Comparative Studies of Vitamin C Contents in Different Processed and Un-Processed Milk Samples

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Summary: Vitamin C contents were measured in various milk samples including powder, liquid packs and animal milks using UV-VIS spectrophotometric techniques. In all the milk samples, high concentration of Ascorbic acid was found in the powder milk (Poland SMP) 57.02 mg/100g followed by Milk pack sample i.e. Haleeb Tea Max 26.80 mg/100g. Thus as a whole the general order of vitamin C contents was Powder > Liquid milk packs > Animal milk. The purpose of the study was to know the contents of vitamin C in various milk samples.

Introduction

Vitamin C or ascorbic acid is widely required in the metabolism of living being. In humans this substance is not synthesized during the metabolic process [1]. Thus it is necessary to be constituent of the alimentary diet because it participates in the redox mechanism, allowing the hydrogen transport in the cellular respiratory chain level. According to U.S. minimum daily requirement of 60 mg ascorbic acid per 250 mL container is required [2].

Ascorbic acid increases the organism resistance against microorganisms and participates in the antibody formation. Its deficiency provokes fatigue and debility against blood vessel, teeth, bones some escorbated indication, difficult the hurt cicatrisation, the growth, the reproduction and the lactation [3-5].

Vitamin C is used as medicine and is also added in manufactured food to act as antioxidant to conserve the product for a long time [6]. However, the excess of vitamin C can cause gastric irritation and diarrhoea, giving as metabolic product the oxalic acid, which can cause renal problems [1, 7]. Thus vitamin C determination is very important for biological and food industry.

Therefore, there is a need to find an accurate, rapid, reliable and easy-to-implement method for measuring the amount of ascorbic acid in the sample. However, there have been some difficulties in quantifying ascorbic acid due to its instability in aqueous solution. The instability of ascorbic acid is due to its oxidation to dehydroascorbic acid, which is a reversible reaction, and subsequently to 2, 3-diketo-L-gulonic acid. The later reaction is irreversible.

Ascorbic acid is highly sensitive to heat, alkali, oxygen, light and contact with traces of copper and iron [8]. Copper (II) and Iron (III) can greatly speed up the oxidation of ascorbic acid in aqueous solution.

Various methods have been employed for the analysis of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma etc. These include titration [9-10], HPLC [8, 11-12], UV [13-15], fluorimetry [16-17] etc. Tititration is a classic and time consuming method along with experimental errors. The HPLC and fluorimeteric methods have demonstrated good sensitivity and specificity as compared to titration method.

Tulley has invented a UV method for the analysis of ascorbic acid [13], but its procedure is complicated and only applies to the analysis of plasma samples. A UV method for assaying ascorbic acid based on its stability studies has been reported [14]. This is very useful for the ascorbic acid analysis in the presence of other vitamins. However, this method is time dependent and need strict carefulness to achieve reliable and reproducing results.

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Recently, Kwakye [15] has developed a UV method for analyzing ascorbic acid in commercial tablets by adding thiosulphate (0.04 % w/v) to the analysis of ascorbic acid in the multivitamin-mineral formulations containing interfering copper [8].

The oxidation of ascorbic acid during the sample preparation has caused much attention [8, 11] especially in the presence of copper (II). An aqueous sample containing about 0.2 ppm of Copper (II) only obtained about 50 % of recovery if copper (II) was not effectively chelated [8]. To date, however, there has not been any systematic research into effect of copper (II) concentrations on the oxidation of ascorbic acid in aqueous solution. Also, there have not been any quantitative studies on the oxidation of ascorbic acid during the sample preparation.

Vitamin C degrades quickly [18] and therefore there is special concern regarding the shelf life of these fortified foods. Consequently there has been considerable interest in alternative methods of determining the ascorbic acid content of food products.

Since spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories; a great number of colorimetric methods have been proposed for the determination of vitamin C [11, 15, 19-24]. Majority of these methods are based on its oxidation-reduction properties or its ability to couple diazotized aniline derivatives. Some of these methods are time consuming and suffer from lack of specificity or good sensitivity especially if they are used for the analysis of beverages, fruits or even some pharmaceutical preparations where coloring matter can interfere with its determination. Tedious pretreatment is often needed to remove possible interferences [25, 26]. Therefore, the need for a low cost, fast and selective method is obvious, especially for routine quality control analysis of ascorbic acid containing products.

The standard method for measuring ascorbic acid in foods is by titration with 2, 6-dichlorophenol-indophenol [27].

The amount of ascorbic acid tends to decrease during the storage of fruits and vegetables [28, 29].

Furthermore, small variation in temperature and light exposure during extraction significantly affected ascorbic acid recovery [30].

In the present study, efforts have been made to analyze different types of milk (powder, milk packs and animal) for their vitamin C contents. The study will be very useful for public awareness about the maximum or minimum concentration of vitamin C in the milk frequently available from the market. It is also of particular importance, to know the daily uptake of vitamin C using milk as its major source.

Results and Discussion

The solubility of ascorbic acid in aqueous solution is determined by four factors i.e. pH, oxygen, time and temperature.

The acidity of ascorbic acid is based on enol group ionization and from C^3 and C^2 atoms, with their PKa values 4.17 and 11.57 respectively [31]. The undissociated ascorbic acid present in solution with pH lower than 2, has maximum absorbance at 243 nm. At pH 4, above 50% of the molecules are dissociated and a maximum absorbance obtained at 250 nm. While from pH 5 to 10 almost all ascorbic acid are completely dissociated [32].

Ascorbic acid values for 24 samples of aseptically packed, powder and animal milks were determined with UV-VIS spectrophotometer.

It can be seen from Table-2 that the high concentration of vitamin C in dry milk samples was found in Poland SMP and Nestle nido both having similar concentration of vitamin C i.e. 57.02 mg/100g while the lowest content of vitamin C was found in Millac unilac 53.1 mg/100g. The rest of dry milk samples have the vitamin C content between 53.1-57.02 mg/100g.

Among the liquid milk pack samples the highest concentration of vitamin C was observed in Haleeb Tea Max i.e. 26.80 mg/100g followed by Candia Skimz 26.19 mg/100g while Nurpur milk pack shows the lowest concentration of vitamin C 25.57 mg/100g. The rest of the liquid milk pack samples have vitamin C content between 26.80 mg/100g - 25.57 mg/100g.

It can be seen from Table-2 that among these three categories of milk, animal milk samples have the lesser amount of vitamin C than powder and pack milk. However, among the animal milk samples, the high vitamin C content was found in goat milk 9.262 mg/100g followed by cow 9.114 mg/100g. Both these samples have almost similar vitamin C concentration

Table-1: Different standard solutions and their concentrations at (mg/100g)

S. No	Absorbance	Concentration
1	0.311	20
2	0.593	40
3	1.122	80
4	1.835	120
5	2.282	160
6	3.600	180

while the buffalo milk contained lower vitamin C value 8.701 mg/100g as compared to the two animal milk samples (goat, cow).

It can be concluded from the data given in Table-2 that the vitamin C content among these three categories of milk samples i.e. powder or dry, pack and animal is in the order of Dry milk > Pack milk > Animal milk.

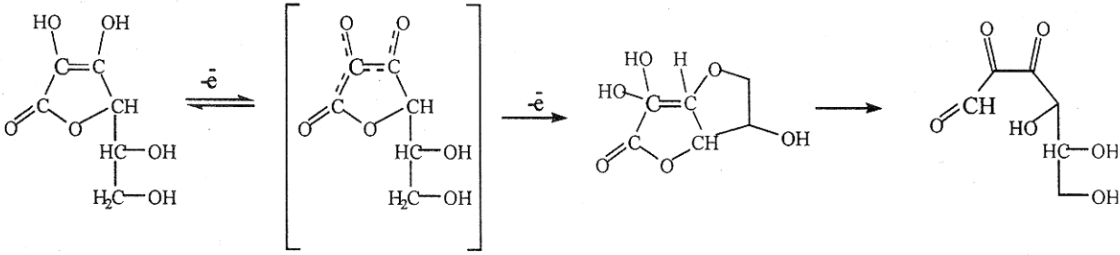
As in our samples, among the dry milk samples the vitamin C content is high while in liquid milk samples it is low which may be due to the fact:

The stability of vitamin C in aqueous solution

Sensitivity towards heat and light

In solution vitamin is oxidized due to dissolving oxygen.

As it is already discussed that the dry milk samples have highest vitamin C content followed by the liquid milk packs which support the conclusion of the work of Suner et al., (1956) [1], Ogata *et al,* (1968) [33], in which they concluded that the ascorbic acid is stable in solid form but is oxidized in solution by dissolved oxygen according to the equation.



L-Ascorbic Acid Ascorbic free Radical Dehydroascorbate 2,3-Diketogulonic acid

Some factors such as temperature, solvent, pH, light and metal ions (Cu^{+2}, Ag and Fe^{+3}) affect this reaction [33]. At temperature of 10 ^{0}C, the aqueous solution of ascorbic acid is stable and is not affected by pH nor oxygen, however at 25^{0}C the ascorbic acid is stable only in the absence of oxygen. In relation to solute pH, in the presence of oxygen at 25^{0}C, a decomposition of the ascorbic acid of 14% and 24% after 60 minutes was observed for pH 5 and 5.6 respectively.

Table-2: Concentration of Vitamin C in Different Milk Samples (mg/100 g)

S.No	Sample	Absorbance	Concentration
Dry milk samples			
1	Haleeb ISMP	0.102	56.18
2	Nirala Lahore(SMP)	0.088	54.48
3	Russian	0.099	55.82
4	Skimz Candia	0.084	53.98
5	Poland SMP	0.108	57.02
6	Dairy America	0.086	54.22
7	Millac King F.C	0.088	54.48
8	Russian 2 SMP	0.095	55.34
9	Millac Unilac	0.077	53.1
10	Dairy Crot F.C	0.078	53.14
11	Nestle Everyday	0.084	54.00
12	Russian 1 SMP	0.100	56.02
13	Nestle Nido	0.108	57.02
14	Green Pak Vegi. fat	0.081	53.60
15	Millac ISMP Powder	0.083	53.82
Milk pack samples			
16	Haleeb	0.068	25.93
17	Nestle Milk Pak	0.071	26.16
18	Candia Milk	0.067	25.87
19	Nestle everyday	0.069	25.99
20	Candia skimz	0.072	26.19
21	Dairy Queen	0.067	25.87
22	Nirala Doodh	0.069	26.02
23	Nurpur	0.062	25.57
24	Haleeb Tea Max	0.081	26.80
Animal milk samples			
25	Goat	0.067	9.262
26	Cow	0.063	9.114
27	Buffalo	0.054	8.701

From the Table-2 it can be seen that animal milk samples have least amount of vitamin C which is as a result of heating of these milk samples and it supports the conclusion of Anna, *et al* (2002) [30] that heating of milk or exposure to light decrease the vitamin C content of milk and food products etc.

With increasing Cu (II) concentrations, the rate of oxidation of ascorbic acid obviously increases, which demonstrate that copper (II) can greatly accelerate the oxidation of ascorbic acid in aqueous solution [8]. Within 10 min, 64.7 % of ascorbic acid in aqueous solution with 1.0 ppm Cu^{+2} is oxidized. Within 1 h, almost all ascorbic acid (98.5 %) in aqueous solution with 1.00 ppm Cu^{+2} is oxidized [34].

Ascorbic acid in aqueous solution is oxidized slowly by O_2 in air. Traces of common metal ions such as Fe^{+3} and Cu^{+2} can serve as catalyst to accelerate the oxidation of ascorbic acid.

As it is clear from the results (Table-2) that the animal milk samples have the lowest vitamin C content. The mean concentration of copper (II) in drinking water is 60 µg/L [35]. As it is already known that water is abundant in animal milk samples. This might explain why the ascorbic acid in animal milk, containing water as a major content, was oxidized so fast.

Experimental

Reagents

All the reagents were of analytical grade. Ethylenediamine tetraacetic acid (EDTA) and sulphruic acid were purchased from Merk BDH, ammonium molybdate AR (Winlab), oxalic acid (E. Merk, Germany) and were used as such without any further purification. A USP grade ascorbic acid reference standard (RS) were purchased from May and Baker Ltd. (England). Deionized distilled water was used throughout the experiment.

Instrument

A Hitachi UV-VIS Spectrophotometer (model U-2000 Japan), with a 1.0 cm optical path quartz cell was used for spectrophotometric measurements at a wavelength of 760 nm.

Sampling

About 27 different milk samples were taken for analysis. Amongst these 15 were dry, 9 were milk packs obtained from supermarket in Peshawar while 3 animal milk samples were obtained directly from the animals (Goat, Cow and Buffalo) and were processed for their vitamin C contents.

Preparation of Stock solutions

Ammonium molybdate (5%w/v) solution

Take 5 g of ammonium molybdate and dissolve it in a 100 mL of distilled water.

Oxalic acid (0.05 M) -EDTA (0.02 M) solution

Weigh required quantity of oxalic acid, freshly prepared solution, containing 0.02M EDTA and then make up the volume 100 mL with distilled water.

Sulphuric acid (5 % v/v) solution

Weigh 5 mL of concentrated sulphuric acid and add distilled water to make up the volume 100 mL.

Meta phosphoric acid with acetic acid solution

Dissolve with shaking 3 grams of meta phosphoric acid pellets in 15 mL of acetic acid and then make up the volume 100 mL with distilled water.

Standard L-ascorbic acid (0.1 % w/v) solution

Weigh 0.1 g of L-ascorbic acid and dissolve in oxalic acid (0.05 M) solution freshly prepared and make up the volume 100 mL.

Preparation of different standard solutions

Take 0.5-4.5 mL standard L-ascorbic acid (0.1 % w/v) solution in separate 25 mL volumetric brown flask. Then add 4.5, 4, 3, 2 and 0.5 mL of oxalic acid (0.05 M) solution in each volumetric brown flask. Add meta phosphoric acid with acetic acid 0.5 mL, sulphuric acid (5 % v/v) solution 1 mL and ammonium molybdate solution 2 mL separately in each volumetric brown flask and make up the volume to 25 mL with distilled water.

Preparation of sample solutions

Accurately weigh 1g of each sample in a 25 mL conical flask and add 10 mL of oxalic acid (0.05 M) solution and place the sample for 24 h.

After 24 hours filter the samples through 0.45µm filter paper. Then transfer 2.5 mL of each sample to a separate 25 mL volumetric brown flask, add 2.5 mL of oxalic acid (0.05 M) solution and the remaining reagents in the same way as in the preparation of standard solution i-e ammonium

molybdate (5 % w/v) sulphuric acid (5 % v/v) meta phosphoric acid with acetic acid and make up the final volume to 25 mL with distilled water [22].

References

1. S. P. Sunar, *Bioquimica, editorial Paz Montalvo Madrid*, 1017 (1956).
2. S. V. Ting, In Citrus Nutrition and Quality; Nagy, S., Attaway, J. Eds.; American Chemical Society: Washington, DC, Chapter 1, pp 1 (1980).
3. H. Vuorela and R. Hinkkanen, *Hitunen, Acta Pharm. Fenn.* **95,** 141 (1986).
4. B. M. Tolbert and J. B. Ward, Ascorbic Acid, Chemistry, Metabolism and Uses, in : P. A. Seib, B.M. Tolbert (Eds.), Advances in Chemistry 200, American Chemical Society, Washington , DC, (1982).
5. C. A. B. Clemetson, Vitamin C, CRC Press, Boca Raton, FL, (1989).
6. J. C. Auerfeind, D. M. Pinkert, Hoffmann-La Roche, Basle, p. 5 (1970).
7. A. Hodgkinson, Academic Press, London, (1977).
8. L. D. Muller, *J. Pharm. Biomed. Anal.*, **25**, 985 (2001).
9. British Pharmacopeia, British Pharmacopeia Commission, (2000).
10. USP 24/NF19; USP Convention Inc., (2000).
11. J. Lykkesfeldt, *Anal. Biochem.*, **282,** 89 (2000).
12. E. S. Wagner, B. Lindley, R. D. Coffin, *J. Chromatogr.* **153**, 225 (1979).
13. R. T. Tulley, *US Patent*, **5**, 441, 872 (1995).
14. J. K., Kwakye, *West Afr. J. Pharm.*,. **65** (1997).
15. J. K., Kwakye, *Talanta*, **51**, 197 (2001).
16. T. Egberg, *J. Assoc. Off. Anal. Chem.*, **60**, 126 (1977).
17. B. Vuilleumier, *J. Micronutr, Anal.*, **5** 25 (1989).
18. O. H. A. Mageed, P. Y. Khashaba, H. F. Askal, O. A. Saleh and I. H. Reffat, *Talanta*, **42**, 573 (1995).
19. S. Morton, W. P. Charles, and G. W. Ernest, *Anal. Chem.*, **25**, 1486 (1953).
20. M. Hashmi, (ed.) p. 286.Wiley-Interscience, New York, (1972).
21. T. Kamangar, A. B. Fawzi, and R. H. J Maghssoudi, *J. Assoc. Off. Anal. Chem.*, **60**, 528 (1977).
22. K. L. Bajaj; and G. Kaur, *Analyst,* **106**, 117 (1981).
23. K. Florey, (ed.), Analytical Profiles of Drug Substances, Vol. **11**, and p. 76: Academic Press, (1982).
24. J. Augusten; B. P. Klein; D. Becker and P. Venugopol, "Methods of Vitamin Assay", p. 303. Wiley-Interscience, New York, (1985).
25. R. C. Williams, D. R. Baker and J. A. Schmidtt, *J. Chromatogr. Sci.*, **11**, 618 (1973).
26. M. I. Karayannis and D. I. Farasoglou, *Analyst,* **112**, 767 (1987).
27. AOAC Official Methods of Analysis, **31**st ed.; Association of Official Analytical Chemists; Washington, DC, p.746 (1980).
28. I. T. Agar, J. Streif and F. Bangerth, *Postharvest Biology and Technology*, **11**, 47 (1997).
29. K. K. Lee and A. A. Kader, *Postharvest Biology and Technology*, **20**, 207 (2000).
30. R. Anna, B. Ada and V. Sabrina, *Food Chemistry*, **77**, 257 (2002).
31. Catalog Fluka Chemika-Biochemika Analytica. Fluka Chemie AG Industrietrasse **25** CH-9471 Buchs.Switzerland, p. 156 (1995-96).
32. J.C.B Fernandes *Analytica Chemica*, **366**, 11 (1998).
33. Y. Ogata, Y. Kosugi and T. Morimota, *Tetrahedron,* **24,** 4057 (1968).
34. D. R. Lide, Handbook of Chemistry and Physics, **78**th ed., CRC Press, Florida, 1997-1998.
35. W. Zeng, F. Martinuzzi, *J. Pharm. Biomed. Anal.* , **36,** 1107 (2005).