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The ascorbic acid contents of five green leafy vegetables stored in refrigerator, freezer and sun-dried for five days: 1

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ABSTRACT

Five green leafy vegetables (*Basella alba, Vernonia amygdalina, Talinum triangulare, Manihot esculenta* and *Corchorus olitorius*) were analysed for their moisture, crude protein and ascorbic acid contents. The vegetables were earlier differently sun-dried, stored in the refrigerator and stored in the freezer for five days each before the ascorbic acid contents were determined. Moisture and crude protein were high in all the samples. The between the sample variations in the ascorbic acid contents were high in the fresh (zero day) with values ranging from 234 mg/100 g (recorded in *T. triangulare* and *C. olitorius*) to 1041.3 mg/100 in g *V. amygdalina*. The highest between the day variations in the values of each sample were recorded in the samples kept in the freezer (45.7 % -131.3 %) but lowest in the sun-dried samples (23.6 %-75.7 %); the rate of value change per sample per day also followed this observed trend. The ascorbic acid values in the sun-dried samples were significantly different at $p \leq 0.05$. The percentage loss of ascorbic acid contents in the five days was found to be sample as well as storage condition dependent.

Introduction

Vitamins are important, indeed literally vital constituents of foodstuffs; but were not discovered until early in the 20th century and have not been fully explored[1].

They betrayed their presence through illnesses befalling groups of people who for one reason or another were deprived for one of them. The prevalence of scurvy, which results from a deficiency of vitamin C, might well have started the process of discovering the vitamins. But scurvy was a special problem that arose mainly on long sea voyages and seldom affected the population at large. In general it would be true to say that most people in Europe, up to the 19th century, obtained what vitamins they needed from their diets, even if these diets were rather monotonous. However, the advent of processed food in the 19th century brought with it, along with some advantages, new risks of vitamin deficiency, and set the stage for serious and systematic work on the causes of such deficiency diseases as beriberi and pallagra[1].

Vitamin C was proved to exist by a long series of experiments on guinea pigs begun in 1907 by the Norwegians Holst and Frölic. Actually they were trying to induce beriberi, but the animals went down with what was obviously scurvy. (This was a double fluke. Guinea pigs, apes, monkeys and vampire bats are the only animals apart from humans which cannot make their own vitamin C.) The vitamin was isolated in 1925, by the Hungarian researcher Albert Szent-Györgi[1].

The chemical name or vitamin C is ascorbic or L-ascorbic acid, or simply ascorbate (the anion of ascorbic acid), systematic (IUPAC) name 2-Oxo-L-threo-hexono-1, 4-lactone-2, 3- enediol or (R) -3, 4-dihydroxy-5 ((S) -1, 2- dihydroxyethyl) furan-2(5H)- one. It is required in greater amounts than all the other viatmins[2] and its need has been reported to increase in some diseases, particularly tuberculosis[3].

A survey at Igbo-ora in Western Nigeria[4] found that the intake of ascorbic acid there varied from 280 % of the recommended allowance in children 3-5 years old to about 400 % in pregnant women. On the other hand, Nicol[5] found that lack of fruit and leaves in the Northern Region of Nigeria resulted in varying degrees of dietary deficiency of ascorbic acid. It is therefore concluded that green vegetables must be a good source of ascorbic acid[6].

Ascorbic acid is multifunctional and is involved in many aspects of human physiology. A small molecule of L-ascorbic acid incorporating the endiol structure –C = C -HO OH interferes with a broad spectrum of oxidation-reduction reactions. In this way, vitamin C influences the synthesis of collagen, carnitine and neurotransmitters; the transformation of cholesterol into bile acids; biotransformation of xenobiotic substances; absorption of iron, formation and scavenging of oxygen free redicals. The functions of central nervous, immune and cardiovascular systems, the periodontal tissue as well as the detoxification function of the liver, are negatively influenced by vitamin C deficiency[7].

Some workers have reported losses in the vitamin C contents of various vegetables as a result of blancing[8, 6, 9], standing in various environmental temperatures[2], cooking after various periods of wilting[10] and effect of processing[9,11]; but there are scanty records of losses due to sun-drying, storage in refrigerators and storage in freezers. It is therefore necessary to answer the question of what storage system secures the best quality of vitamin C.

Materials and methods

Samples

The vegetables used in this investigation were *Basella alba* (Indian spinach), *Vernonia amydalina* (Bitter leaf), *Talinum triangulare* (Water leaf), *Manihot esculenta* (Cassava leaf) and *Corchorus olitorius* (Jew's malow). Their vernacular (Yoruba) synonyms are Amununtutu, Ewuro, Gbure, Ewe ege and Ewedu respectively.

Sampling

The vegetables were obtained fresh from farms located around the College of Education Campus at Ikere –Ekiti,

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Nigeria in the morning to avoid loss of vitamin C accompanying wilting[12]. They were quickly brought to the laboratory, washed with tap water to rid them of sand and other impurities and then allowed to drain for about seven minutes.

Sample treatment

The fresh vegetables were treated as follows:

A. After draining, each sample was cut into pieces as it would be prepared for soup in the kitchen, and minced 5 g samples were then taken immediately for vitamin C content determination.

B. The remainder of the samples from A above was divided into three equal parts.

(i) One part of each sample was spread in different plastic trays and solar dried.

(ii) 5g of each sample in five times (representing five days) was weighed into transparent polythene bags tied and kept in the refrigerator (about 5^{o}C).

(iii) The process in B (ii) was repeated but the samples were kept in the freezer (about -18^{o}C).

C. The moisture content of the samples was determined in the fresh vegetables.

D. The crude protein content was determined in the dry vegetables.

E.Results were subjected to statistical analysis[13].

Moisture and crude protein determinations

The moisture content of the samples was determined using the method of AOAC[14]. The nitrogen content was determined by the micro-Kjeldahl method described by Pearson[15] and was converted to crude protein multiplying by 6.25.

Ascorbic acid determination

Vitamin C was determined using the method of Pearson[15]. Standard ascorbic acid was prepared from standard sample obtained from British Drug House (BDH) using metaphosphoric acid-acetic acid solution, while 2, 6 – dichloroindophenol solution was prepared from its sodium salt by dissolving 50 mg of it in 50 cm^3 of distilled water containing 42 mg sodium carbonate, shaken vigorously to dissolve the dye and diluted to 200 cm^3 with distilled water and filtered. The indophenol solution was standardized by titration against the standard ascorbic acid solution and the two standard solutions were stored in refrigerator. The vegetable samples were pulverized by gentle grinding, added HPO_3^--HOAc/5g and filtered. Sample aliquots were titrated with the standard indophenols solution. Blank titrations were carried out using equivalent of extraction solution.

Results and discussion

Moisture and protein contents

The results in Table I show the values obtained for moisture and protein contents of the samples. The values for the moisture contents varied from about 80-8-95.1 g/100 g in *Manihot esculenta* and *Talinum traingulare* respectively. This observation is similar to the results obtained for *Talinum traingulare* by Oke[6] and Faboya[11]; other moisture contents fall within the results of these two authors. All the samples contained high moisture contents. The level of protein in the dried samples ranged from 17.4 g/100 g in *T. traingulare* to 38.8 g/100 g in *B. alba.* The results of the crude protein here have good similarities with the results of Oshodi[12]. This drying method may serve as an alternative method of obtaining leaf protein concentrate[12] and can be used to improve the protein intake during the dry season when farmers in developing countries are usually short of leafy vegetables due to lack of refrigeration facilities. It is observed that the dried form of these leaves were richer in protein than many dried cereals[16]. In addition, it has been shown that the essential amino acid composition of leafy protein is comparable with other good quality sources. Therefore, some of the dry leafy vegetables with high protein content may be used as protein concentrate for the production of animal rations.

Variations in the ascorbic acid contents of the fresh vegetables

Column two in Tables II, III and IV show the values of the ascorbic acid contents of the fresh vegetables. The values ranged from 234.0 mg/100 g recorded in both *Talinum triangulare* and *Corchorus olitorius* to 1041.3 mg/100 g in *Vernonia amygdalina* with a grand mean of 610.7 mg/100 g and a coefficient of variation of 55.9 %. This meant that the intersample variation in the fresh leaves was not very high. Comparison with values in literature is difficult because reported results were obtained from dry samples. The results here showed that all the samples were good sources of vitamin C.

Effect of sun-drying on the ascorbic acid contents of the vegetables

Table II shows the effect of sun-drying on the vegetables for a period of five days. The most obvious result was the loss of vitamin C on daily basis for all the samples although *T. triangulare* maintained the value of 187.2 mg/100 g on days 1-3. The between the day variations were high in *B. alba* (54.0 %) and *M. esculenta* (75.7 %) while such variations were less than 50.0 % in the other three samples with *T. triangulare* having the least coefficient of variation of 23.6 %. The rate of ascorbic acid change per day in the samples was highest in *B. alba* (152.1 mg/100 g) followed by *V. amygdalina* (119.3 mg/100 g) and then *M. esculenta* (103.0 mg/100 g) while the least position in the rate of change per day was shared by *T. traingulare* and *C. olitorius* with a value of 28.1 mg/100 g. The intersample variations in the ascorbic acid contents for each day were high with coefficients of variations ranging between 52.7-76.4 %.

The values obtained in Table II even at the 5th day of sun-drying was much higher than the results of Oke[6] who also determined the ascorbic acid contents of some vegetables similar to those under discussion. Fafunso and Bassir[10] got values which are similar to the average values in this report for *T. triangulare* and *C. olitorius* but other average values in this report were higher than their results. These results, however, fall within the wide range of 29-643 mg reported for the ascorbate contents of several Malaysian leafy vegetables purchased over a period of several months[10]. Various reasons could be used to account for the difference in values, such as differences in values, such as differences in leaf maturity and the fact that quite different ascorbic acid assay methods were employed [17, 18]. The differences in the ascorbic acid contents of the five leaves under report was not due to chance as significant differences was observed at $p \leq 0.05$.

The percentage losses of ascorbic acid due to sun-drying is depicted in Fig.I. *V. amygdalina* had the least loss of ascorbic acid at the end of the first day with a value of 6.72 % but moved very high to 42.68 % on the second day but changing very slowly with a loss of 50.5 %- 57.3 % in days 3-5. *T. triangulare* lost only 20.0 % of its total ascorbic acid in the first three days, lost 25.0 % on the fourth day and 60.0 % on the fifth day. *C. olitorius* lost 20.0 %, 25.0 %, 30.34 %, 40.0 % and 60.0 % from day one to day five making it very nearer to *T. traingulare* in vitamin loss rate during sun-drying. The daily loss of ascorbic acid in *B. alba* for five days were 35.8 %, 48.14 %, 60.49 %, 75.31 % and 80.25 % respectively. The behavior of *M. esculenta* followed the pattern of *B. alba* with loss ranging from 58.85 % to 86.23 %.

Table I. Moisture and crude protein contents of the leafy vegetables

Sample number	Local names	English names	Botanical names	[a]Moisture in fresh sample (g/100 g)	[a]Crude protein in dried samples (g/100 g)
1.	Amununtutu	Indian spinach	*Basella alba*	93.4± 1.6	38.8±0.8
2.	Ewuro	Bitter leaf	*Vernonia amygdalina*	87.3±0.4	32.0±0.6
3.	Gbure	Water leaf	*Talinum triangulare*	95.1±0.3	17.4±0.6
4.	Ewe ege	Cassava leaf	*Manihot esculenta*	80.8±1.0	23.6±0.5
5.	Ewedu	Jew's malow	*Corchorus olitorius*	89.7±0.6	25.0±0.0

[a]Determintion was in triplicate.

Table II. The ascorbic acid contents (mg/100 g)[a,b] of leafy vegetables sun-dried for five days

Sample number	Fresh 0	Sun – dried Day (s) 1	2	3	4	5	Grand mean	CV %	Rate of change per day
1.	947.7 (11.7)[c]	608.4 (0.0)	491.4 (0.0)	374.4 (0.0)	234.0 (0.0)	187.2 (0.0)	473.9 (255.8)	54.0	152.1
2.	1041.3 (11.7)	971.1 (11.7)	596.7 (11.7)	514.8 (0.0)	456.3 (11.7)	444.6 (0.0)	670.8 (243.1)	36.2	119.3
3.	234.0 (0.0)	187.2 (0.0)	187.2 (0.0)	187.2 (0.0)	175.5 (11.7)	93.6 (0.0)	177.5 (41.9)	23.6	28.1
4.	596.7 (11.7)	245.7 (11.7)	187.2 (0.0)	140.4 (0.0)	117.0 (0.0)	81.9 (11.7)	228.2 (172.8)	75.7	103.0
5.	234.0 (0.0)	187.2 (0.0)	175.5 (11.7)	163.8 (0.0)	140.4 (0.0)	93.6 (0.0)	165.8 (42.9)	25.9	28.1
Grand mean	610.7 (341.5)	439.9 (308.5)	327.6 (179.9)	276.1 (145.3)	224.6 (122.4)	180.2 (137.6)	343.2 (145.1)	42.3	86.1
CV (%)	55.9	70.1	54.9	52.6	54.5	76.4	52.7	-	-

[a]Determination was in duplicate. [b]Values were significant at p < 0.05. [c]Standard deviations are in parentheses.

Table III. The ascorbic acid contents (mg/100 g) of leafy vegetables stored in the freezer for five days

Sample number	Fresh Freezer Storage Day (s) 0	1	2	3	4	5	Grand mean	CV (%)	Rate of Change per day
1.	947.7 (11.7)	315.9 (11.7)	152.1 (11.7)	105.3 (11.7)	81.9 (11.7)	46.8 (0.0)	275.0 (312.9)	113.8	180.2
2.	1041.3 (11.7)	163.8 (0.0)	128.7 (11.7)	93.6 (0.0)	81.9 (11.7)	81.9 (11.7)	265.2 (348.2)	131.3	191.9
3.	234.0 (0.0)	210.6 (0.0)	128.7 (11.7)	93.6 (0.0)	70.2 (23.4)	46.8 (0.0)	130.7 (69.7)	53.3	37.4
4.	596.7 (11.7)	269.1 (11.7)	140.4 (0.0)	93.6 (0.0)	81.9 (11.7)	46.8 (0.0)	204.8 (189.0)	92.3	110.0
5.	234.0 (0.0)	140.4 (0.0)	105.3 (11.7)	93.6 (0.0)	81.9 (11.7)	70.2 (0.0)	120.9 (55.2)	45.7	32.8
Grand mean	610.7 (341.5)	220.0 (65.1)	131.0 (15.5)	95.9 (4.7)	79.6 (4.7)	58.5 (14.8)	199.3 (191.1)	95.9	110.4
CV (%)	55.9	29.6	11.8	4.9	5.9	25.3	29.7	-	-

Table IV. The ascorbic acid contents (mg/100 g) of leafy vegetables stored in the refrigerator for five days

Sample number	Fresh Freezer Storage Day (s) 0	1	2	3	4	5	Grand mean	CV (%)	Rate of Change per day
1.	947.7 (11.7)	315.9 (11.7)	175.5 (11.7)	93.6 (0.0)	46.8 (0.0)	46.8 (0.0)	271.1 (316.5)	116.7	180.2
2.	1041.3 (11.7)	187.2 (0.0)	140.4 (0.0)	93.6 (0.0)	81.9 (11.7)	81.9 (11.7)	271.1 (346.5)	127.8	191.9
3.	234.0 (0.0)	210.6 (0.0)	187.2 (0.0)	152.1 (11.7)	93.6 (0.0)	81.9 (11.7)	159.9 (56.8)	35.5	30.4
4.	596.7 (11.7)	456.3 (11.7)	269.1 (11.7)	140.4 (0.0)	81.9 (11.7)	46.8 (0.0)	265.2 (201.4)	75.9	110.0
5.	234.0 (0.0)	187.2 (0.0)	152.1 (11.7)	105.3 (11.7)	93.6 (0.0)	93.6 (0.0)	144.3 (52.6)	36.5	28.1
Grand mean	610.7 (341.5)	271.4 (104.0)	184.9 (45.3)	117.0 (24.5)	79.6 (17.2)	70.2 (19.6)	222.3 (186.7)	84.0	108.1
CV %	55.9	38.3	24.5	20.9	21.6	27.9	23.7	-	-

These results showed that exposure to sun before analysis caused an appreciable destruction of the ascorbic acid with loss values ranging between 57.3 % to 86.23 % at the end of five days.

Most of these leaves usually keep their lush appearance on the open market stalls up to about 10 h after harvest from various farms, after which physical and visible wilting occurs. At this time, they no longer entice the housewives and other buyers and are thus discarded[10]. Exception to this is *T. triangulare* which wilt more slowly, as they still retain their fresh appearance up to 24 h after harvest.

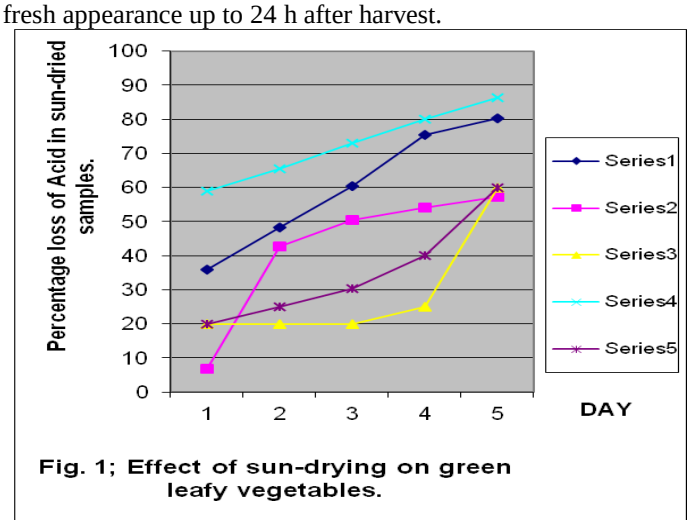

Fig. 1; Effect of sun-drying on green leafy vegetables.

Effect of storage in the freezer on the ascorbic acid contents of the vegetables

Table III shows the effect of freezer storage on the ascorbic acid values of the vegetables for five days. There was a downward ascorbic acid change in all the samples with the rate of change higher than the observation in the sun-dried samples. The between the day changes in the ascorbic acid values were very high in *B. alba, V. amygdalina, T. triangulare* and *M. esculenta* with coefficient of variations of 113.8 %, 131.3 %, 53.3 % and 92.3 % respectively; the coefficient of variation in *C. olitorius* was 45.7 %. However, the between the sample coefficient of variation as observed in columns 3, 4, 5, 6, 7 were 29.6 %, 11.8 %, 4.9 %, 5.9 %, 25.3 % and 29.7 % respectively. Since such wide differences occur in the coefficient of variations in between the day changes and between the sample changes per day in ascorbic acid values, it may be concluded that the effect of the freezer storage is a function of the particular type of vegetable. Also, the rate of change per day in the ascorbic acid values for each samples was correspondingly higher in the freezer samples than in the sun-dried samples. Such values were 180.2 mg/100 g (*B. alba*), 191.9 mg/100 g (*V. amygdalina*), 37.4 mg/100 g (*T. triangulare*), 110.0 mg/100 g (*M. esculenta*) and 32.8 mg/100 g (*C. olitorius*). Like the observation in Table II, both *T. triangulare* and *C. olitorius* had the lowest rate of change per day. No significant difference ($p \leq 0.05$) was observed among the different ascorbic acid values in the different vegetables under this condition.

The percentage losses of ascorbic acid due to freezer storage is depicted in Fig.II. *T. triangulare* recorded the least loss at the end of the first day with a value of 10.0 %; in Fig. I, V. *amygdalina* had the lowest with a value of 10.0 % in day one to 80.0 % in the fifth day, this value is more than the 60.0 % recorded in the sun-dried samples in the 5th day. The percentage loss in *C. olitorius* rose from 40.0 % in the first day to above 60 % in the 5th day. The percentage losses in *B. alba, V. amygdalina* and *M. esculenta* were high with values ranging from 66.65 %-95.06 %, 85.4 %-92.12 % and 54.93 %-92.16 % respectively; this means that these three vegetables would have lost more than 90.0 % of their ascorbic acid contents at the end of five days storage in the freezer. The high rate of percentage loss in ascorbic acid may be due to the chilling effect in the freezer. *T. triangulare* is a succulent vegetable and this may account for the low loss (10.0 %) of ascorbic acid at the end of the first day.

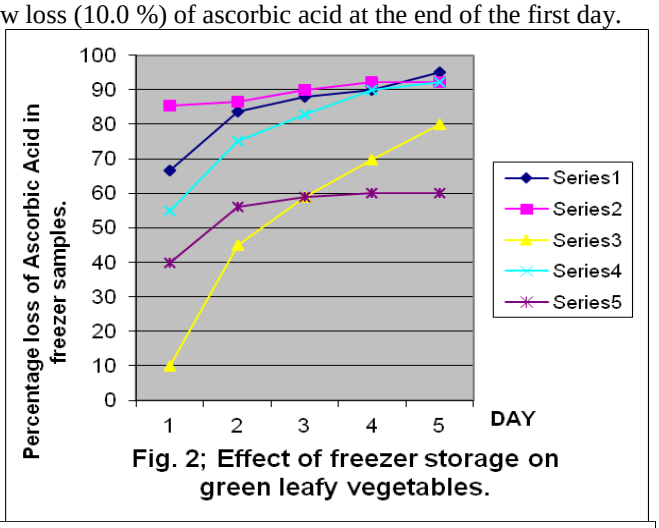

Fig. 2; Effect of freezer storage on green leafy vegetables.

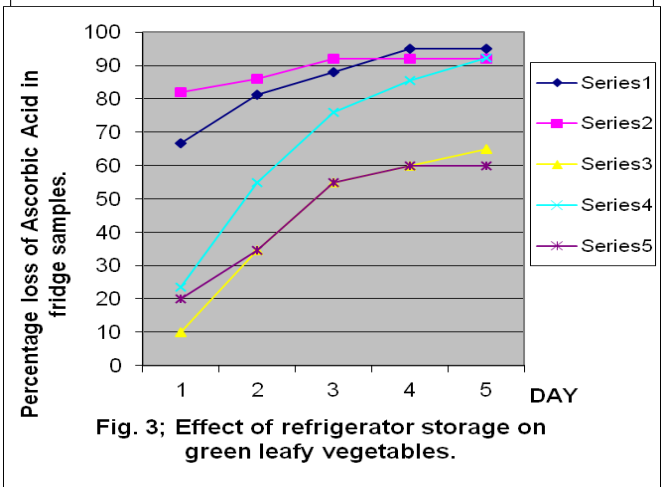

Fig. 3; Effect of refrigerator storage on green leafy vegetables.

Effect of storage in the refrigerator on the ascorbic acid contents of the vegetables

Table IV shows the effect of household refrigerator storage on the ascorbic acid value of the vegetables for five days. The between the day ascorbic acid value changes in each sample closely followed the trend as seen in Table III with very high coefficients of variation for three of them and low CV (%) for the rest two . For example, the CV (%) for *B. alba, V. amygdalina* and *M. esculenta* were 116.7, 127.8 and 75.9 respectively while the CV (%) values for *T. triangulare* and *C. olitorius* were 35.5 and 36.5 respectively. Also the variation per day between the samples showed low CV (%), (although slightly higher than the values in Table III) with values ranging between 20.9-38.3. These results showed that storage in freezer and household refrigerator may be having similar effect on these vegetables (Tables III and IV). No significant difference ($p \leq 0.05$) was observed among the sample results. The rates of change per day in the ascorbic acid contents in *B. alba, V. amygdalina* and *M. esculenta* were found to be similar in Tables III and IV with respective values of 180.2 mg/100 g, 191.9 mg/100 g and 110.0 mg/100 g but such values in *T. triangulare* and *C. olitorius* were 30.4 mg/100 g and 28.1 mg.100 g respectively which were slightly lower than the results in Table III.

The percentage losses of ascorbic acid due to refrigerator storage is depicted in Fig.III. The T. *triangulare* had the lowest

loss of 10.0 % at the end of the first day (like in Fig.II) and lost about 65.0 % in the 5th day. Again, *B. alba, V. amygdalina* and *M. esculenta* recorded comparatively higher losses with values ranging from 66.68 %- 95.06 %, 82.02 %-92.12 % and 23.46 %-92.16 % respectively, this is the same trend as observed in Fig. II. This means reasonable amount of ascorbic acid contents were lost when these vegetables were stored in the refrigerator for five days.

Conclusions

The effect of sun-drying on the vegetables led to significant differences in their ascorbic acid contents with variations of 52.6 %-76.4 %, the variation of ascorbic acid contents in the five days in each sample varied between 23.6 %-75.7 %. The effects of freezer and refrigerator storage appeared to be similar in all the vegetable samples (Tables III and IV; Figure II and III), with high losses of ascorbic acid contents. To conserve vitamin in them, it is recommended that these vegetables should be processed immediately after harvesting or purchasing[11]. On the other hand, if preservation is contemplated, sun-drying is recommended for them all or storage of *C. olitorius* and *T. triangulare* in the refrigerator or storage of C. olitotius only in the freezer. On the 5th day, *B. alba, V. amygdalina* and *M. esculenta* were in colloquial terms, not a 'vegetable' any more but can be consumed as a source of protein and calories[19].

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