Vitamin C Levels in Lemons (*Citrus Limon*) Grown in Zambia; an Opportunity for Utilisation in Fish Feeds

Alexander Shula Kefi
Department of Fisheries
P. O. Box 350100, Chilanga, Zambia

Chrisanty Chama
Zambia Agriculture Research Institute
P. O. Box Mufulira, Zambia

Lumbwe Kalumba

Ian Bbole

Patience Chungu

Majorie Mbambara

National Aquaculture Research and Development Centre
P. O. Box 22797, Kitwe, Zambia

Abstract

A study was conducted to determine the amount of ascorbic acid in two Citrus limon varieties (Eureka and Lisbon) collected from three agro – ecological regions of Zambia. The ascorbic acid was determined by back titration method. Location and variety of lemon affected the moisture, crude fibre, crude protein, ash, carbohydrates and energy independently while crude protein level in the lemon fruit were influenced by both. Location did affect ($P < 0.05$) the amount of ascorbic acid in the lemon. However, the amount of vitamin C was not significantly different ($P > 0.05$) between the lemon varieties. Polynomial regression suggested a linear relationship ($Y = 0.086X + 7.617$, $r = 0.5$) between the weight of the Eureka lemon and vitamin C with a lot of variations. It is recommended that the possibilities of using lemons especially Lisbon variety as a source of vitamin C in fish feeds are explored.

Keywords: Lemon, Eureka, Lisbon, Vitamin C, Fish, Feeds

1. Introduction

Lemons have been described to grow well in both tropical and semi tropical climate conditions with the major production restricted to sub-tropical regions although their origins are uncertain (Mansfield, 2003). This probably is the main reason why the lemons are grown in Zambia which has cool winters and warm to hot summers the conditions associated with good production of lemons. Furthermore, lemons require both dry and humid conditions and are known to tolerate very infertile or poor soil (Morton, 1987). Of the lemon varieties grown, Eureka and Lisbon are common and cultured in all the three agro – ecological regions of Zambia. Eureka lemon is elliptic to oblong. The peel is yellow when ripe, longitudinally ridged, slightly rough because of sunken oil glands and medium-thick. The fruit has a greenish-yellow pulp, usually with about 10 segments, juicy fruit and is very acidic. The Lisbon fruit is almost identical to Eureka in all other aspects except that it is barely rough, faintly pitted and has few or no seeds (Morton, 1987). The literature on lemon production in Zambia is scanty probably due to little research that has been directed to the citrus fruit.

Njoku, Ayuk, & Okoye (2011) found lemons to contain vitamin C, also known as ascorbic acid, only behind oranges and grapes but more than in lime fruits at varying storage temperatures. Vitamin C has antioxidant and therapeutic properties in many organisms.

It helps the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defence system against reactive oxygen species and free radicals, thereby preventing tissue damage (Okieiet al., 2009). Amongst the vitamins essential for fish growth, vitamin C has been found to be an important element for the growth, reproduction and physiology of fish. This is because; unlike other animals fish lack an enzyme gulonolactone oxidase necessary in the last step in the biosynthesis of ascorbic acid (ChatterJee, 1973; Dabrowaski, 1990; Merchie, Lavens, & Sorgeloos, (1997). Deficiency of Vitamin C in fish causes broken back described by Meyer (1975) as an anomaly characterized by damaged spinal column, depigmented skin areas, reduced growth rate and increased mortality. Other deficiency syndromes include distorted gill filaments, mottled hyperplasia of sclerotic cartilages, hemorrhages, impaired collagen biosynthesis and delayed wound healing (Agrawal and Mahajan, 1980).

Based to the authors' knowledge, there has been no study that has been conducted to determine the amount of ascorbic acid in the fruit grown in Zambia. Though many of these lemons have some commercial value in their unprocessed forms and usually find their way into urban markets, their potential as industrial raw products is largely unexploited (Mingochi, 1998). Attempts to utilise the fruit in the production of livestock and fish feeds has not been explored too. The experiment attempts to determine the amount of vitamin C and other nutritional composition in relation to the tree and fruit size in the two varieties (Eureka and Lisbon) of lemons grown in Zambia in all the three agro – ecological regions. The aim is to determine the amount of vitamin C in the Eureka and Lisbon varieties of the lemon and their suitability as feed ingredient in the fish feeds.

2. Materials and method

The lemons of each variety were collected from the three sites that represented the agro ecological regions of Zambia. From region I, lemons were collected from three (3) farmers in Mambwe District of Eastern Province. From region II the lemon fruits were specifically collected from the Zambia Agriculture Research Institute (ZARI) situated in Nanga, Southern Province. From region III, collection was done from five (5) farmers in Mufulira and two (2) from Kitwe Districts of the Copperbelt Province.

Before the fruits could be plucked from the trees, the physical appearance of the fruit and of the tree were observed, age of the plant and diameter of the plant were taken. Thereafter, the tree heights were measured using a clinometer (Sunto). Furthermore, Global Positioning Satellite (GPS) coordinates were taken for the sample areas too. Soil samples were obtained near the lemon tree at the depth of 30cm using a soil auger and were packed in plastics for fertility analysis in the laboratory. The management practices and utilization of the lemons were collected too using a structured questionnaire.

Forty lemon fruits were plucked and classified by colour for ripeness from each tree variety with both unripe (green) and ripe (yellow) collected in equal numbers. Each fruit was then weighed and diameter measured using a scale and vernier calliper respectively. The lemons were packed in plastics and preserved in cooler boxes before they were transported to the laboratory at National Aquaculture Research and Development Centre (NARDC).

2.1 Laboratory analysis

In the laboratory, the lemons were weighed and their lengths measured before and after peeling in both axes (longer and shorter) since the lemons are ellipsoid. The peeled lemon was then inserted in the juice maker (Philips) for juice extraction. The extracted juice volume was determined using the measuring cylinder before its pH was determined using the portable pH meter (Checker®).

Vitamin C was determined following the back titration method as described by Terpstra (2005). Starch indicator solution was made by mixing one tablespoon of starch and few drops of water to make a paste and a further 250 ml of water was added before the mixture was boiled. Iodine solution (0.005 mol/L) was prepared by adding 2ml of iodine solution into a beaker followed by 98ml of distilled water and stirring to mix evenly. Ten (10) drops of the made solution was added to 75 ml of hot water while stirring. Twelve (12) drops of 2% iodine solution was added to the solution. Ten (10) ml of the made solution was poured into a 50ml volumetric flask. The extracted juice was titrated with the sample until the indicator solution changed from blue to colourless. The amount of vitamin C in the samples was calculated.

Furthermore, forty eight (48) lemons for each variety and region in equal numbers were taken for proximate analysis according to the methods described by AOAC (2002). Dry matter of lemons was determined by drying samples in an Advantech electric furnace maintained at $105^{o}C$ for 5 hours. Crude protein levels were determined indirectly from the analysis of total nitrogen by the Micro-Kjeldahl method after acid digestion.

The amount of protein in the lemon sample was calculated by multiplying the amount of nitrogen by 6.25. The ash was determined as total inorganic matter by incineration of the sample in an advantec electric furnace at 550^{o}C for 5 hours. The crude fat was determined by extraction with petroleum ether for 16 hours in a Soxhlet apparatus. After drying the ether, the flasks containing the fat were dried in an oven for 8 hours at 85^{o}C. The ether was evaporated and the crude fat weighed. Crude fibre was determined by subjecting the residue from ether extraction to boiling in dilute sulfuric acid (1.25%) for 30 minutes, followed by boiling in dilute sodium hydroxide (1.25%) for another 30 minutes. The nitrogen-free extract (carbohydrates) was calculated by subtracting the percentages calculated for each nutrient from 100.The amount of vitamin C contained in the lemon was calculated as described by Smith (http://chemistry.about.com/od/demonstrationsexperiments/ss/vitctitration_3.htm#step-heading).

2.2 Statistical analysis

General Linear Model (GLM), multivariate analysis and independent T - test were used to test the interaction and differences in the amount of vitamin C, juice pH, soil parameters and proximate nutrients of lemon among the sites, between the varieties and ripeness of the lemons which were deemed significant at $P < 0.05$. If interactions did not exist the independent variables (site that represented the agro ecological regions, lemon varieties and ripeness of the lemons) were treated independently. Post hoc analysis was performed by Duncan Multiple Range Test (Duncan, 1955). Correlation analysis was also performed between vitamin C and weight of lemon, and pH, and regression analysis was conducted if the correlation was strong (r > 0.5) and significant ($P < 0.05$) after polynomial regression was applied to determine the best regression model between the vitamin C and lemon size, and soil mineralogy. Before analysis, normality was tested using Shapiro–Wilk test and the homogeneity of variance by Levene's test for equality of variances for GLM. For T – Test, significance level for variances assumed was only used if it was not significant ($P > 0.05$).

3. Results

There were no significant differences ($P > 0.05$) observed in the selected soil nutrients determined among the agro ecological regions except that of calcium among the regions. Region III had a significantly ($P < 0.05$) lower calcium level than the other two regions. A two way analysis of Variance (ANOVA) revealed that location and variety of lemon affected the moisture (%), crude fibre (%), crude protein (%), ash (%), carbohydrates (%) and energy (kcal/100g) independently while crude protein (%) level in the lemon fruit seemed to be influenced by both location and variety of the lemon. Eureka had a significantly ($P < 0.05$) higher moisture content than Lisbon. The rest of the parameters were not significant ($P > 0.05$) between the two varieties.

Fruit moisture, crude fat, crude protein, crude fibre and energy were not significantly ($P > 0.05$) different in all the three locations sampled from. However, the lemon fruit found in region I had a significantly ($P < 0.05$) lower levels of carbohydrates than the lemons collected from regions II and III. However, lemons collected from regions I and II had similar ($P > 0.05$) ash levels but significantly ($P < 0.05$) higher ash levels than in region III (Table 3).

Variety, location and ripeness of the lemon fruit acted independently in the amount of vitamin C contained in the lemon since interactions were insignificant ($P > 0.05$). Therefore, the independent variables (variety, ripeness of the fruit and location) were treated independently. Significant ($P < 0.05$) differences were observed in vitamin C amount among location and ripeness of the lemons with the ripe lemons containing significantly more vitamin C than the smaller and unripe lemons. The lemons had similar amounts of vitamin C (Table 5).

A significant interaction ($P < 0.05$) among the variety, location and ripeness of the lemon was observed in the pH of the juice extracted. Splitting data according to location and the means separated by ripeness and variety of the lemon, significant differences were observed in region I where the ripe lemon was found to have lower pH than the unripe lemons. No significant differences were observed in the variety of the lemons.

Combining both varieties, a weak relationship was found to exist (r = 0.17, n = 141, $P < 0.05$), therefore, regression analysis was not performed. Similarly, a weak positive relationship was observed in the Lisbon variety (r = 0.2, n = 92, $P < 0.05$). Therefore, the regression analysis was not conducted for the variety. There was a positive significant correlation between the amount of vitamin C and the weight of the Eureka variety (r = 0.5, n = 141, $P < 0.05$) indicating that the bigger the Eureka lemon the more vitamin C it is contained. Polynomial regression suggested a linear relationship (Y = 0.086X + 7.617, r = 0.5) between the weight of the lemon and vitamin C with a lot of variations (Figure 1).

4. Discussion

To the best of our knowledge, there has been no study that has been conducted in profiling the amount of the vitamin C and the nutritional value of the lemon varieties grown in Zambia. Comparisons can then only be made to studies conducted outside the country. Therefore, this study provides a novel literature in Zambia.

The study reveals that soil nutrients were similar in all the sites where the lemons were collected except that for the calcium which was significantly lower in the region III than in other regions sampled. Lemons collected from region III had significantly ($P < 0.05$) higher vitamin C than those in other regions which coincided with the lowest soil calcium. Similar results were found by Ochmian (2012) on Higbush Blueberry fruits where application of calcium fertilizers had an adverse effect on the content of vitamin C. This is because cultural practices influence the amount of vitamin C (Lee &Kader, 2000). In this experiment, the study did not reveal any differences in the amount of vitamin C in lemon varieties. However, the amount of vitamin C in lemons collected from region III had higher amounts of vitamin C compared to other regions. The amount of the vitamin C found of 21.06, 22.89 and 38.66 mg/100g for region I, II, III respectively is comparable to the findings of other authors. Esch, Friend & Kariuki (2010) found a concentration of 26.2 mg/100g in conventionally grown lemons. Babashahi – Kouhanestani *et al*. (2014) determined 42mg/100g ascorbic acid in the fresh lemon.

The study reveals that the unripe lemons have significantly lower vitamin C than the ripe ones. This contradicts the findings of Nagy (1980) who found that immature citrus fruits contained the highest concentration of vitamin C, whereas ripe fruits contained the least. The study reveals a linear positive relationship between the vitamin C and weight of the Eureka lemon indicating that the bigger and heavier the lemon the more vitamin C is contained. Therefore, it is possible to estimate the amount of vitamin C given the weight of eureka lemon variety.

Proximate analysis showed that lemons have very low crude protein and fat but a higher crude fibre compared to both soya bean and maize meal the most widely used plant sources in fish feeds (Harmon et al., 1969). Therefore, the use of lemons as the source of protein and lipid is limited. The utilisation of lemon fruit as the source of vitamin C in fish feeds should be explored. This is because lemon plants are grown across the country with less utilisation of the fruit. It may provide the cheaper source of vitamin C which can be fortified into the fish feed. In the studies, salient issues for storage and handling should be considered as vitamin C is very easy to be lost if poorly handled.

5. Acknowledgement

We are indebted to the National Science and Technology Council of Zambia for funding the study under the Strategic Research Fund (SRF).Gratitude is also directed to the Ministry of Fisheries and Livestock (MFL), through the National Aquaculture Research and Development Centre, which falls under the Department of Fisheries, for providing the research facilities. Many thanks go to farmers and Zambia Agriculture Research Institute (ZARI) stations for accepting to collect the lemons from their farms.

6. References

Agrawal, N. K., &Mahajan, C. L. (1980). Nutritional deficiency disease in an India Mojor Carp. Cirrlu'rmIririgala Hamilton due to avitaminosis C during early growth. Journal of Fish Diseases, 3, 231 – 248.

AOAC (Association of Official Analytical Chemists). (2002). Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Inc., Arlington.

Babashahi-Kouhanestani, M., Salehi, M., Mazloomi, S. M., &Almasi-Hashyani, A. (2014). Quantitative evaluation of vitamin C in industrial lemon juice by titration method. Journal of Biology and today's World, 6, 139 – 141.

ChatterJee, I. B. (1973). Evolution and biosynthesis of ascorbic acid. Science, 182, 1271 – 1272.

Dabrowski, K. (1990). Ascorbic acid status in the early life of whitefish (*Coregonuslavaretus* L.). Aquaculture, 84, 61 – 70.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11, 1 – 42.

Esch, J. R., Friend, J. R., &Kariuki, J. K. (2010). Determination of the Vitamin C Content of Conventionally and Organically Grown Fruits by Cyclic Voltammetry. International Journal of Electrochemical Science, 5, 1464 – 1474.

Harmon, B. G., Becker, D. E., Jensen, A. H., & Baker, D. H. (1969) Nutrient Composition of Corn and Soybean Meal. Journal of Animal Science, 28, 459-464.

http://chemistry.about.com/od/demonstrationsexperiments/ss/vitctitration_3.htm#step-heading. Retrieved on 28th February 2015.

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.

Merchie, G., Lavens, P., & Sorgeloos, P. (1997). Optimization of dietary Vitamin C in fish and crustacean larvae: a review. Aquaculture, 155, 165 – 181.

Meyer, F. P. (1975). The pathology of major diseases of catfish. In: The pathology of fishes (Ribelin, W. E and Migaki, G., ed.) p. 275 – 286, The University of Wisconsin Press, Madison, W. S.

Mingochi, D. S. (1998). Review of the status of fruit research in Zambia. World Conference on Horticultural Research 17 – 20 June 1998.

Morton, J. (1987). Fruits of warm climates, Pg 160–168 Miami, FL.

Nagy, S. (1980). Vitamin C contents of citrus fruit and their products: a review. Journal of Agriculture and Food Chemistry, 28, 8–18.

Njoku, P. C.,Ayuk, A. A., &Okoye, C. V. (2011). Temperature Effects on Vitamin C Content in Citrus Fruits. Pakistan Journal of Nutrition, 10, 1168-1169.

Ochmian, I. (2012). The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the 'Duke' Cultivar. Not Bot Horti Agrobo, 40, 163 – 169.

Okiei, W., Ogunlesi, M., Azeez, L.,Obakachi, V., Osunsanmi, M., & Nkenchor, G. (2009). The Voltammetric and Titrimetric Determination of Ascorbic Acid Levels in Tropical Fruit Samples. International Electrochemcal Science, 4, 276 – 287.

Tables

Table 1: Selected soil mineral nutrients determined in three agro ecological regions (mean±SE)

	Agro Ecological Region		
Soil parameter	**I**	**II**	**III**
pH (1:5H_2O)	6.3±0.157	6.6±0.033	6.5±0.052
Nitrogen (%)	0.019±0.001	0.017±0.001	0.019±0.001
Potassium (ppm)	21.208±5.738	18.833±1.965	15.861±2.984
Calcium (me/100g)	1.383±0.145^{b}	1.333±0.524^{b}	0.678±0.074^{a}
Magnesium (me/100g)	7.508±0.566	6.200±1.150	6.994±0.632
Organic Carbon (%)	1.721±0.186	1.006±0.117	1.427±0258

Values with different superscript in a row are significant different ($P < 0.05$)

Table 2: Selected proximate analysis parameters according to lemon variety

	Variety	
Proximate analysis parameter	Eureka	Lisbon
Moisture (%)	8.688±0.336^{b}	7.234±0.283^{a}
Crude Fat (%)	3.484±0.808	2.460±0.878
Crude Fibre (%)	12.403±0.766	14.206±0.860
Crude Protein (%)	5.471±0.420	4.096±0.752
Ash (%)	4.533±0.277	5.080±0.202
Carbohydrates (%)	65.417±0.656	66.811±1.364
Energy (Kcal/100g)	314.886±6.708	305.136±6.875

Values with different superscript in a row are significant different ($P < 0.05$)

Table 3: Selected proximate analysis parameters according to location

	Region		
Proximate analysis parameter	**I**	**II**	**III**
Moisture (%)	8.383±0.437	7.948±0.674	7.9350±0.334
Crude Fat (%)	2.445±1.110	2.672±0.897	3.795±1.139
Crude Fibre (%)	14.470±1.160	12.553±1.332	12.978±0.500
Crude protein (%)	6.543±0.713	4.575±0.791	4.050±0.330
Ash (%)	5.068±0. 073[b]	5.213±0.233[b]	4.135±0.305[a]
Carbohydrates (%)	62.983±0.834[a]	66.895±0.701[b]	67.188±1.212[b]
Energy (Kcal/100g)	300.036±10.667	309.925±7.880	318.370±7.211

Values with different superscript in a row are significant different ($P < 0.05$)

Table 4: The amount of vitamin C according to location, variety and ripeness of the lemon fruit

Variable		**Vitamin C (mg/100g)**
	I	21.056±1.029[a]
Location	II	22.885±1.716[a]
	III	38.659±1.331[b]
Variety	Eureka	29.892±1.609
	Lisbon	23.093±1.135
Ripeness	Green	26.244±1.236[a]
	Yellow	33.280±2.437[b]

Values with different superscript in a column within a variable are significant different ($P < 0.05$)

Table 5: The pH according to location, variety and ripeness of the lemon fruit

	Ripeness of the lemon		Variety of the lemon	
Region	Green	Yellow	Eureka	Lisbon
I	2.968±0.031[b]	2.778±0.058[a]	2.928±0.039	2.863±0.049
II	2.783±0.015	2.733±0.042	2.778+0.014	2.743±0.048
III	2.906±0.035	2.870±0.038	2.917±0.031	2.826±0.045

Values with different superscript in a column within Ripeness are significant different ($P < 0.05$)

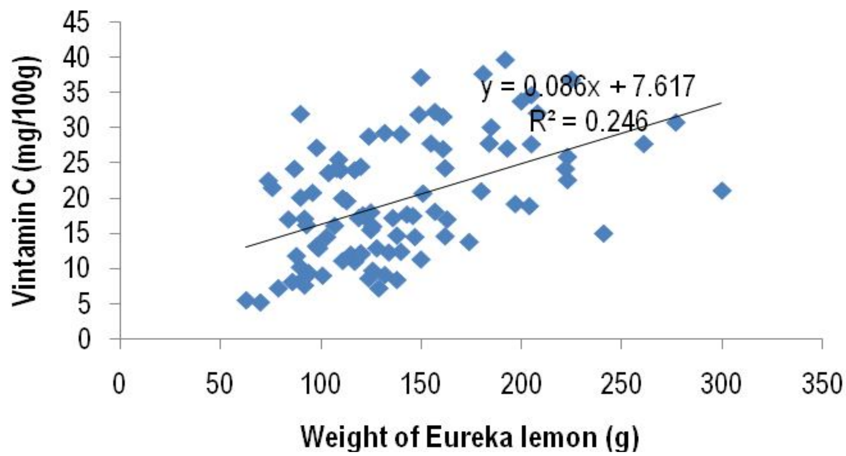

Figure 1: Scatter plot for the relationship between weight of the Eureka Variety and vitamin C (explain the variation)