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ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.5, pp 3211-3219, Aug-Sept 2014

Loss of l- Ascorbic acid in milk powder for infants caused by storage time, Humidity and light after opened the package.

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Abstract: This research aims to determination of the amount of vitamin C in four varieties of dry milk for infants imported to Syria of the same brand.
The research also aims to study moisture effect (kitchen's weather) and light on the amount of Vitamin C in the four categories of baby milk, from opening the package until the fourth weeks.
The results showed that decreasing in concentration of vitamin C with increasing time of exposure to moisture (H= 55 ± 5%) and light in all varieties of imported milk. The amount of vitamin 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 (without light). The amount of vitamin C in the milk of A and C were less than the allowable limit for children at this age after the third week, while it was less in milk B and D after the two weeks(with light). This leads to the injury of children to vitamin C deficiency.
Keywords: Dry milk- moisture-light-viamin C- HPLC.

1- Introduction

L-Ascorbic acid, also known as L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreo hexuronic acid lactone, antisorbutic vitamin and vitamin C, the chemical formula is $C_6H_8O_6$ and a molecular weight of 176.12. Fig. 1 [1, 2].

This water-soluble vitamin is important in forming collagen, a protein that gives structure to bones, cartilage,muscle, and blood vessels. It also helps maintain capillaries, bones, teeth and aids in the absorption of iron.

Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As as acavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen [3].

Deficiency

Deficiency of vitamin C can lead to anemia, infections, bleeding gums, muscle degeneration, poor wound healing, atherosclerotic plaques, capillary hemorrhaging and neurotic disturbances [4], and can cause sudden death [5].

Vitamin C toxicity:

The FAO/WHO [6] report pointed out that the potential toxicity of excessive doses of supplemental ascorbic acid relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of

2–3 g/day of ascorbic acid produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people. Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose. Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. [7,8] .

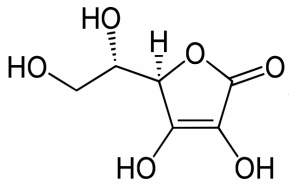


Fig. 1. Structure of Vitamin C

Human milk is recognised as the optimal milk source for infants at least throughout the first year of life. It is recommended as the sole nutritional milk source for infants during the first 4 to 6 months of life. IOM [9] estimated the AI for infants based on the average volume of milk intake of 780 ml and an average concentration of ascorbic acid of 50 mg/l in human milk. For infants 0-6 months, 40 mg per day was the estimated AI and for the 7-12 months infants, the AI was 50 mg per day, taking into consideration the amount of ascorbic acid from solid foods consumed at this stage [10,11,12].

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 [13, د ملك]. Therefore , the goals of this study were to compare L-ascorbic acid concentration in four kinds (A, B, C , D) of infant baby milk powder witch are imported to Syria . Loss of L- ascorbic acid in milk powder for infant caused by storage time , humidity and light was also investigated.

2- Materials and Methods

2.1. Samples

Four kinds of infant baby powder milk are imported to Syria (Table 1), five gram of all samples placed in climatic test cabinets at (Temperature = 23 +2 ºC, Humidity = 55+ 5 %) without light and with light (12000 lux) for four weeks.

Table 1. The Composition of four dry milk samples for infants

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

2.2. Chemicals and reagents

Vitamin C (extra pure, MERCK), methanol, acetonitrile, of HPLC grade (Sigma), meta phosphoric acid (extra pure, 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 ºC, Humidity =55± 5 %) without light, and with light (12000 lux), TK 252, Turkey.
2- High-performance liquid chromatographic (HPLC), connected with an ultraviolet-diode array detector (PDA), Shmizu. column ODS- C18 (250×4.6 mm).

3- Extraction 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 and preparing the samples in a darkened room[14]. Then it was dilute up to the mark by the mobile phase solution. The obtained solution is filter and centrifuged at 4000 rpm for 15 minutes, after what the supernatant solution is used for determination vitamin C [15]. 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 [16, 14]. The loss rate in vitamin C were determinant as [17].

4- Results and discussion

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 is methanol, acetonitrile, metaphosphoric acid (15:10:75), The detection wavelength is set at 254 nm and flow rate is 1.8 ml/min

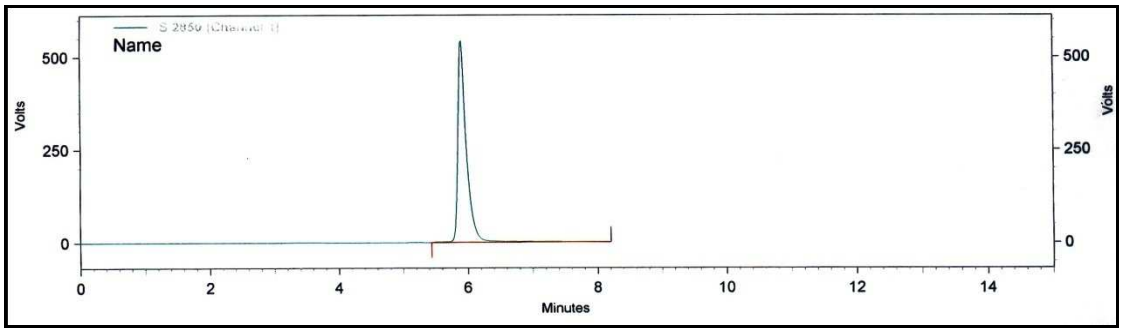


Figure 2. The chromatogram of ascorbic acid standard (10PPm)

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.

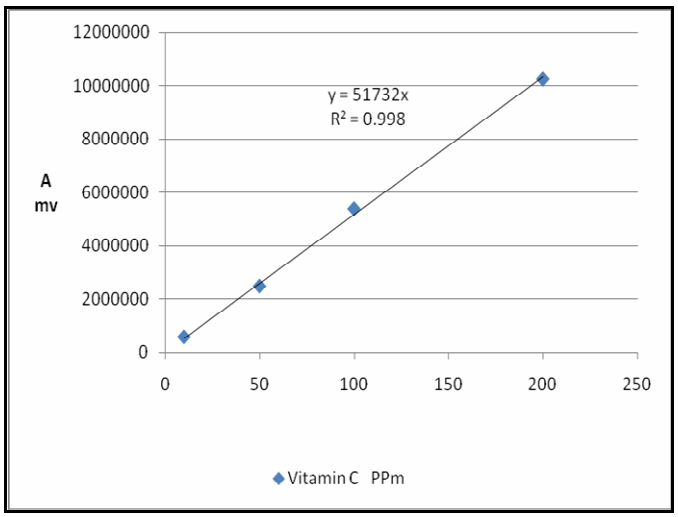
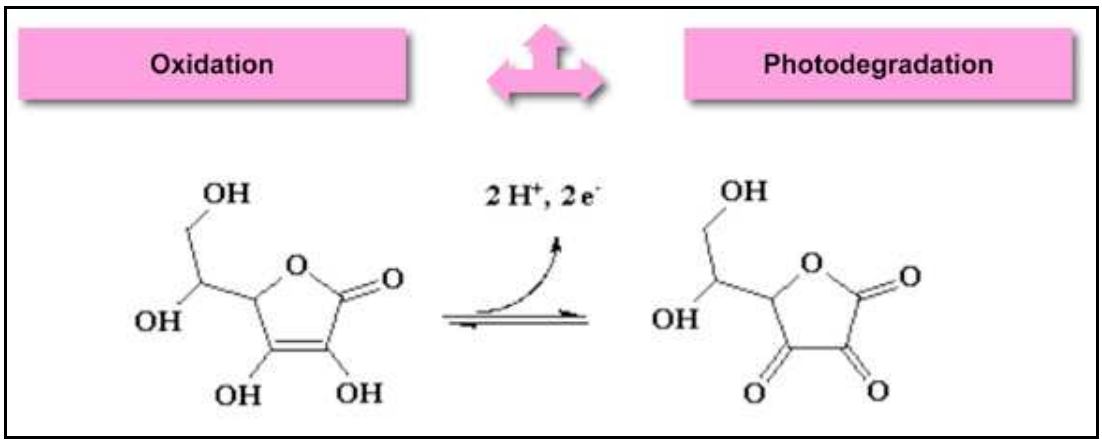


Figure 3. calibration curve of L- ascorbic acid

Discussion

Milk is not rich in vitamin C, but it is used as an indicator because of its sensitivity to light, which is the greatest among the other milk components[18]. Exposure of milk to sunlight or fluorescent light does not only

affect the flavor quality, but also decreases the nutritional value [19]. L- ascorbic acid is extremely unstable to heat, oxygen, light, pH, moisture content[20,21]. L-Ascorbic acid is easily and reversibly oxidized to dehydro ascorbic acid,(figure 4)[22]. Dehydroascorbic acid in solution at neutral $_{p}H$ undergoes a non reversible oxidation to form the biologically inactive, straight–chained compound, 2,3- diketogulonic acid. [23,2]



Dehydroascorbic acid **L -** ascorbic acid

Figure.4 . Mechanism of Vitamin C degradation by exposure to air and light

Table2. 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, D respectively (87, 78, 87, 86.6 mg/100gr) these amounts may be resulted by storage conditions [1].

Table 2 . vitamin C content in milk samples during storage

Vitamin C content in milk sampls* X± SD mg/ 100 g				Time Weeks
D	C	B	A	
85.599±0.30	86.23±0.32	75.743±0.20	85.901±0.11	Open the package (0)
70.331±0.21	78.151±0.22	62.151±0.12	77.731±0.21	After one week
58.861±0.11	64.182±0.12	52.779±0.11	63.658±0.10	After two weeks
41.891±0.03	48.057±0.10	37.362±0.10	47.635±0.22	After three weeks
30.813±0.02	37.362±0.02	26.691±0.03	38.106±0.04	After four weeks

* (Temperature = 23 ±2 ° C , Humidity = 55± 5%, without light)

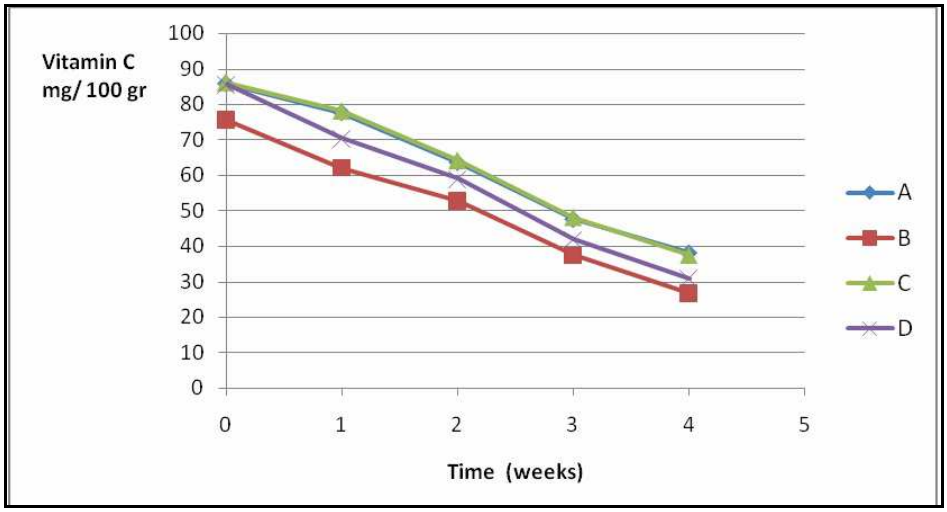


Figure 5. Humidity affect on vitamin C content during storage milk samples (Temperature = 23 ±2 ° C , Humidity = 55± 5%,without light)

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 till the fourth week. Amount of vitamin C 's decrement increases during storage period at room temperature, humidity exposure results increasing in vitamin decrement [24,25], (H = 55± 5%) is chosen humidity , which it is rising humidity average in house kitchen between summer and winter conditions (observed by Electronic Hygrometer)[26].

From table 3 and figure 5 decrement ratio 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 [27, 28, 26].

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

Time* Week	Loss ratio of vitamin C %			
	A	B	C	D
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 º C , Humidity = 55± 5% without light)

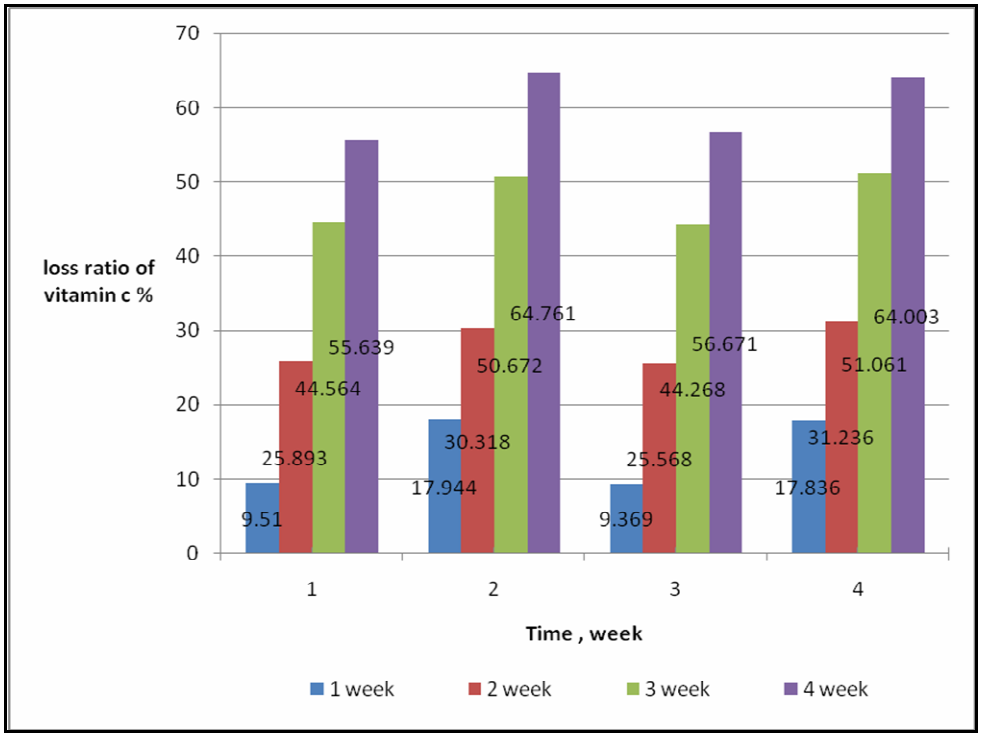


Figure 6.The increasing of Loss ratio of vitamin C in milk samples during storage (1(A), 2(B), 3(C), 4(D))

Table 2 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 package and recommended not to have milk after it),40mg/day is the allowed limitation for this age (0-6 month) [19]. 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)[12]. This leads to the injury of children to vitamin C deficiency**.** [29]

The effect of light

According to table 4 and figure 7, vitamin C amount decreasing during time when samples exposure to humidity and light. Decrement of vitamin C ratio was relatively proximate in samples C and A. It was (68.724 , 69.857 mg/100g) after first week respectively. Decrement of vitamin C increases to (28.106, 26.872 mg / 100gr) after the fourth week , while it was relatively proximate in samples D and B after the first week (66.261 , 59.628 mg /100 gr) respectively and it decreased after the fourth week to (18.519 , 15.502 mg / 100gr) [30, 24].

Tables 2, 4 and figures 5, 7 clarify that losing ratio of vitamin C while exposure for light was higher than being in darkness at same humidity (H = 50 ±5 %) [30].

Table 4. vitamin C content in milk samples during storage

Vitamin C content in milk sampls* X± SD mg/ 100 g				Time Weeks
D	C	B	A	
85.599±0.30	86.230±0.32	75.743±0.20	85.901±0.11	Open the package (0)
66.261±0.41	68.724±0.41	59.628±0.60	69.857±0.54	After one week
46.791±0.23	58.067±0.27	40.74±0.32	58.564±0.43	After two weeks
26.808±0.16	33.848±0.11	23.064±0.21	34.689±0.23	After three weeks
18.519±0.12	28.106±0.11	15.502±0.13	26.872±0.14	After four weeks

*(Temperature = 23 ±2 º C, Humidity = 55± 5%, light = 12000 lux)

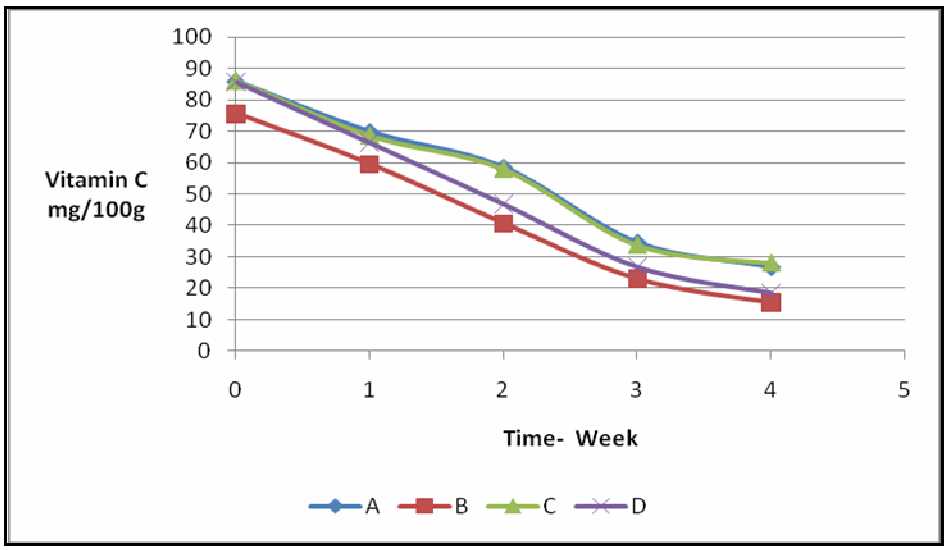


Figure 7. Light affect on vitamin C content during storage milk samples
(Temperature = 23 ±2 º C, Humidity = 55± 5%, light = 12000 lux)

Losing ratio of vitamin C while exposure for humidity in the darkness in samples C and A after the first week was (9.369, 9.510%) while it was (20.301, 18.683 %) during exposure for humidity and light respectively. An extreme increasing in losing ratio of vitamin C when exposure for light was recognized (68.717, 67.405%) after fourth week in comparison with it in darkness(55.639 ,56.671)% .

After the first week of exposure for humidity and light, losing vitamin ratio in samples D and B equals (22.591, 21.275%) . It increased after the fourth week to (78.365, 79.533 %) [31] , this is the highest ratio for both samples in the dark for same humidity ratio, (17.836, 17.944), (64.003, 64.761) after first week and after the fourth week respectively. It is noticed that losing ratio of vitamin was higher in samples B and D than in samples C and A.

Increasing of losing ratio of vitamin C during exposure for light and humidity results a decrement in the amount of vitamin to make it under the international standard limitations of vitamin existing in children milk, and that may make children liable to vitamin C decrement.

Vitamin in samples C and A becomes less than allowed limitations for children for this age (40mg/ 100gr , 0-6 month) after the third week (which is the allowed period for drinking milk), (33.848 , 34.689 %) was vitamin amount in samples C and A after the third week. While Vitamin in samples D and B becomes less than allowed limitations for children for this age (50mg/ 100gr , 6-12 month) before the third week , (46.791, 40.740 %) was vitamin amount in samples D and B after the second week [12].Vitamin C is sensitive to humidity and light and it is destroyed over time when exposed to atmospheric oxygen [30, 32, 33, 34].The result in this study are agreement with reported data on [22], figure 4.

Milk must be reserved in tight closed package and kept away of kitchen humidity and light to ensure give children the vitamin amount required for good health and growth.

Table 5. Loss ratio of vitamin C in milk samples during storage

Time* Week	Loss ratio of vitamin C %			
	A	B	C	D
After one week	18.683	21.275	20.301	22.591
After two weeks	31.823	46.212	32.666	45.336
After three weeks	59.617	69.549	60.746	68.681
After four weeks	68.717	79.533	67.405	78.365

*(Temperature = 23 ±2 º C , Humidity = 55± 5%, light = 12000 lux)

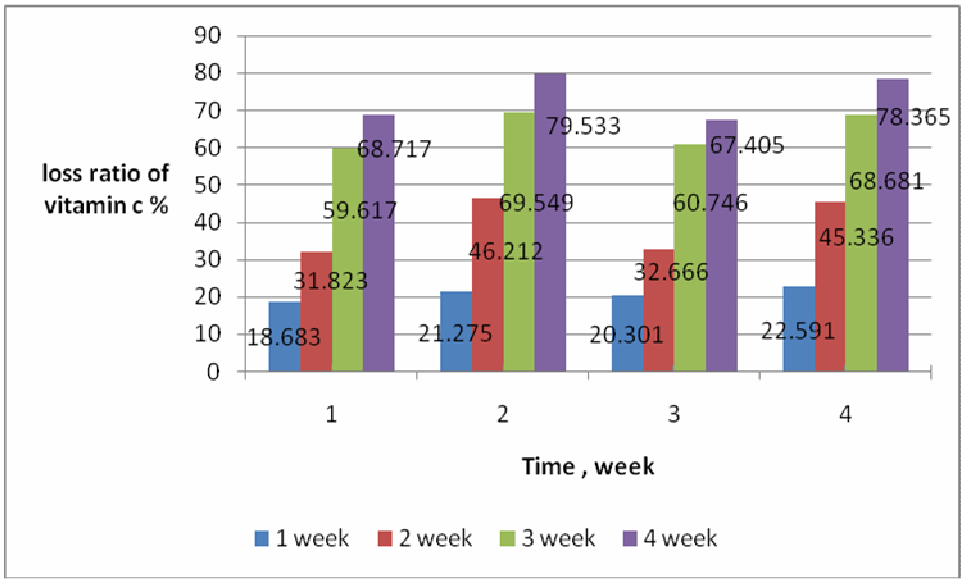


Figure 8. The increasing of Loss ratio of vitamin C in milk samples during storage (With light = 12000 lux) (1(A), 2(B), 3(C), 4(D))

5 – Conclusion

Increasing exposure for light and humidity decreases vitamin C amount during time and increases losing ratio. While losing ratio increases during exposure for light at the same humidity when exposure for darkness and it increases to be under the allowed limitation of losing.

The results of the analysis showed that: Long exposure to light, ambient temperature, and house kitchen humidity (air –conditions) may affect vitamin C stability. Vitamin C content in all samples decreased gradually during storage. The maximum vitamin C was in the milk stored under dark conditions, while the minimum content was in the milk samples stored under light (12000 lux). The results of the analysis showed that, Increasing exposure to room temperature, house kitchen humidity (kitchen's weather) and light results decrement in allowed vitamin C amount limitations in infant's milk. Results show that the largest for keeping baby formula A, C when exposed to moisture and without light for three weeks, either with light conceding for a period of less than three weeks. While saves baby milk B, D when exposed to moisture and without light for a

period of two weeks, either with light conceding for a period of less than two weeks .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 .Milk must be reserved in tight closed package and kept away of kitchen humidity and light to ensure give children the vitamin amount required for good health and growth, and it can be stored safely for long periods of time.

Acknowledgements

The author thanks Dr. Malak ALjoubeh (Department of Chemistry, Faculty of Sciences, Damascus University, Syria) to assist in completing research scientifically, linguistically and laboratory research.

6- References

1. ANDREA, S.T., EMÍLIA, 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.
2. George, F.m. 2006. Vitamins in foods, Analysis, Bioavailability, and Stability. Taylor and Francis group. New York.
3. Bender, D.A., 2003. Vitamin C (ascorbic acid). In: Bender, D.A. (Ed.), Nutritional Biochemistry of the Vitamins. 2nd ed. Cambridge University Press, Cambridge, UK, pp. 357–384.
4. Kinsman, R.A. & Hood,J. 1971. Some behavioral effects of ascorbic acid deficiency. Am. J. Clin. Nutr.,24: 455.
5. Zieve, D. 2010. In Vitamin C: MedlinePlus Medical Encyclopedia. Retrieved, from *http://www.nlm.nih.gov/ medlineplus/ency/article/002404.htm*.
6. FAO/WHO .2002. Vitamin C. In: *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 73-86.
7. Khalid I., Alam K. & M. Muzaffar, A. 2004.Biological Significance of Ascorbic Acid (Vitamin C) in Human Health – A Review. Pakistan Journal of Nutrition 3 (1): 5-13.
8. Wandzilak, T.R., DAndre , & Williams H.E.1994. Effect of high dose vitamin C on urinary oxalate levels. J. Urol., 151: 834-837.
9. IOM .2000. Ascorbic acid. In: *Dietary Reference Intakes for Ascorbic acid, Vitamin E, Selenium, and Carotenoids.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; chapter 5, pp 95-185.
10. Sizer F & Whitney El.1997. Nutrition Concepts and Controversies (7th ed.). West/Wadsworth International Thomas Publishing Company.
11. Mayo Clinic Staff. 2010. In Vitamin C (ascorbic acid). Retrieved June 1, from *http://www.mayoclinic.com/ health/vitamin-c/NS_patient-vi taminc*
12. Dietary Reference intake for vitamin C (DRIS) The essential guide to nutrient Requirements. 2001. Washington : The National Academy Press.
13. Food and Nutrition Board, Institute of Medicine, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academies Press,Washington, DC, USA.
14. 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.
15. Albala-Hurtadom S., Vecianna-Nogues MT., Izqierdo-Pulido M., & Marine-Font A.1997. Determina tion of water-soluble vitamins in infant milk by high-performance liquid chromatography. 778:247-253.
16. 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.
17. Enemo R.E., Ekpunobi,U.E.,Nnubia D., & Onuegbu, T. U.2010. Comparative Study on the Ascorbic Acid Content of Some Common Nigerian Vegetables (*Solanum gilo, Gnetum africanum, Gongomera latifolium,* and *Vernonia amygalina*). *The Pacific Journal of Science and Technology* ,Vol. 11: 2.
18. Metin M.: S.t Teknolojisi. Ege .niv. M.hendislik Fak. Yay. No:33, Üzmir. 1998; 332-334.
19. Olsen J.R. & Ashorr S.H.: An assessment of light induced off flavors in retail milk. J. Dairy Sci., 1986; 70: 13]\62-1370.
20. Lee, S. K. & Kader, A. A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology, 20, 207-220.

21. Lin, T. M.; Durance, T. D. & Scaman, C. H. 1998. Characterization of vacuum microwave, air and freeze-dried carrotslices. Food Research International, 31:2, 111-117.
22. Banzakour G, Fathi M et al. 2005. Influence of temperature, light and plastic material on vitamin c, stability in total parenteral Nutrition Administration sets. JPEN, 29: 125- 130.
23. Rose J. K. C.1987. Solubility properties of reduced and oxidized ascorbate as determinates of membrane permeation, Biochim .Biophys.Acta, 145, 685.
24. Nyyssonen K., Salonen J.T. & Parviainen M.T., 2000. Ascorbic acid. In: Leenheer, A.P.D., Lambert, E., Bocxlaer, J.F. (Eds.), Modern Chromatogragraphic Analysis.
25. Favell, D.J.1998. Acomparison of the vitamin C content of fresh and frozen vegetables, food chem., 62,59.
26. Ozkan M., Ikirca A. & Cemeroglu B. 2004.Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. Food chem., 88,591- 597.
27. DaDavid M .2007. Home moisture problems. Brook. Former extension agent, Multnomah country, Oregon state university .
28. BeiBeizadea E. 2009. Fortification of wheat flour. Romanian Society of Biotechnol. lett. Vol. 14, No. 2.pp. 4300-4306.
29. Mansour R and Aljoubeh M. The effect of storage time and humidity on vitamin C level in infant's baby milk powder after opening the package. International Journal of ChemTech Research.CODEN (USA): IJCRGG ISSN : 0974-4290 Vol.6, No.2, pp 1124-1130, April-June 2014.
30. Song A. & 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.
31. OeOey I.,Verlinde P. & *etal*. 2006. Temperature and pressure stability of L – ascorbic acid and / or 5-methyltetrahydrofolic acid : A kinetic study . Eur food Res Technol , 223, 71- 77.
32. Tawali A.B., Zainal D. 2004. *Mempelajari Perubahan Fisik dan Kimia Buah Pir Selama Penyimpanan. Jurnal Sains dan Teknologi*. 4(3), 130 – 138.
33. Van den Broeck, I., Ludikhuyze, L., Weemaes, C., Van Loey, A., Hendrickx, M.: Kinetics for isobaric-isothermal degradation of Lascorbic acid. J. Agric. Food Chem., 1998; 46: 2001-2006.
34. Pavlovka G, and Tanevsk S. 2010. Influence of temperature and humidity on the degradation process of ascorbic acid in vitamin C chewable tablets. Journal of Thermal Analysis and Calorimetry. Vol.111:3, pp 1971-1977.

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