INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS) VOL. 1, No. 1: pp. 26 – 29, 4 TABLES.

Microbiological and physicochemical assessment of some brands of Vitamin C Syrup.

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ABSTRACT

The physical, chemical and microbiological qualities of one hundred and sixty samples of four different brands of Vitamin C syrup were assessed using standard methods. The assessment involved determining the viable microbial load, type of microbial contaminants, absence or presence of turbidity, type of seal, leakage, Vitamin C contents and pH of the different brands. Inspite of the age of the syrups examined, no turbidity was observed. A large proportion, up to 100% in some brands, of the syrup containers did not have a perfect seal. This imperfect seal lead to the leakage of syrup from the sides of the cap of some of the containers. All the syrups were acidic having a pH range of 2.2-3.5. The Ascorbic acid content of the syrups examined depended upon the age of the syrup, the seal and the brand. The syrups contained between 14.6 and 116.4% of the stated Ascorbic acid content. Exposure of the syrup to the atmosphere after first opening resulted in varying degrees of loss (65-97%) of Ascorbic acid. None of the brands were spared the presence of microorganisms. Only *Bacillus* species were the bacterial contaminants isolated. No fungi were isolated. These findings emphasize the need for proper packaging, preservation and storage of Vitamin C syrups.

Keywords: Vitamin C, microbiological, physicochemical, evaluation.

INTRODUCTION

Vitamin C or Ascorbic acid is an important nutritional compound required in health and disease. Diets poor in Vitamin C result in scurvy (Enstrom *et al*., 1992). Other functions known to be associated with Vitamin C are the enhancement of cell mediated immunity and the building of bones. Furthermore, Vitamin C supplements are used to treat and prevent many diseases including coronary heart diseases (Losonozy *et al*., 1996). Vitamin C boosts immunity by increasing the production of B and T cells and other white blood cells (Hueser and Vojdani, 1997). Vitamin C aids the body's absorption of iron by helping to convert dietary iron to a soluble form (Watanabe *et al*., 1988). As an antioxidant, Vitamin C helps to prevent oxidative damage in the body such as may be caused by strenous exercise which increases the level of free radicals in the body (Alessio *et al*., 1997).

The official dosage form of Vitamin C recommended by the British Pharmacopoea (1988) is the Tablet form. Choice of this dosage form is informed mainly by the instability of Vitamin C in an aqueous medium, oxygen liability and photo-oxidative degradation. Storage of Vitamin C is therefore carried out in amber-coloured, well-closed, non-metal containers.

The presentation of pharmaceutical preparations in aqueous media increases the likelihood of microbial contamination. It must also be borne in mind that such preparations are formulated with biodegradable compounds and as such, offer ideal sources of microbial nutrients unless they are securely preserved (Bloomfield *et al*., 1988). Such instability factors present in Vitamin C syrup necessitated the assessment presented in this study.

MATERIALS AND METHODS

Vitamin C syrup samples

One hundred and sixty samples (bottles) of four different brands of Vitamin C syrup were purchased from requisite retail outlets. All the samples had a shelf-life of 24 months. The samples purchased aged between one and twenty two months. For descriptive purposes, the four brands examined are coded AKP, BNK, CMZ and DEV.

Physicochemical Examination of Syrups

The physical examination of the Vitamin C syrups included visual examination of the presentation of each product with regard to whether the syrup was presented in amber coloured glass or plastic bottle, the approximate age of the syrup, the clarity of the content of each bottle and the level of sealing offered by the cap to each bottle. The effectiveness or otherwise of each seal was determined by laying the bottle horizontally on a level slab for one hour and checking for any leakage of syrup from the sides of the cap.

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Manuscript received by the Editor September 2, 2004; Revised manuscript accepted July 21, 2005.
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The chemical examination of each Vitamin C syrup entailed determining the Ascorbic acid content of the syrup. The Ascorbic acid content was assayed by titrating a solution consisting of $10cm^3$ aliquot of the syrup in $160cm^3$ of carbon dioxide free distilled water acidified with $20cm^3$ one molar sulphuric acid against 0.5M volumetric solution of iodine standardized with pure Ascorbic acid. $1cm^3$ starch solution was used as indicator. The titration was continued until a persistent violet blue colour was obtained. The volume of syrup solution at neutralization point was utilized in calculating the Ascorbic acid content. The calculation involved using the equivalence of 0.008805g Ascorbic acid per unit volume of titre (British Pharmacopoea, 1973).

To examine the effect of poor sealing on the Ascorbic acid content of syrups, aliquots of $45cm^3$ were aseptically dispensed into sterile amber coloured glass bottles with tight fitting screw caps. These bottles and those from which the aliquots were taken were screw-capped as tightly as possible and left in the dark at 28^oC. Thereafter, the stored syrups were assayed weekly for Ascorbic acid content for a minimum of 12 weeks.

Microbiological Examination of Syrups

Microbiological contaminants of the syrups were cultured using either the diluted or undiluted syrup. The syrup was diluted 10^{-1}, 10^{-2} or 10^{-3} times with sterile physiological saline (0.5M NaCl). Plating was done on Blood Agar (Oxoid, CM35), MacConkey Agar (Oxoid, CM9), Sabouraud Dextrose Agar (Oxoid, CM41) and Tryptone Soya Agar (Oxoid, CM131).

Viable microbial count was performed on syrup dilutions of 10^{-1}, $10^{-2} – 10^{-6}$ in physiological saline using the above listed solid growth media. All bacterial culture plates were incubated at 37^oC for 24-48h while the fungal culture plates were incubated at 28^oC for 2-5 days.

Discrete colonies on incubated plates were picked for characterization and identification according to the scheme of Cowan and Steel (1974).

RESULTS

General physicochemical features of the syrups examined are given in Table 1. This Table reveals the fact that all the syrups examined were not turbid.

Except brand DEV, all the syrups examined exhibited leakage of syrup from the sides of the sealing cap. The proportion of each brand exhibiting the leakage was least in brand CMZ (10%) and highest in brand BNK (40%).

Turning the cap of a syrup bottle through 0.5-0.75 circle led to the breakage of the cap to a very high proportion (80-100%) of the tested syrup bottles. However, a small proportion (10-20%) of the caps to brands AKP and BNK bottles would not break following a full circle turn (Table 1). Infact, extra force like cutting, was required to break such caps. Brand DEV offered the best seal protection. The syrups examined were highly acidic having pH values between 2.2 and 3.5.

Table 2 portrays the microbiological condition of the Vitamin C syrups that were tested. Clearly, a high proportion of the samples up to 60% were contaminated with microorganisms. Only bacterial contaminants (*Bacillus spp.*) were seen. The viable count of the syrups were relatively low falling within the range $0.91 \times 10^2 – 11.15 \times 10^2$ viable cells per unit volume (cm^3) of syrup. Brand DEV had the lowest proportion (30%) of bottles of syrup contaminated with bacteria. SDA yielded no growth and as such, no fungi was isolated from the syrups.

Table 1. Physicochemical conditions of some brands of Vitamin C syrups

Condition of Brand	Proportion (%) of Brand			
	AKP	BNK	CMZ	DEV
Turbidity	0	0	0	0
Leakage of Syrup	30	40	10	0
Cap breakage by ½ - ¾ circle turn.	90	80	100	100
Cap not breaking at full circle turn.	10	20	0	0
Amber glass bottle container	100	100	100	100
pH	2.5-2.8 (2.0)*	2.8-3.5 (2.9)*	2.5-3.0 (2.7)*	2.2-2.4 (2.3)*

* Mean pH value.

Table 2. Microbiological Conditions of some brands of Vitamin C syrup.

Brand	Proportion (%) contaminated	Viable Count (cells cm^{-3})		Isolate
		Range (x 10^2)	Mean (x 10^2)	
AKP	50	2.30 – 20.00	11.15	*Bacillus subtilis*
BNK	60	0.10 – 1.9	1.00	*Bacillus subtilis*
CMZ	40	2.00 – 15	8.50	*Bacillus subtilis*
DEV	30	0.12 – 1.70	0.90	*Bacillus subtilis*

Table 3 gives the Ascorbic acid content of the different brands of Vitamin C syrup tested. The majority (91.5%) of the syrup samples did not comply with the British Pharmaceutical Codex (BPC), (1994) standard of not less than 99% of the stated content of $C_6H_8O_6$ (Ascorbic acid). The Ascorbic acid content of the syrups varied with the brands and the age of the syrups. The variation in Ascorbic acid content was least (± 1.8) in brand DEV and highest (± 51.4) in brand CMZ.

DISCUSSION

The qualities of Vitamin C syrup with regard to microbial load, physical and chemical characteristics have been examined in this study.

The observation that the syrups were not cloudy is indicative of the absence of undesirable chemical and physical changes as well as the absence of visible microbial growth in the syrups. The absence of visible microbial growth does not preclude the presence of micro-organisms in the syrups as other characteristics of the syrups suggested the likelihood of microbial contamination of the syrups.

Table 3. Ascorbic acid content of different brands of Vitamin C syrup.

Brand	Age of syrup (months)		Ascorbic acid content (%)	
	Range	Mean	Range	Mean (± SD)
AKP	1-5	3	74.5 – 116.4	96.46 (21.5)
BNK	1-16	5.6	14.6 – 96.5	50.52 (38.9)
CMZ	5-13	7	28.7 – 129.5	80.19 (51.4)
DEV	10-22	16	96.2 – 98.8	98.75 (1.8)

This may be misleading to the casual observer since microorganisms were actually present in the syrups. The absence of visible microbial growth also suggests effective formulation and preservation of the syrups although no preservative was indicated on the labels of the different brands. The benefits and untoward effects of the preservation of aqueous preparations have been emphasized by Bloomfield *et al* (1988).

The effect of poor sealing on the Ascorbic acid content of the syrups is evident from the data in Table 4. Clearly, the syrup in the poorly sealed bottles lost, on average, 75% of the Ascorbic acid content within 12 weeks of storage with periodic opening and closure of the bottles. Conversely, the well sealed bottles under the same conditions as the poorly sealed bottles lost 48% of the Ascorbic acid content. Statistical analysis of variance between the values of Ascorbic acid content of these two types of bottles revealed the fact that statistically significant difference exists between the values (Table 4).

The poor sealing and leakage of syrup from the sides of the sealing cap on the syrup bottles is disappointing. Aside from the loss of syrup, the leakage permits the ingress of air, other gases and microorganisms into the syrup leading eventually to the spoilage of the syrups. It is therefore imperative for the manufacturers of the syrups to provide more effective seals for the syrup. Providing effective seals for the syrups should not jeopardise the opening of the syrups as suggested by the finding that some of the caps to the syrups needed to be cut, by means other than turning, to gain access to the syrup.

The statistically significant loss of Ascorbic acid from the stored syrups was not surprising. Not with the poor seal on the bottles. Allwood (1984) has studied extensively the factors influencing the stability of Ascorbic acid in total parenteral nutrition infusions. Such factors as air and water exist in these syrups, hence the degradation of the Ascorbic acid in the syrups. The differences in the level of degradation of Ascorbic acid in the poorly and properly sealed syrups buttress the requirement of effective seal on the syrup containers. It is however commendable that all the syrups were contained in amber glass bottles as a means of discouraging the degradation of the Ascorbic acid in the syrups.

Table 4. Effects of sealing and exposure to air on the ascorbic acid content of some Vitamin C syrups.

Initial Status		Ascorbic acid content (%) at 12th week of storage			
Age (months)	Assay (%)	Reassay from original bottle and cap (% loss)		Reassay from aliquots in well sealed bottles (% loss)	
1	116.4	20.7	(82)	65.4	(44)
2	96.4	31.2	(68)	53.8	(44)
3	74.5	25.6	(66)	36.7	(51)
4	114.5	35.2	(69)	64.0	(56)
5	96.6	30.8	(68)	58.2	(40)
6	85.0	29.2	(66)	53.1	(318)
10	97.7	20.8	(79)	58.7	(40)
13	28.7	3.9	(86)	10.8	(62)
16	14.3	2.8	(80)	6.2	(57)
22	98.6	15.6	(84)	52.8	(46)
Mean	82.27	21.58	(75)	45.97	(48)
Variance Ratio (F)*		2.570		0.358	

*At p = 0.05, F > 0.520

The observation that none of the syrups complied with the compendial standards in terms of Ascorbic acid content is unacceptable. This calls for a more stringent quality control profile in the manufacture of the syrups.

The fact that microorganisms were found in the syrups is further proof of the poor seal on the syrup bottles. It is gratifying to note that very low levels of microbial contamination of the syrups were observed. These low levels of microbial contamination are suggestive of two things. Firstly, the presence of preservatives in the syrups, though not stated and secondly, the highly acidic nature of the syrup and the inability of microbial contaminants to grow in them. Only acidophilic, sporogenous and hardy organisms can survive in such syrup environment. This is probably why only bacterial contaminants were found in the syrup.

CONCLUSION

It is instructive from the foregoing to suggest inclusion of appropriate chemical preservative(s) in Vitamin C syrup, providing effective seal for the syrup bottles and ensuring strict quality control during manufacture, distribution and storage of the syrups.

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