International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.6, No.2, pp 1124-1130, April-June 2014

The effect of Storage Time and Humidity on Vitamin C level in Infant's baby milk powder after opening the package

*[1]Rita Mansour and [2]Malak AlJoubbeh

[1]Department of Basic Sciences, Faculty of Agriculture, Damascus University, Syria
[2]Department of Chemistry, Faculty of Sciences, Damascus University, Syria.

*Corres.author: ritamansoor@yahoo.com

Abstract: The goals of this study were to compare L-ascorbic acid concentration in four different kinds (A, B, C, and D) of infant baby milk powder that are imported to Syria of the same brand. The research also aims to study the effect of storage time and humidity (kitchen's weather) on vitamin C level in the four categories of baby milk, from opening the package until the fourth week. The results showed that decreasing inconcentration of vitamin C with increasing time of exposure to moisture (H=55± 5%) in all varieties of imported milk. The amount ofvitamin C in the milk of A and C were less than the allowable limit for children at this age after the fourth week, while it was less in milk B and D after the third week. This leads to the injury of children due to vitamin C deficiency.
Keywords: Dry milk, Humidity, Vitamin C, HPLC.

1. Introduction

Vitamins are group of organic complex compounds that the body needs in small quantities. Vitamins must be supplies from outside, as the human body cannot synthesize them. One such vitamin is Vitamin C. The exact amount of Vitamin C the body needs is unknown, and is thought to be anywhere from 45 to 75 mg a day[1].Vitamin C, or ascorbic acid, is a vitamin that can be found in various fruits and vegetable. Vitamin C is a white, crystal-shaped organic compound, and can be synthesized from glucose or extracted from certain natural sources such as orange juice. Vitamin C was first isolated from lime juice by SzentGyorgy in 1928[2].

Vitamin C plays a vital role in our lives; firstly, it contributes to the synthesis of collagen tissue around bones, teeth, cartilage, skin, and damaged tissue. Second, this vitamin is needed to activate various enzymes related to the nervous system, hormones, and detoxification of medicine and poison in the liver. Third, its role as an antioxidant is well-known in society; its solubility enables it to work as antioxidant within our bodily fluids. Fourth, Vitamin C increases the rate of absorption of iron, calcium, and folic acid. Fifth, it reduces allergic reactions, boosts the immune system, stimulates the formation of bile in the gallbladder, and facilitates the excretion of various steroids[3]. Vitamin C is important to the functioning of the brain, as the brain contains a large amount of Vitamin C. A study by two researchers at the Texas Woman's University found that high

school students with high blood Vitamin C rates produced better IQ test results as compared to students with low rates[4].

Deficiency of this vitamin can lead to anemia, scurvy, infections, bleeding gums, muscle degeneration, poorwound healing, atherosclerotic plaques, capillary hemorrhaging and neurotic disturbances[5].

Severe deficiency of vitamin C causes scurvy. Although rare, scurvy includes potentially severe consequences, and can cause sudden death[6].

Vitamin C toxicity: Toxicity, normally, does not occur since vitamin C is water-soluble and is regularly excreted by the body. Excess ascorbic acid excreted in the urine gives a false-positive test for sugar. High levels of vitamin C interfere with copper absorption[7]. Vitamin C should be avoided by those who suffer from kidney stones, as it can convert to oxalate[8]. However some research suggests that vitamin C only undergoes this transformation in urine after the urine has left the body[9-11].

Vitamin C is highly soluble in water and alcohol, and is easily oxidized. The oxidation of Vitamin C occurs very quickly in a base environment at high temperatures. Light and heat damage Vitamin C in fruits and vegetables[12]. Vitamin C also decreases if food is left warm or hot for too long[13]. Vitamin C amount is significantly reduced if stored at room temperature[14].

Milk is rich in several nutrients that are essential for health, especially in babies. Babies can obtain ascorbic acid by drinking milk[16]. Breast milk has a higher level of vitamin C than commercial milk, but sometimes forced to use the kinds of private commercial milk for infants. Therefore, the goals of this study were to compareL-ascorbic acid concentration in four kinds (A, B, C, D) of infant baby milk powder witch are imported to Syria . The effect of storage time and humidity after opening the package on L-ascorbic acid levels in baby milk powder was also investigated[17].

L-ascorbic acid ($C_6H_8O_6$) is the trivial name of Vitamin C. The chemicalname is 2-oxo-L-threo-hexono-1, 4-lactone-2, 3-endiol, Figure 1[18,19].

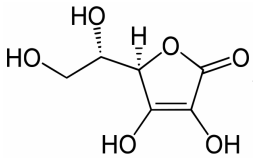

Figure 1.Structureof Vitamin C (L-ascorbic acid)

The best way to get the daily requirement of essential vitamins, including vitamin C, is to eat a balanced diet that contains a variety of foods from the food guide pyramid. Vitamin C should be consumed every day because it is not fat-soluble and, therefore, cannot be stored for later use. The Food and Nutrition Board at the Institute of Medicine recommends the following amounts of vitamin C:Infants and Children(recommended daily allowance (RDA)[20-22].

- 0 - 6 months: 40 milligrams/day (mg/day)
- 7 - 12 months: 50 mg/day
- 1 - 3 years: 15 mg/day
- 4 - 8 years: 25 mg/day
- 9 - 13 years: 45 mg/day

2. Materials and Methods

2.1. Samples

Four kinds of infant baby powder milk are imported to Syria(Table 1),fivegram of allsamples placed in climatic test cabinets at (Temperature = 23 +2º C,Humidity = 55+ 5 %) without light for four weeks.

Table 1. The Composition of four drymilk samples for infant's milk before opening the package

Composition	Milk samples			
	A	B	C	D
Vitamin C mg/100 g	87	78	87	86.6
Humidity %	2	2	2	2
Age (month)	0 - 6	6-12	0-6	6-12

2.2. Chemicals and reagents

VitaminC (extra pure,Merck), methanol, acetonitrile, of HPLC grade (Sigma), metaphosphoricacid (extrapure, BDH). Deionized water and Solvents of LC grade was obtained by filtering through 0.45μm filter membrane and degassed for 20 minutes by ultra sonic.

2.3. Instrumentation

1- Climatic test cabinets at (Temperature = 23 ± 2 º C, Humidity = 55± 5 %) without light, TK 120, Turkey.

2- High-performance liquid chromatographic (HPLC), connected with an ultraviolet-diode array detector (PDA),Shmizu.

Column ODS- C18 (250×4.6 mm).

2-4 Extractions of Vitamin C

Five grams of sample(milk) were homogenized with 25 mL of mobile phase solution, and it was quantitatively transferred into a 50 mL volumetric flask and shaken gently to homogenize solution(the samples were protected from light by wrapping tubes and flasks with aluminum foil andpreparing the samples in a darkened room[23]. Then it was diluted up to the mark by the mobile phase solution. The obtained solution is filtered and centrifuged at 4000 rpm for 15 minutes, after what the supernatant solution is used for determination vitamin C[24]. Vitamin C content was analyzed using HPLC, Ascorbic acid was identified by comparing the retention time of the sample peak with that of the ascorbic standard at 254 nm[23,25]**.** The loss rate in vitamin C were determined as[25].

3. Results and discuss ion

Calibration curve and linearity

The chromatogram for ascorbic acid (10 ppm) is seen in figure 2, it shows the relative retention time with HPLC system is (6) minutes. The mobile phase used ismethanol, acetonitrile, metaphosphoric acid **(15:10:75),** the detection wavelength is set at 254 nm and flow rate is 1.8 ml/min.

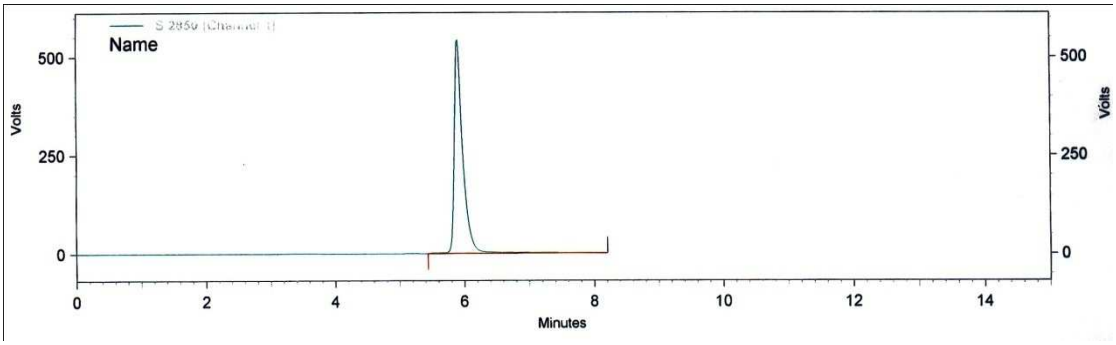

Figure 2. The chromatogram of ascorbic acid standard (10PPm)

The linearity of the method was determined at four concentrations levels ranging from (10) to (200) ppm for ascorbic acid at the room temperature. The calibration curve was constructed by plotting response factor against concentration of the ascorbic acid (figure 3). The stop and intercept value for calibration curve was Y=51732X, R^2 = 0.998.

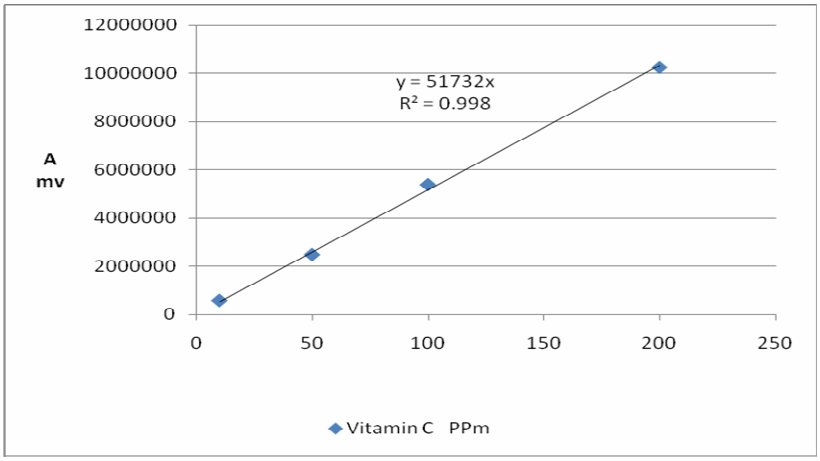

Figure 3.Calibration curve of L- ascorbic acid

4 Discussion

There are many factors affect the levels of vitamin C (Humidity, temperature, sun light, storage). L-Ascorbic acid is easily and reversibly oxidized to dehydroascorbic acid, forming the ascorbyl radical anion as an intermediate (figure 4)[19]. Dehydroascorbic acid in solution at neutral pH undergoes anonreversible oxidation to form the biologically inactive, straight – chained compound, 2, 3- diketogulonic acid[27,19].

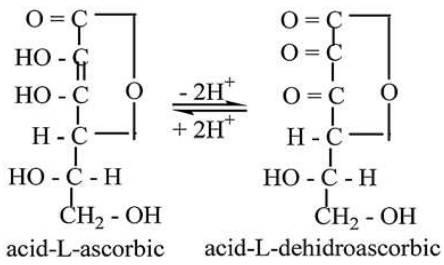

Figure 4. Vitamin C oxidation

Table 2 Showed that Vitamin C value (accounted according to extraction method) was slightly less than the value on the package (table 1). Accounted Vitamin C amounts in A, B, C and D samples are (85.901,

75.743, 86.230, 85.599 mg/100gr) while the amounts on package for A, B, C and D respectively (87, 78, 87 and 86.6 mg/100gr) these amounts may be resulted by storage conditions[18].

Table 2. Vitamin C content in milk samples during storage

Time Weeks	Vitamin C content in milk samples* X± SD mg/ 100 g			
	A	B	C	D
Open the package(0)	85.901±1.11	75.743±1.20	86.23±1.32	85.599±1.30
After one week	77.731±1.21	62.151±1.12	78.151±1.22	70.331±1.21
After two weeks	63.658±1.10	52.779±1.11	64.182±1.12	58.861±1.11
After threeweeks	47.635±1.22	37.362±1.10	48.057±1.10	41.891±1.03
After four weeks	38.106±1.04	26.691±1.03	37.362±1.02	30.813±1.02

* (Temperature = 23 ±2 ° C , Humidity = 55± 5%)

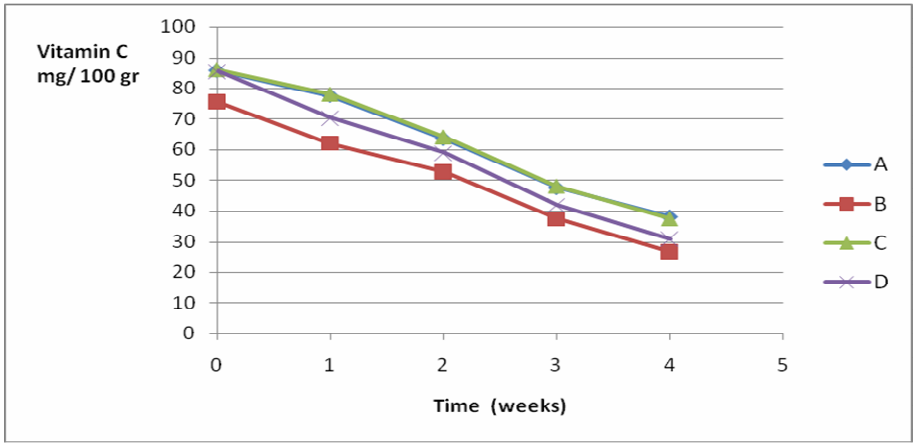

Figure 3. Humidity effecton vitaminC content during storage milk samples (Temperature = 23 ±2 ° C, Humidity = 55± 5%)

According to table 2 and figure 4 we notice decrement of vitamin C quantity in the four milk types since the first week of opening the package until the fourth week. Amount of vitamin C's decrement increases during storage period at room temperature humidity exposure results increasing in vitamin decrement[28,29], (H = 55± 5%) is chosen humidity, which it is rising humidity average in house kitchen between summer and winter conditions (observed by Electronic Hygrometer) [30].

From table 3 and figure 5decrement rate of vitamin in samples C and A was approximate with humidity exposure at room temperature after the first week (9.396, 9.510% respectively), in the second week (25.568, 25.893 %) but after the third week (44.268, 44.546 %) and after the fourth week (55.639, 56.671%). In the other hand, it is noticed that decrement rate of vitamin in samples B and D was approximate in the four weeks, decrement rate was (17.944, 17.836% respectively) after first week (31.236, 30,318%) after second week (51.061, 50.672%) after third week (64.761, 64.003%) after fourth week[31,32,30].

Table 3. Loss ratio of vitamin C in milk samples during storage

Time* Week	Loss ratio of vitamin C %			
	A(1)	B(2)	C(3)	D(4)
After one week	9.510	17.944	9.369	17.836
After two weeks	25.893	30.318	25.568	31.236
After three weeks	44.546	50.672	44.268	51.061
After four weeks	55.639	64.761	56.671	64.003

*(Temperature = 23 ±2 ° C , Humidity = 55± 5%)

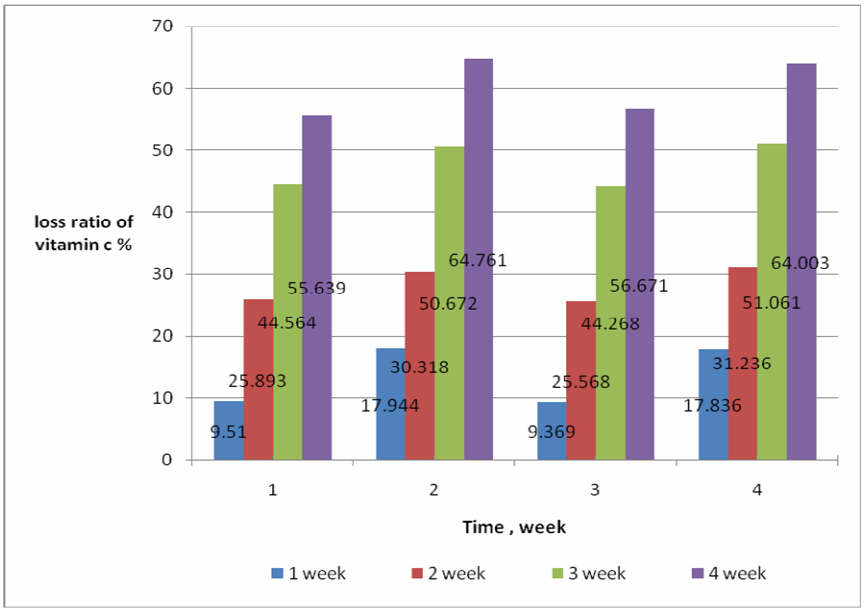

Figure 5.The increasing of Loss ratio of vitamin C in milk samples duringstorage (1(A), 2(B), 3(C), 4(D))

Table2 clarify that vitamin C amount is (47.635, 48.057 mg/100gr) in samples A and C after the third week (which is the period written on the tin and recommended not to have milk after it), 40mg/day is the allowed limitation for this age (0-6 month) [21]. But after the fourth week vitamin amount was less than the allowed limitation (38.106, 37.362 mg/100gr). Vitamin C amount in samples B and D was less than the allowed limitations (37.362, 41.891 mg/100gr) after the third week (50 mg/day for 6-12 month) [22]. This leadsto theinjury of childrento vitamin Cdeficiency**.**

5 Conclusion

The results of the analysis showed that, increasing exposure to room temperature and house kitchen humidity (kitchen's weather) results decrement in allowed vitamin C amount limitations in infant's milk. Decrement of vitamin C down to the allowed limitations in infants' milk make infant a purpose for vitamin lack symptoms which may cause sudden death.

6 References

1. DeMan, J. M. 1997. *Principles of Food Chemistry*. Penerjemah Kosasih Padmawinata Dalam Kimia Makanan. Institut Teknologi Bandung, Bandung, 408 – 414.
2. Marsetyodan Kartasaputa. 2004. *Ilmu Giz iKorelasi Gizi, kesehat and anproduktifitas Kerja*, Rineka Cipta, Jakarta, 78 – 80.
3. Nurfatimah. 2008. *Konsumsi Vitamin C "Majalah Percikaniman*. www.percikan-iman.com/mapi/indeks.phd.html [12 Februari 2008]
4. Sitti R, W., Bunbun, B. 2009. Kinetics of The Oxidation Of Vitamin C. *Prosiding Seminar Kimia Bersama UKM-ITB VIII 9-11* .P.535 .
5. K- Kinsman, R. A. and Hood, J. 1971. Some behavioral effects of ascorbic acid deficiency. Am. J. Clin. Nutr., 24: 455.
6. Zieve, D. 2010. In Vitamin C: Medline Plus Medical Encyclopedia. Retrieved, from *http://www.nlm.nih.gov/medlineplus/ency/article/002404.htm*.
7. Finley, E. B. and F. L. Cerklewski, 1983. Influence of ascorbic acid supplementation on copper status inyoung adult men. Am. J. Clin. Nutr., 37: 553-556.
8. Piesse, J. W. 1985. Nutritional factors in calcium containing kidney stones with particular emphasis on vitamin C. Int. Clin. Nutr. Rev., 5: 110-129

9. Khalid, I., Alam, K., and M. Muzaffar, A. 2004. Biological Significance of Ascorbic Acid (Vitamin C) in Human Health – A Review. Pakistan Journal of Nutrition 3 (1): 5-13.
10. Wandzilak, T. R., D'Andre, and Williams, H. E. 1994. Effect of high dose vitamin C on urinary oxalate levels. J. Urol., 151: 834-837.
11. Tarin, J. J., Brines, J., and Cano, A. 1998. Is antioxidant therapy apromising strategy to improve human reproduction? Antioxidants may protect against infertility. Human Reproduction, 13 (6), 1415- 1424.
12. Song, A. and Tamer, T. 2005. Influence of Different Light Sources, Illumination Intensities andStorage Times on the Vitamin C Content in Pasteurized Milk. Turk J Vet Anim Sci.29, P.1097-1100
13. Wills, J. 2008. *Supaya Bahan Makanan Tidak Kehilangan Gizi "Dinas Kesehatan Jawa Timur" Artikel*. www.dinkesjatim.co.id/berita-detail.html /berita-detail.html [13 Februari 2008.
14. Tawali, A. B., and Zainal, 2004. *Mempelajari Perubahan Fisikdan Kimia Buah Pir Selama Penyimpanan. Jurnal Sainsdan Teknologi*. 4(3), 130 - 138
15. Kirby, C. J., Whittle, C. J., Rigby, N., Coxon, D. T. and Law, B. A. 1991. Stabilization of ascorbic acid by microencapsulation in liposomes. International Journal of Food Science and Technology, *26*, pp. 437-449.
16. Padayatty SJ, Katg A, Wang Y and *et al.*, 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J.Am. coll. Nutr. 22, 1835.
17. Parker RO. Introduction to food science. Rich Parker: Delmer thoms on learning. 2003.
18. Andrea, S. T., Emilia, L. E. 2006. Vitamin C degradation during storage of fortified foods. Journal of Food and Nutrition Research Vol. *45*, No. 2, pp. 55-61. J. Food Nutr. Res.
19. George, F.m. 2006. Vitamins in foods, Analysis, Bioavailability, and Stability. Taylor and Francis group. New York.
20. Sizer, Frances and Whitney, Eleanor. 1997. Nutrition Concepts and Controversies (7th ed.). West/Wadsworth International Thomas Publishing Company.
21. Mayo Clinic Staff, 2010. In Vitamin C (ascorbic acid). Retrieved June 1, from *http://www.mayoclinic.com/health/vitamin-c/NS_patient- vitaminc*
22. Dietary Reference intake for vitamin C (DRIS) The essential guide to nutrient Requirements. 2001. Washington: The National Academy Press.
23. Romeu-nadal M, Morera-Pons S, Castellote AI, Lopez-Sabater MC.2006. Rapid highperformance liquid chromatographic method for Vitamin C determination in human milk versus an enzymatic method. J Chromatography. 830:41-46.
24. Albala-Hurtadom, S., Vecianna-Nogues, MT., Izqierdo-Pulido, M., Marine-Font, A. 1997. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. 778:247-253
25. Dionnex corporation. 2009. Determination of water – and fat – soluble vitamins in functitional waters by HPLC with UV- PDA Detection. Application Not e 261, LPN 2145, Sunnyvale, CA.
26. Enemo, R. E., Ekpunobi, U. E., Nnubia, D. and Onuegbu, T. U. 2010. Comparative Study on the Ascorbic Acid Content of Some Common Nigerian Vegetables (*Solanumgilo, Gnetumafricanum, Gongomeralatifolium,* and *Vernoniaamygalina*). *The Pacific Journal of Science and Technology* ,Vol. 11. N2
27. Rose, J. K. C.1987. Solubility properties of reduced and oxidized ascorbate as determinates of membrane permeation ,Biochim .Biophys.Acta , 145, 685.
28. Favell, D. J. 1998. Acomparison of the vitamin C content of fresh and frozen vegetables, food chem. 62,59.
29. Ozkan, M., Ikirca, A., and Cemeroglu, B. 2004. Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. Food chem., 88,591- 597.
30. David, M. 2007. Home moisture problems. Brook. Former extension agent, Multnomah country, Oregon state university.
31. Beizadea, E. 2009. Fortification of wheat flour . Romanian Society of Biotechnol. lett. Vol. 14, No. 2.pp. 4300-4306.
32. Oey, I.,Verlinde, P. and *et al*., 2006. Temperature and pressure stability of L – ascorbic acid and / or 5-methyltetrahydrofolicacid: A kinetic study. Eur food Res Technol, 223, 71- 77.

*****