Influence of Storage Temperature on Vitamin C in Camel [Camelus dromedaries] Milk

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DEDICATION

To my lovely Kids

To the soul of my father

To my lovely mother

To my sisters and brothers

To my friends

To all those whom I love
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# LIST OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>1</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>II</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td>III</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>V</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>VI</td>
</tr>
<tr>
<td>ENGLISH ABSTRACT</td>
<td>VII</td>
</tr>
<tr>
<td>ARABIC ABSTRACT</td>
<td>VIII</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
</tr>
</tbody>
</table>

## CHAPTER ONE: LITERATURE REVIEW

1.1 Classification of camel                                 | 4    |
1.1 Nature of camel's milk                                   | 4    |
1.2 Fresh camel's milk                                       | 5    |
1.3 The colostrums                                          | 5    |
1.4 General composition of camel's milk                      | 6    |
1.5 Chemical composition of camel's milk                     | 9    |
1.6 Milk protein content of camel's milk                     | 9    |
1.7 The amino acid composition                               | 11   |
1.8 Lactose content of camel's milk                          | 11   |
1.9 The minerals content of camel's milk                     | 12   |
1.10 The vitamins content of camel's milk                    | 12   |
1.10.1 Vitamin C sources                                     | 13   |
1.10.2 Vitamin C and formation of collagen                   | 14   |
1.10.3 Vitamin C as an anti-oxidant                          | 14   |
1.10.4 Synthesis of vitamin C                                | 15   |
1.10.5 The history of vitamin C ...............................................................16
1.10.6 Vitamin C intake ...........................................................................16
1.10.7 The benefits of vitamin C .............................................................18
1.10.8 Importance of camel's milk for human nutrition .........................19
1.11 Vitamin C and cancer ....................................................................20
1.12 Vitamin C and blood pressure ......................................................21

CHAPTER TWO:
MATERIALS AND METHODS
2.1 Materials .............................................................................................23
2.1.1 Experimental animals .....................................................................23
2.1.2 Blood sampling ................................................................................23
2.1.2.1 Milk sampling ...............................................................................24
2.2 Reagents ...............................................................................................24
2.3 Methods Determination of Ascorbic Acid ............................................25
2.4 Standard Sample preparation ...............................................................25
   Methods ................................................................................................26
2.5 Chemicals ............................................................................................26
2.6 Apparatus ............................................................................................26
2.7 Statistical analysis ................................................................................26
2.8 Method of ascorbic analysis by HPLC ..................................................27
2.9 Determination of Ascorbic Acid concentration ....................................27

CHAPTER THREE
RESULTS ..................................................................................................31

CHAPTER FOUR:
DISCUSSION .............................................................................................44
CONCLUSIONS ..........................................................................................47
RECOMMENDATIONS ................................................................................47
REFERENCES ..............................................................................................48
Appendix ....................................................................................................53
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Relation of ascorbic acid concentration in serum and Camel fresh milk</td>
<td>33</td>
</tr>
<tr>
<td>2-</td>
<td>Correlation between ascorbic acid concentrations in Serum and camel fresh milk</td>
<td>34</td>
</tr>
<tr>
<td>3-</td>
<td>The relation of ascorbic acid concentration in fresh milk, fresh milk after 7 days in refrigerator at 4 °C, Fermented milk and fermented milk after 7 days in refrigerator at 4°C in refrigerator</td>
<td>35</td>
</tr>
<tr>
<td>4-</td>
<td>The relation of ascorbic acid concentration in fresh milk, fresh milk after 7 days in refrigerator at 4°C, Fermented milk and fermented milk after 7 days in refrigerator at 4°C (HPLC)</td>
<td>36</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>Figure No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Ascorbic acid reduced form</td>
<td>22</td>
</tr>
<tr>
<td>2-</td>
<td>Sample detected in HPLC</td>
<td>29</td>
</tr>
<tr>
<td>3-</td>
<td>HPLC Standard curve</td>
<td>30</td>
</tr>
<tr>
<td>4-</td>
<td>Concentration of ascorbic acid in serum and camel fresh milk.</td>
<td>37</td>
</tr>
<tr>
<td>5-</td>
<td>The relation of ascorbic acid concentration in fresh milk, fresh milk after 7 days in refrigerator at 4 °C, fermented milk and fermented milk after 7 days in refrigerator at 4 °C.</td>
<td>38</td>
</tr>
<tr>
<td>6-</td>
<td>The relation of ascorbic acid concentration in fresh milk, fresh milk after 7 days in refrigerator at 4 °C, fermented milk and fermented milk after 7 days in refrigerator at 4 °C (HPLC).</td>
<td>39</td>
</tr>
<tr>
<td>7-</td>
<td>HPLC result paper</td>
<td>40</td>
</tr>
<tr>
<td>8-</td>
<td>HPLC result paper</td>
<td>41</td>
</tr>
<tr>
<td>9-</td>
<td>HPLC result paper</td>
<td>42</td>
</tr>
<tr>
<td>10-</td>
<td>HPLC result paper</td>
<td>43</td>
</tr>
</tbody>
</table>
Abstract

The aim of this study is to detect the amount of ascorbic acid in milk and blood serum of camel, and determine the effect of storage temperature on ascorbic acid concentration of camel milk.

This study was carried out in camel unit of Khartoum University Farm. 27 samples of blood serum and 54 milk samples were collected from nine healthy she camel at age between 4-7 years which grazed naturally, their milk yield was 1-3 kilogram /day for one animal. Samples were taken under veterinary supervision and subjected to laboratory analysis in Shambat central laboratories where analyzed by Spectrophotometer and HPLC to confirm the result. All chemicals used were annular grade.

The result of the study was that the mean of ascorbic acid concentration in serum was 17.08±3.02mg/dl, in fresh camel milk was 65.57±9.26mg/dl at 37 C,° in fermented camel milk(after one day in room temperature 25C°) was 41.01±11.16mg/dl, the concentration of ascorbic acid in milk after 7 days in refrigerator at 4C° was 57.53±7.65mg/dl, the ascorbic acid concentration in fermented camel milk after 7 days in refrigerator at 4C° was 25.86±6.65mg/dl this readings are highly significant at 0.05 level.
The Pearson correlation between ascorbic acid concentration in serum and ascorbic acid concentration in fresh camel milk was 0.792 that means correlation is significant at the 0.05 level. The study conducted that fermentation reduce the level of Vitamin C significantly compared to the non fermented milk that kept in the same temperature for the same period. Also storage even in cool temperature result in very low level of ascorbic acid compared to fresh milk.
الخلاصة

الهدف من الدراسة هو تحديد كمية فيتامين سي في سيرم الدلم ولبن النباق وكذلك دراسة اثر درجة حرارة حفظ اللبن على تركيز فيتامين سي في لبن الأبل.

اجربت هذه الدراسة في وحدة الأبل بمزرعة جامع الكريتومم.

اخذت 27 عينة من سيرم الدلم و 54 عينة من اللبن من 9 نباق معافاة واعمارها بين 4-7 سنوات وغذاؤها على المرعى الطبيعي انتاج الحليب يتراوح بين 1-3 كيلوجرام في اليوم للقيقة الواحدة. اخذت العينات تحت اشراف بيطرى وأجري التحليل في المعامل المركزي بسمبات. كل المواد المستخدمة كانت قياسية. وقد خلصت هذه الدراسة إلى ان متوسط تركيز فيتامين سي في سيرم دم الأبل 3.02+17.08 ملمج/دسل.

ومن المستقرز لزيادة فيتامين سي في اللبن الطازج في درجة حرارة 37 م ل 9.2+57.6 ملمج/دسل. أما متوسط تركيز فيتامين سي في القدر 3 ملليمتر لمدة 7 أيام كان 7.3+57.6 ملمج/دسل. أما متوسط تركيز فيتامين سي في اللبن القارص بعد حفظه عند درجة حرارة 4 م لمدة 7 أيام فهو 6.65+25.86 ملمج/دسل.

وهذه القراءات ذات فروق معنوية عالية عند مستوى 0.05. معامل ارتباط بيرسون بين نسبه فيتامين سي في سيرم الدم ولبن الأبل الطازج كان 0.0792، وهذا دليل على ان الارتباط موجب وذو قيمة معنوية عالية.

خلصت الدراسة الى أن التخمير يؤدي الى نقصان معنوي في مستوى فيتامين سي في اللبن المتخمر مقارنة بالبن الطازج والمتحرك في نفس درجة الحرارة وفي نفس المدى الزمني.

وأيضا خلصت الدراسة الى ان الحفظ في المبرد للبن الطازج والبن المتخمر يؤدي الى نقصان في مستوى فيتامين سي في اللبن المتخمر مقارنة بالبن الطازج.
Introduction

The milk of dromedaries, or domesticated one-hump camels, is high in both protein and vitamin C, and low in cholesterol. Loaded with minerals such as sodium, potassium, and magnesium, camel milk is easily digestible by those with lactose intolerance and has been hailed as a possible cure for everything from diabetes to Cohn's disease. Camels are capable of producing remarkable amounts of this ultra-nutritious foodstuff in some of the most challenging climatic conditions on earth, Matthew (2006).

Camel milk is rich in vitamin C. This is important from the nutritional standpoint in areas where fruit and vegetables containing vitamin C are scarce. The vitamin C content of camel milk varies between 5.7 and 9.8 mg percent. As lactation progresses, the vitamin C content increases. Vitamin C levels are three times that of cow milk and one-and-a-half that of human milk, Zafar (2004).

Ascorbic acid is a sugar acid with antioxidant properties. Its appearance is white to light-yellow crystals or powder, and it is water-soluble. One form of ascorbic acid is commonly known as vitamin C. The name is derived from a- (meaning "no") and scorbutus (scurvy), the disease caused by a deficiency of vitamin C. At the time of its
discovery in the 1920s, it was called hexuronic acid by some researchers, Svirbelf et al (2007).

Ascorbate usually acts as an antioxidant by being available for energetically favorable oxidation. Many oxidants (typically, **reactive oxygen species**) such as the hydroxyl radical (formed from **hydrogen peroxide**), contain an unpaired electron, and, thus, are highly reactive. This can be highly damaging to humans and plants at the molecular level due to their possible interaction with **nucleic acids**, proteins, and lipids. These free radical interactions are so damaging since they result in a whole chain of free radical reactions. More specifically, the interaction of an initial free radical (often reactive oxygen species) with another molecule changes that molecule itself into a free radical, which than reacts with other molecules and also turning them into free radicals. Ascorbate can terminate these chained radical reactions by being a stable electron donor in interactions with free radicals, being first converted into an ascorbate radical then **monodehydroascorbate** and then **dehydroascorbate**. The oxidized forms of ascorbate are relatively stable and un-reactive, and do not cause cellular damage and can be reversed back to ascorbate by cellular enzymes. Valpuesta et al (2004).
Objectives:

1- To detect present of vitamin C in camel milk and blood serum.
2- To detect the effects caused by storage temperature on vitamin C concentration of camel milk.
CHAPTER ONE
Literature Review

1.1 Classification of camels

All the members of the camel family are found in the order of the Artiodactyla (even-toed ungulates); suborder: Tylopoda (pad-footed); family: Camelidae. The old-world genus is the Camelus, having the two species of the Bactrianus (two-humped) and Dromedarius (one-humped). The new-world genus of the Lama has three species, while the genus of Vicugna has only one species. Yagil & Etzion, (2000).

1.2 Nature of camel milk:-

Most camel milk is drunk fresh. It is also consumed when slightly sour or strongly soured. Camels' milk is generally opaque white. Yagil and Etzion,( 2000). Normally it has a sweet and sharp taste, but sometimes it is salty. Rao et al (1970). At times the milk tastes watery. In certain countries there are prejudices among the urban population concerning camel milk. It is considered as having an unpleasant taste Yasin (1990). It is frothy when shaken slightly . Shalash (1979). The changes in taste are caused
by the type of fodder and the availability of drinking water. Majid (2000).

1.3 Fresh camels' milk:

Camel milk is generally opaque white. Normally it has a sweet and sharp taste, but sometimes it is salty. Sometimes the milk tastes watery. Fresh camels' milk has a high pH. The pH of milk is between 6.5–6.7. This is similar to the pH of sheep's milk. When camel milk is left to stand, the acidity rapidly increases. The lactic acid content increases from 0.03 percent after standing 2 hours to 0.14 percent after 6 hours Shalash (1979).

1.4 The colostrums:

The first milk, the colostrum, is white and slightly diluted as compared with the colostrum of cow Yagil and Etzion, (2000). Other studies on the composition of the milk, depending on the stage of lactation, confirm these data. It was found that 3 hours post-partum total solids averaged 30.4 percent. The T.S. declined to 18.4 percent during the first 2 days of lactation. This decline in T.S. was not caused by a variation in fat content, as initially the fat percentage was low, at 0.2 percent, and then greatly
increased to 5.8 percent; rather the decline in total proteins and minerals was responsible.

In Somalia the colostrum (dumbar) is used by some as a food, but is generally only taken as a laxative Ismael, (1986). However, in most countries where camels are kept, the colostrum is considered unsuitable for drinking (Shalash, 1979). It is even considered as unsuitable for the calf and is milked onto the ground. However, as colostrum contains large amounts of antibodies and is beneficial for digestion in the newborn calves, it is advisable to use it for the calves, if it is not palatable for human consumption.

1.5 The general composition of camels' milk:-

Milk analyzed at monthly intervals until the 6th month of lactation, then analyzed in the 12th month, and at the end of the 14–17 months total lactation period, showed that the average composition observed during the first month of lactation remained constant for the first 6 months Ismael, (1986).

The specific gravity of camel milk is less than that of cow, sheep or buffalo milk Shalash, (1979). The most important factor in camel milk is water content. Young camels, and especially the humans living in drought areas, are in need
of fluid to maintain homeostasis and thermo neutrality. The water content of camel milk fluctuates from 84 percent to 90 percent Knoess,(1977). When examining only the effects of the lack of drinking water on camel milk, the diet remaining unchanged throughout the year, great changes in water content of milk were found Yagil and Etzion, (2000). The camels were allowed ad libitum drinking water only during the winter. From spring until the end of summer the mothers and calves were allowed to drink only once a week for one hour. With water freely accessible, the water content of the milk was 86 percent, but when water was restricted the water content of milk rose to 91 percent. These changes reflect the range presented in the literature and thus makes it important as to when the milk was sampled by the various investigators. Water content of fodder would also affect water content of milk. Thus, it would appear that the lactating camel loses water to the milk in times of drought. This could be a natural adaptation in order to provide not only nutrients, but necessary fluid to the dehydrated calf. Another explanation can be found when examining the mechanism of sweating in man when exposed to heat Conte.et al, (1984). Adaptation to heat causes secretion of a profuse watery sweat. This is caused by secretion of endogenous ADH (anti-diuretic hormone, secreted from
the neurohypophysis) because man process the same water sweat when injected with ADH. Thus man loses water from his sweat glands, allowing him to maintain thermo neutrality. As the mammary glands have the same embryonic origin as the sweat glands Conte. et al, (1984), and as ADH secretation is elevated in the dehydrated camel Yagil and Etzion,( 2000), it could happen that the loss of water into the milk is due to the action of this hormone. Injections of ADH into lactating laboratory rats exposed to heat for 8 hours a day also caused increased water content in milk Yagil and Elzion (2000). Even the milk of slightly dehydrated cows, not under desert conditions, shows such an increase Omer (2001). It is also of importance to note that the other hormone of the neurohypophysis is oxytocin, the hormone that is essential for the letdown of milk. Stimulation of suckling and milking could possibly influence the neurohypophysis and induce secretion of both hormones and so lead to a dilution of the milk. Whatever the explanation, the diluted milk at times of water deprivation makes an excellent food for man. It also explains the Bedouin tales of taking a lactating camel along on long trips through the desert . Musa (2003).
1.6 Chemical composition of camel milk:

Fat 5.38% - SNF 7.01% - Protein 3.01% - Lactose 3.36% - Ash 0.7% - Density Water. Omer (2001)

With the increase in water content of milk produced by thirsty camels, there was a decrease in the fat content, from 4.3 to 1.1 percent. Yagil, Etzion (2000). In the literature, the percentage of milk fat of camels varies from 2.6 to 5.5. Again, the hydration status of the animals would determine the fat content of the milk, as well as the type of forage eaten Yasin (1990).

The milk fat is also different from that of other animals. When left standing, fat is distributed as small globules throughout the milk. Yagil and Etzion (2000). The fat globules are very small 1.2–4.2 microns in diameter Omer (2001). The ratio of fat to total solids averages 31.6 percent, This is much lower than that of the buffalo, which is 40.9 percent Shalash(1979). The fat appears to be bound to the protein. This would explain why it is difficult to extract fat by the usual method of churning sour milk Rao et al., (1970). This difference in milk fat necessitated saponification of camel milk in order to extract vitamin A and carotene Petroleum ether extraction, as used in milk of other animals, was not efficient enough for camel milk Musa (2003).
Compared to cow, buffalo and ewe milk fat, camel milk fat contains less short-chained fatty acids, but the same long-chained fatty acids can be found. Ismael (1986) claim that the value of camel milk is to be found in the high concentrations of volatile acids and, especially, linoleic acid and the polyunsaturated acids, which are essential for human nutrition. The molar percentage distribution of the glycerides in camel milk fat is as follows: Fully saturated glycerides is 25.6. Mono-oleo unsaturated glycerides 37.8. The total saturated acids in whole fat was 62.6 percent mole Ismael (1986).

1.7 Milk protein content of camel milk:-

Milk protein content of camel milk ranges from 2 to 5.5 percent. Yasin (1990). The total protein in camel milk is similar to that of cow milk. Ismael (1986) reported the casein content of dromedary and Bactrian milk as 2.7 and 0.89 percent respectively and that of albumin as 3.8 and 0.97 percent respectively. Examined four breeds of camels and found the value for total protein to vary from 3.5 to 3.8 percent and casein from 2.7 to 2.9 percent. Egyptian camels had low casein, 2.6 percent. Camel milk casein and their fractions were found to be poor in crude protein when compared with cow milk. Yasin (1990).
Milk from the dehydrated camel has a severely decreased protein percentage. Yagil and Etzion (2000). Again, this demonstrates the direct effect of drinking water on the composition of milk. It must be stressed that protein content of the feed will also directly affect that of milk.

1.8 The amino acid composition:-

The amino acid composition of Bactrian milk declines as lactation advances. Omer (2001). The contents of methionine, valine, phenylalanine, arginine and leucine are greater than in cow milk. The nitrogen content of camel milk was found to be 15.6 gr/100 gr. The following amino acids were present: alanine 3.05; arginine 3.15; asparagine 7.65; glycine 1.57; glutamine 23.4; histidine 2.5; isoleucine 6.4; leucine 10.4; lysine 7.6; methionine 3.5; phenylalanine 5.7; proline 13.3; serine 5.9; threonine 6.9; tyrosine 5.8; valine 7.4. Omer (2001).

1.9 Lactose content of camel milk:-

Omer (2001) found that the lactose content of camel milk remained unchanged from the first months up to the end of lactation. The concentrations in milk vary from 2.8 percent to 5.8 percent. These were approximately the same range as found between the hydrated and dehydrated animals. The changes in lactose
concentration would account for the milk being described as sometimes sweet and other times bitter Yagil and Etzion (2000).

1.10 The mineral content of camel milk:-

The total ash content of camel milk varies greatly, and the lowest percentage of ash was found in the milk produced by dehydrated camel. Camel milk is rich in chloride. Although milk from the dehydrated camel showed decrease of fat, protein and lactose content, that of sodium and chloride increased Yagil and Etzion, (2000). This would account for the salty taste.

Both concentrations of calcium phosphate and magnesium decline in the milk of dehydrated camel. However, these concentrations are still adequate for human nutrition and are similar to the values presented by Yassin (1990).

1.11 Vitamin content of camel milk:-

Camel milk is rich in vitamin C. Musa (2003). This is important from the nutritional stand point in areas where fruit and vegetables containing vitamin C are scarce. Ismael (1986) found the vitamin C content of camel milk to vary between 5.7 and 9.8 mg percent. As lactation progresses, the vitamin C content increases The vitamin C
levels are three times that of cow milk and one-and-a-half
that of human milk. Vitamin $B_{12}$ in camel milk declined
from 3.9 ug/l at 1.5 months lactation to 2.3 ug/l at the
fourth month of lactation. Vitamin $B_1$ and Vitamin $B_2$
concentrations are adequate and are higher than those of
Afar sheep. Vitamin $B_2$ content in camel milk is also
higher than in Afar goat milk, but the vitamin $B_1$ is lower in
camel milk. Carotene concentrations in the milk declined
from 0.46 mg/kg at 1.5 months lactation to 0.16 mg/kg at 4
months lactation (Ismael, 1986). The vitamin A content
has been reported as being as little as 0.037 mg percent
to 1.264 mg/l Yagil and Etzion (2000) found an average of
7.57 µg/ml of vitamin A and 9.4 ug/ml of carotene.

1.11.1 Vitamin C sources:-

Vitamin C (or ascorbic acid) is found in many fruits and
vegetables. Good sources of vitamin C include broccoli,
brussel sprouts, cauliflower, cabbage, green leafy
vegetables, red peppers, parsley, blackcurrants,
strawberries, kiwi fruit, guavas and citrus fruit. The highest
amounts of vitamin C are found in citrus fruits and green
vegetables, because vitamin C is quickly lost in cooking
and processing, fresh fruits are often a better source of
nutrients. In food, vitamin C can be partially or completely
destroyed by overcooking or long periods of storage, as it is sensitive to heat, light and oxygen. N.R.C. (1998)

1.11.2 Vitamin C and formation of the protein collagen:-

Vitamin C participates in reactions that are required for the formation of the protein collagen. When collagen is produced a series of events occur inside and outside of the cell. Vitamin C is an active inside of the cell and hydroxylates to the amino acids proline and lysine. These help form a precursor molecule called procollagen that is later modified into collagen outside of the cell. Without vitamin C the formation of collagen is disrupted, causing a wide variety of problems throughout the body. Collagen is found wherever tissues require strengthening, especially in those tissues with a protective, connective or structural function. Collagen is critical to the maintenance of bone and blood vessels and is essential for the healing of wounds. Vitamin C aids red blood cell formation helping preventing hemorrhaging and fighting bacterial infections. Mazzotta. (1994)

1.11.3 Vitamin C as an antioxidant:-
Vitamin C can act as an antioxidant by donating electrons and hydrogen ions and reacting with reactive oxygen species or free radicals. Vitamin C is important for the absorption of iron and reduces ferric iron to its ferrous form and is beneficial in the treatment of those suffering from the iron deficiency anaemia. Vitamin C is vital for the function of the immune system especially for the function of lymphocytes. Svirbelf et al (2007)

1.11.4 Synthesis of vitamin C:-

Vitamin C can be prepared by synthesis from glucose or extracted from plant sources such as rose hips, blackcurrants or citrus fruits. Humans are among the few mammals that can not synthesis their own vitamin C, and so must ingest it. Many foods are known for their ability to provide a source of vitamin C, but a number of factors - such as processing method, storage conditions, and exposure to light and heat - determine how much is actually present by the time the product is consumed. In addition to its nutritional benefits, vitamin C is used as a photographic developing agent in alkaline solutions and is used industrially as a reducing agent. Valpuesta et al (2004).
1.11.5 The history of vitamin C:-

Vitamin C was first isolated in 1928 by the Hungarian biochemist Albert Szent-Gyorgyi. In 1937, Szent-Gyorgyi received the Nobel Prize in physiology and medical science for discoveries in the area of biological combustion processes, particularly with regard to vitamin C and fumaric acid catalysis. Linus Pauling was the first to realize the value of vitamin C towards the maintenance of a healthy immune system and, in 1970, proposed that a regular intake at far higher levels than the recommended daily allowance (RDA) could help prevent and shorten the duration of the common cold. The medical community immediately voiced their strong opposition to this theory, but many ordinary people followed the advice of Pauling and noticed a great reduction in the frequency and severity of their colds. Certain recent medical advice does seemingly confirm Pauling's original idea that vitamin C can markedly reduce the severity of a cold and help prevent secondary viral or bacterial complications. Slain, et al (2003)

1.11.6 Vitamin C intake:-

The European Community RDA of vitamin C currently stands at 60 milligrams per day. All of us require vitamin C, but some people need more than others - such as
those that have low nutrient diets, who are at risk from vitamin C deficiency. Many elderly men consume low levels of fruits and vegetables and are also at risk. Studies conducted have shown that 20% of elderly men have low levels of vitamin C. In addition, smokers and those individuals exposed to cigarette smoke generally have lower levels of vitamin C, and may need to consume roughly twice as much vitamin C as non-smokers. Pauling recommended a vitamin C intake of 1000 milligrams per day or more as opposed to the current RDA. One should realize that the RDA is not based on what is required for optimum health, but is the amount required to avoid the most obvious deficiency disease. Schectman (1993).

Many eminent medical and scientific experts are of the opinion that the value is currently too low to provide optimum health and protection against disease, especially as evidence continues to emerge about the important health benefits at higher levels than those once considered adequate to prevent scurvy. Vitamin C has been used as an alternative therapy for many years and numerous doctors do not hesitate to recommend doses of 1-5 grams per day for certain individuals. A team of researchers at the National Institute of Health in the United States recently completed a study to determine the vitamin C requirement of healthy, young adults. It was
found that a minimum intake of 1000 milligrams per day was required to completely saturate the blood plasma with vitamin C. The studies recommended that the vitamin C should be taken in several doses throughout the day, as urinary excretion increased rapidly for doses above 500 milligrams. National Research Council, (1998).

1.11.7 The benefits of vitamin C:-

The Scottish physician James Lind first advocated the use of fresh vegetables and ripe fruits back in 1753 to prevent scurvy, with the British Navy adopting his advice some 40 years later. The navy men were later nicknamed Limeys as they took lime juice on long sea voyages to ward off scurvy. A severe deficiency of vitamin C can lead to the onset of scurvy, a disease that causes swollen gums, loose teeth, a tendency of wounds to not heal and excessive bleeding as well as bone malformations in infants. Fortunately, scurvy is rare nowadays in the UK due to the wide availability of foods that are rich sources of vitamin C. Research conducted by the National Institute on ageing reported that elderly people who took vitamin C and E supplements had a 50% lower risk of dying prematurely from disease than those individuals that did not use such supplements.
1.11.8 Importance of camel milk for human nutrition:-

The milk of all four quarters appears to have the same composition. Musa (2003). Camel milk is very similar to goat milk and compares very favorably with human milk I. This again stresses the importance of camel milk for human nutrition. Camel herders living only on milk in Kenya and in the Ahaggar region of the Sahara. Majid, (2000) are healthy and vigorous. Camel milk is renowned for its health-giving qualities, which includes good bone growth. Some camel herders living an camel milk only show a change in the colour of their hair to red but this returns to normal when a more balance diet is resumed. Majid (2000),

From all the data presented it is clear that the camel produces nutritious milk for human consumption. It is also evident that the taste and quality of milk is directly affected by the amount of water drunk, and the amount and quality of feed eaten. The fluctuations in fat, protein, fat and salt are determined by the amount of water drunk Yagil and Etzion, (2000) and by changes in pasture. Grazing on Atriplex halimus gives a salty taste to the milk, and grazing on Schouwia purpurea gives a cabbage smell to the milk. Majid (2000).
1.12 Vitamin C and cancer:

Numerous studies have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, lung, mouth, prostate, and stomach. Pauling and his colleagues at the Vale of Leven Hospital in Scotland carried out clinical studies on cancer patients subjecting them to large daily doses of vitamin C in addition to their regular treatment. Rees, et al (1993).

They concluded that cancer patients who received doses of greater than 10 grams per day lived longer, suffered less pain and had in general, a better quality of life than similar patients who did not receive the vitamin C. They believed that vitamin C combats cancer by promoting collagen synthesis and thus preventing growing tumors from invading adjacent tissue. Many researchers now believe that vitamin C prevents cancer by deactivating free radicals before they can damage DNA and initiate tumors growth, while others think that vitamin C acts as a pre-oxidant helping the bodies own free radical defense mechanism destroy tumors in their early stages. Whatever the mechanism, it seems apparent that vitamin C may be effective in preventing cancer, alleviating its symptoms
and in certain cases halting its progress for some individuals. The work of Pauling and his peers concerning the benefits of vitamin C has not been without controversy, and for many years has divided medical opinion and it is not unreasonable to suggest that the debate will continue for many more years to come.

1.13 Vitamin C and blood pressure:

Research published in the medical journal Lancet found that a 500 milligram daily supplement of vitamin C could significantly reduce high blood pressure in hypertensive patients. High blood pressure is a serious health problem in much of the world and a key risk factor in heart disease and strokes. The studies, performed by Boston University School of Medicine and Linus Pauling Institute at Oregon University (2005), found that vitamin C supplements reduced blood pressure without side effects, were inexpensive, and could yield reductions comparable to certain prescription drugs used to treat hypertension. Patients were given 500 milligrams of vitamin C daily and after a month the systolic, diastolic and mean blood pressures had decreased by about 9%, enough to reduce a moderately high blood pressure back to a normal level.
Dehydro ascorbic acid (oxidized form)

Ascorbic acid (reduced form)

FIG (1) Reduced and oxidized forms of L- ascorbic acid
CHAPTER TWO
Materials and Methods

This work has been conducted in camel unit of Khartoum University Farm and central laboratory unit in Shambat.

2.1 Materials

2.1.1 Animals

Nine clinically healthy she camels (*Camelus dromedaries*), weighting 350 – 450 Kg and six to nine years of age were used in this experiment. The animals were brought from Alrashida area. (Kasala province) to Khartoum University Farm in (August 2008). Their yield of milk from 1 – 3 Kg/day to one animal.

The animals were naturally grazed. Blood and milk samples were taken on the same day of collection, blood and milk samples were placed immediately in an ice thermos jug before taken to the laboratory for the estimation of the Ascorbic acid content.

2.1.2 Blood sampling

Blood samples (10 ml) were withdrawn from the jugular vein into test tube free from anti coagulant after using all
the precautions of handling the animal (shaving, sterilization and avoiding samples from an exciting animal. After 45 minutes the blood was clotted and the serum was centrifuged at 3000 r.p.m for 15 minutes. Then the test was done for estimation of Ascorbic acid in the sample.

2.1.3 Milk sampling
10ml of milk were taken from clean healthy udder. The samples kept into thermo jug, the sampling was collected after sun set to avoid the sun heat. The samples were taken immediately to the laboratory for estimation of Ascorbic acid in the samples. 15ml let out over night at 37°C to be fermented and then the next day the Ascorbic acid was measured in it. After 7 days other estimation was done for the two samples (fermented and fresh) milk after storing in refrigerator AT 4°C for 7 days to measure the changes.

2.2 Reagents:
Determination of vitamin C:
Phosphotungstate reagent prepared periodically as it is used up (suspension of 150g sodium tungstate molybdenum free and 60g sodium hydrogen phosphate anhydrorous in 240ml deionizer water mix.
With heating to dissolve and add slowly 145ml 3.7M sulphuric acid heat the solution for 2 hours with reflex condenser not allowing it to boiling; after cooling the solution down adjust pH to 1.0 adding drop wise concentrated sulphuric acid the reagents should be light greenish yellow, a darker one is useless. 56.8µM vitamin C (1- ascorbic acid) stander solution made with use 50m solution of oxalic acid as a solvent.

2.3 Methods

Determination of Ascorbic Acid

Rutkowski et al (1999) measure 1ml of analyzed liquid into the centrifugal test tube add 1.0ml of the phosphotungstate reagent mix thoroughly and leave it at room temperature for 30 minutes centrifuge the tube (7000 r/10 minutes) and collect the whole of the separated supernatant with a pipette the supernatant is a test sample for spectrophotometer measurement.

Standard sample Preparation

As above (using 1.0 ml of the standard solution in stead of the analyzed liquid without centrifugation measure the absorbance of the test. Sample Ax and of the standard sample As at 700 nm against the mixture PR 50 m M Solution of oxalic acid =1:1[v/v] as a reference sample
Calculate concentration $C_x$ of vitamin C [mg/dl] in the analyzed liquid, using the formula;

$$C_x = \frac{A_x}{A_s \cdot C_s}$$

2.4 Chemicals

All chemicals used were annular grade unless otherwise stated.


2.5 Apparatus

. Reflex Apparatus, Heating mantel, Quartz cuvette, Plain container. Analytical Spectrophotometer UV and VIS range (180 – 1000nm) U.K SN =001385, Auto pipette 1000ml, measuring. Cylinder 50\100ml ,Centrifuge Bench 1000 rpm Germany. HPLC (Shimadzu -Japan).

2.6 Statistical analysis

The result were analyzed by T-Test and Pearson Correlation by software SPSS (Statistic package social for
science). Each test was conducted at 0.05 level of significant. Means were separated by multiple comparisons range test

Method of ascorbic acid analysis by HPLC (figure3):
HPLC consisting of an auto sampler binary pump system solvent delivery module, column oven for analysis, 10µl of sample were injected onto a reversed-phase column equipped with a guard column of the same material. Ascorbic acid was carried out with a critic mobile phase consist of 0.1M potassium dihydrogenphosphate at 35 ºC and a flow rate of 0.8ml/min. The retention time of the ascorbic acid was monitored using the photo diode array detector at 254nm, the retention time obtained was compared to that determined using ascorbic acid standard. The data was analyzed using class vp6.14.

Determination of ascorbic acid concentration

Preparation of ascorbic acid standards:
The stock solution (1mg/ml-1000ppm) was prepared by dissolving 100mg of reference standard with water in 100ml volumetric flask standard working solutions was prepared by appropriately diluting 0.2/0.4/1/1.6/2ml from
stock solution to 100ml Volumetric flasks with water to obtain solutions in 2/4/10/20ppm. A calibration curve was generated (Figure 2) by injecting 10µl from each standard working solution and plotting the recorded peak area versus the corresponding mass of the analyte injected. The slope, intercept and least squared fit of the standard curve was computed. Data for the slope and intercept of the calibration curve were used to compute the mass of analyte in unknown sample.
Calibration Info for Ascorbic acid

<table>
<thead>
<tr>
<th>Level No</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>2ppm</td>
<td>4ppm</td>
<td>10ppm</td>
<td>16ppm</td>
<td>20ppm</td>
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<td></td>
<td>45359</td>
<td>80194</td>
<td>222793</td>
<td>383704</td>
<td>482168</td>
</tr>
</tbody>
</table>

FIG (2) HPLC standard curve
FIG (3) Sample detection in HPLC
CHAPTER THREE

Results

In Table (1) The mean concentration of ascorbic acid in serum of camel was 17.07±3.02mg/dl. The concentration of ascorbic acid in camel fresh milk was equal three times that of serum and as the concentration increase in milk it also increase in serum. It is mean was 65.57±9.26mg/dl showed in Figure (4).

In Table (2) The Pearson correlation was found 0.792 that means correlation was significant at the 0.05 level,

In Table (3) The ascorbic acid level in fermented milk over night in room temperature is lower one and half time than that of fresh milk. The range of ascorbic acid concentration was 41.12±11.16mg/dl in fermented milk that was significant. Showed in Figure (5).

The ascorbic acid concentration in camel milk after 7 days in refrigerator at 4ºC is lower than that of fresh milk with 1.1 fold. The range of ascorbic acid was 57.53±7.65mg/dl. Showed in Figure (5)

In Table (3) mean of fermented milk after 7 days at +4ºC was 25.86±6.65mg/dl. The decreasing of concentration from that fresh milk at 4ºC was 1.9 fold which was highly significant.
In Table (3) the decreasing of ascorbic acid concentration between fermented milk over night at room temperature 25°C and that of fermented milk in 4°C after 7 days is 1.3 fold which was significant. Showed in Figure (5).

In Table (4) samples were analyzed by HPLC

The concentration of ascorbic acid in fresh camel milk was 52.70 mg/dl.
The same sample after 7 days at 4°C in refrigerator was found 31.01 mg/dl the decreasing was 1.7 fold which was significant at 0.05 levels.
The fermented camel milk over night in room temperture 25°C concentration was 36.62 mg/dl and same sample after 7 days in refrigerator at 4°C was 20.60 mg/dl the decrease was 1.8 fold which was significant at 0.05 levels.
The decrease in concentration between fresh milk and fermented milk in ascorbic acid was 1.4 fold which was significant at 0.05level.

The concentration of fermented milk after 7 days in refrigerator at 4°C was 20.60 mg/dl and decrease 1.5 fold from milk in refrigerator at 4°C for 7 days was significant at 0.05 level. Showed in Figure (6).
**Table (1) Relation of Ascorbic acid concentration Of serum and fresh camel milk**

<table>
<thead>
<tr>
<th></th>
<th>Fresh mg/dl</th>
<th>Serum mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>65.57*+3.09</td>
<td>17.08*+1.01</td>
</tr>
<tr>
<td>SD</td>
<td>9.26</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Significant *(P<0.05)*
### Table (2) Correlations of ascorbic acid concentration of serum and fresh milk.

<table>
<thead>
<tr>
<th>Ascorbic acid concentration in milk fresh</th>
<th>Person Correlation Sig(2tailed)</th>
<th>N</th>
<th>Ascorbic acid concentration in serum</th>
<th>Person Correlation Sig(2tailed)</th>
<th>N</th>
<th>Ascorbic acid concentration in milk fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>0.792*</td>
<td>0.011</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

- Correlation is significant at the 0.05 level (2-tailed).
Table (3) The relation of ascorbic acid concentration of fresh milk, fresh milk after 7 days, fermented milk and fermented milk after 7 days.

<table>
<thead>
<tr>
<th></th>
<th>Fresh mg/dl</th>
<th>Fresh after 7 days mg/dl</th>
<th>Fermented mg/dl</th>
<th>Fermented after 7 days mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean + SE</td>
<td>65.9*±2.97</td>
<td>57.53*±2.54</td>
<td>41.01*±3.72</td>
<td>25.86**±2.22</td>
</tr>
<tr>
<td>SD</td>
<td>8.93</td>
<td>7.65</td>
<td>11.16</td>
<td>6.65</td>
</tr>
<tr>
<td>Significant</td>
<td>*(P≤0.05)</td>
<td>*(P≤0.05)</td>
<td>*(P≤0.05)</td>
<td>*(P≤0.05)</td>
</tr>
</tbody>
</table>
Table (4) The relation of ascorbic acid concentration of fresh milk, fresh milk after 7 days, fermented milk and fermented milk after 7 days. (By HPLC)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fresh milk mg/dl</th>
<th>Fermented mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (zero time)</td>
<td>52.70*</td>
<td>36.62*</td>
</tr>
<tr>
<td>2 (after 7 days)</td>
<td>31.01*</td>
<td>20.60*</td>
</tr>
</tbody>
</table>

Significant *(P<0.05)*
Figure (4) Relation of Ascorbic acid concentration of serum and fresh camel milk
Figure (5) Relation of ascorbic acid concentration of fresh milk, fresh milk after 7 days, fermented milk and Fermented milk after 7 days.
Figure (6) Relation of ascorbic acid concentration of fresh milk, fresh milk after 7 days, fermented milk and Fermented milk after 7 days. (by HPLC).
--- Shimadzu CLASS-VP Custom Report ---

Sample ID : Ascorbic acid
User Name  : System
Filename  : C:\CLASS-VP\Ascorbic sam1
Method Name : C:\CLASS-VP\Methods\ascorbic acid.met
Sequence Name : C:\CLASS-VP\Sequence\ascorbic.seq
Run Time    : 10/26/2009 5:57:47 PM
Description       : sample 1 (zero time)

**Data Graph**

![Data Graph]

<table>
<thead>
<tr>
<th>Pk #</th>
<th>Name</th>
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<th>Area</th>
<th>ESTD concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>2.624</td>
<td>159684</td>
<td>5.270</td>
<td>Ppm</td>
</tr>
</tbody>
</table>

**FIG (7) HPLC Result Paper**

<table>
<thead>
<tr>
<th></th>
<th>Area</th>
<th>ESTD concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Totals</strong></td>
<td>159684</td>
<td>5.270</td>
</tr>
</tbody>
</table>
Sample ID : Ascorbic acid
User Name  : System
Filename   : C:\CLASS-VP\Ascorbic sam3
Method Name : C:\CLASS-VP\Methods\ascorbic acid.met
Sequence Name : C:\CLASS-VP\Sequence\ascorbic.seq
Run Time    : 10/26/2009 6:14:29 PM
Description       : sample 2(zero time)

**Data Graph**

![Data Graph Image]

1: 254 nm, 8
nm

<table>
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<tr>
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<th>Area</th>
<th>ESTD concentration</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
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<td>2.592</td>
<td>112033</td>
<td>3.662</td>
<td>ppm</td>
</tr>
</tbody>
</table>

Totals

|       |               | 112033         | 3.662  |

FIG (8) HPLC Result Paper
--- Shimadzu CLASS-VP Custom Report ---

Sample ID : Ascorbic
User Name  : System
Filename  : C:\CLASS-VP\ascorb sam1
Method Name : C:\CLASS-VP\Methods\ascorbic acid. met
Sequence Name : C:\CLASS-VP\Sequence\ascorbic2.seq
Run Time    : 11/2/2009 1:28:00 PM
Description       : sample 1(after one week)

Data Graph

<table>
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<th>Area</th>
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<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ascorbic acid</td>
<td>2.677</td>
<td>62732</td>
<td>3.101</td>
<td>ppm</td>
</tr>
</tbody>
</table>

Totals  |       |         | 62732 | 3.101

FIG (9) HPLC Result Paper
Sample ID : Ascorbic
User Name : System
Filename : C:\CLASS-VP\ascorb sam2
Method Name : C:\CLASS-VP\Methods\ascorbic acid.met
Sequence Name : C:\CLASS-VP\Sequence\ascorbic2.seq
Run Time : 11/2/2009 1:34:48 PM
Description : sample 2(after one week)

Data Graph

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>37061</td>
<td>2.060</td>
<td>ppm</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Totals</td>
<td></td>
<td></td>
<td>37061</td>
<td>2.060</td>
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</tbody>
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FIG (10) HPLC Result Paper
CHAPTER FOUR
Discussion

The result obtained in the current study, when the concentration of ascorbic acid of camel serum showed increases; also it is increases in fresh camel milk. The content of ascorbic acid in fresh camel is three times of that in blood serum of camel.

The mean of ascorbic acid in serum was 17.07±1.01 mg/dl. This result agree with record 5.7 to 9.8 mg percent as lactation progresses reported by Zafar (2004).

The mean of ascorbic acid in fresh milk of camel was 65.57±3.09 mg/dl that agree with the normal range. Shahan (2004).

The decrease of ascorbic acid in milk after 7 days in 4°C was 57.53±2.55 mg/dl which shows significant change.

The mean of ascorbic acid in fermented camel milk in room temperature at 25°C over night was 41.02±3.72 mg/dl.

The mean of ascorbic acid concentration in fermented milk after 7 days in 4°C was 25.87±2.2 mg/dl which shows highly significant decreasing.
Camel milk is rich in vitamin C. This is important from the nutritional standpoint in areas where fruit and vegetables containing vitamin C are scarce. The vitamin C content of camel milk varies between 5.7 and 9.8 mg percent. As lactation progresses, the vitamin C content increases. Vitamin C levels are three times that of cow milk and one-and-a-half that of human milk Farah (2004).

Thiagarajan (2006) reported that camel milk has nutritive value with high quantity of vitamin C. According to Noran-org. (2006) vitamin C content of camel milk are three times of cow milk.

The vitamin C content of camel milk 37.4mg/dl and the range is 26.2-61.1mg/dl while the mean in cow is 11.0mg/dl with range 3-23mg/dl Farah (2004).

Comprehensive information on vitamin C content in of the dromedary type camel is not available the only published work comparable with our finding is the report of Sawaya et al (1984) who studied the vitamin content of Saudi dromedaries and found mean values of 24mg/l for vitamin C. The level of vitamin C is in average three times higher than that of cow milk. The availability of a relatively fair amount of vitamin C (average 37.4mg/l) in camel milk is of significant relevance from nutritional standpoint in the arid areas where fruits and vegetables containing vitamin C are scarce Farah (1993).
Ismael (1986) found in comparison of vitamin C content that camel milk is rich in vitamin C content while cow milk is poor in content of vitamin C.
Conclusions
Consisted with the assumption that storage temperature may promote alterations in the concentration of ascorbic acid in camel milk the following is found.
1- The fermented camel milk was decreased from camel fresh milk 1.5 fold which is significant at .0.05 level.
2- Concentration after 7 days in refrigerator at 4ºC was lower 1.4 fold from fresh camel milk which is significant at 0.05 levels.
3- The fermented milk after 7 days in refrigerator at 4ºC is lower 1.9 fold from fresh milk which is highly significant at 0.05 levels.
4- When the concentration of ascorbic acid in fresh milk increases it is also increases in camel serum.

Recommendations
1- Camel milk should be preserved at temperature that does not affect the concentration of vitamin C in the milk.
2- Further studies should be done on vitamins, especially vitamin C and the factors influencing the concentrations.
3- A new means of camel milk preserving must be found.
4- More studies should be carried out on composition and properties of camel milk.
5- The changes in chemical structure of fermented camel milk must be studied.
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Like most vitamins, vitamin C may be obtained in the recommended amount with a well-balanced diet, including some enriched or fortified foods.

Update Date: 3/7/2009
Goat milk (rich source of vitamin C)
Camel and her Calf
Arabian Camel
Rosa canina hips
Rich Source of Vitamin C

Redoxon Vitamin C
HPLC