.14	-
Phenol-1,2-methoxy-4-(1-propenyl)	6.90	-	-	-	0.05	-
Pyridine	7.62	-	-	0.42	-	-
2,6-Adamastanedione-4-iodo	7.8	-	-	0.68	-	-
Butanioc acid	8.23	-	-	-	-	4.15
Hexanoic acid	8.29	-	-	12.55	-	-
4(2-hydroxy ethyl)phenol	8.65	0.64	-	-	-	-
IH,pydizine,hexahydro	9.27	-	-	-	0.38	-
Diethyl phthalate	9.88	-	0.41	4.34	1.67	-
Azelaic acid	10.47	-	-	-	1.11	-
Tetradecanoic acid	11.96	0.31	-	-	-	-
Benzosulfimide	12.42	-	-	-	0.35	9.18
Heptanoic acid-2-methyl ester	13.93	0.92	-	-	-	-
1-(+)-Ascobic acid 2,6-dihexadecanoate	14.32	22.02	34.71	13.57	0.55	10.3
1-propyl-9,12-octa-decadinoate	15.09	57.78	-	-	-	-
Oleic acid	16.08	-	17.24	-	-	17.0
Cyclotriciloxane hexamethyl	44.33	2.31	0.44	8.23	1.49	-

All unknown were removed.

4. CONCLUSION

From this research work, it can be concluded that saccharin was being degraded into its constituent compound 2-sulfonobemzamide which was confirmed using GC-MS analysis. In addition, the spectrophotometric analysis of sobo drink containing saccharin shows that the degradation rate was found to be higher in lower concentrations of saccharin and lower in higher concentration of saccharin. Coliform bacteria and spoilage bacteria were found indicating that, these are responsible for the degradation of saccharin. The use of artificial sweeteners in commercial and homemade drinks are increasing day by day, However, some these sweeteners like saccharin remained unchanged even after consumption and are excreted into the environment and surrounding water. Methods of detecting these compounds are not yet accurate or dependable and there is need to find new approaches to this problem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johnson RJ, Appel LJ, Brands M. Dietary sugars intake and cardiovascular health: A scientific statement from the American Heart Association. Circulation. 2009;120: 1011-1020.
2. Čopikova J, Moravcova J, Wimmer Z, Opletal L, LapčikO, Drašar P. Nahradni sladidla. Chemicke listy. 2013;107:867–874.
3. Velišek J, Hajšlova J. Chemie potravin 2. OSSIS: Havličkův Brod; 2009.
4. National Cancer Institute. Artificial sweeteners and cancer last reviewed: August 5, 2009. Page accessed Feb 29, (2016)
5. FDA Statement on Aspartame, 18 November (1996).
6. Whitehouse CR, Boullata J, McCauley LA. The potential toxicity of artificial sweetener. American Association of Occupational Health Nurses Journal. 2008;56(6):251-259.
7. Claus DC. A standardized gram staining procedure. World Journal of Microbiology and Biotechnology. 1992;8:451-452.
8. Harrigan WF, McCance ME. Laboratory methods in food and diary microbiology. Academic Press, London. 1976;452.
9. Christensen WB. Urea decomposition as a means of differentiating Proteus and Para-colon organisms from each otherand from Salmonella and Shigella types. J. Bacteriology. 1946;52:461.
10. Sunitha BM, Ajai KP, Gupta VK. Extractive spectrophotometric determination os saccharin. Journal of Environmental Sciences and Technology in India. 2008;227.
11. Ibrahim AD, Sani A, Aliero AA, Shinkafi SA. Biocatalysis of H. sabdariffa during 'dawadawan botso' production and biogeneration of volatile compounds. International Journal of Chemical Sciences. 2011;5(5):1922-1931.
12. Sneath PHA, Mar NS, Sharpe ME, Holt JG. Bergey's manual of systematic bacteriology. Volume 2 Williams and Wilkins co. Baltimore; 1986.
13. Omemu AM, Edema MO, Atayese AO, Obadina AO. A survey of the microflora of Hibiscus sabdariffa (Roselle) and the resulting "Zobo" juice. African Journal of Biotechnology. 2006;5(3):254-259.
14. Frazier WC, Westhoff DC. Food microbiology TMH Edition 540pp, (1986).
15. Rhodes A, Fletcher DL. Principles of industrial Microbiology. Pergamon Press, Oxford. 1966;119.
16. Schleheck D, Cook AM. Saccharin as a sole of carbon and energy for *Shingomonas xenophaga* SKN. Archives of Microbiology. 2003;179:191-196.
17. Rein U, Cook AM. Bacterial cleavage of nitrogen to sulfone bonds in sulfamide and 1H-2,1,3 benzothiadiazin-4(3H)-one 2,2-dioxide: formation of 2-nitrobenzamide by Gordonia sp. FEMS Microbiology Letter. 1999;172:107–113.
18. Kertesz MA. Riding the sulfur cycle—metabolism of sulfonates and sulfate esters in gram-negative bacteria. FEMS Microbiology Reviews. 2000;24(2):135–175.
19. Basu TK, Dickerson JWT. Vitamins in human health and disease. Lab International Oxford, **U. K. 1996;125-147.
20. Jackson JV, Moss M, Widop B, Greenfield ES. Handbook of pharamaceutical Excipients. British Pharmacopeia. 1973;69.

21. Nakajima Y, Shautha TR, Bourne CH. Histochemical detection of L gulonolactone. Histochemical. 1969;18: 293-301.

22. Wightman F, Lighty DL. Identification of phenylacetic acid as a natural auxin in the shoots of higher plants. Physiologia Plantarum. 1982;55(1):17–24.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27901