It is with profound joy that I welcome you all to the launch of the newest academic Journal and addition to the already rich academic literature milieu in our part of the world. This Journal came about as a result of the felt need to give academics and practitioners in Food Science, Food Technology, Nutritional, Dietetic Sciences and related study areas, an extra avenue to publish their research work for the benefit of the academic community, the user-food industry sector and the field of Nutrition and Dietetics practice. It will be very useful for graduate students in the same fields as we intend to keep the time from submission of the manuscript to the time of publication of the article as short as is practicable.

I would like to recognize my colleagues Prof. Abdul Faraj of Egerton University, Ms Sheila Kilonzi my colleague at Karatina University, Prof. Arnold Onyango of Jomo Kenyatta University of Agriculture and Technology and other colleagues for believing in me to be able to drive the process of producing this maiden publication of our Journal.

In a very special way, I appreciate the effort of colleagues who contributed the excellent articles to this inaugural publication. Additionally, Mr. Samwel Kumba and Ms Ephline Okoth of the Kenya School of Government are recognized and appreciated for the design of the Journal.

I welcome you all to enjoy the excellent articles contained in this first issue of the Journal of Food and Nutritional Sciences Research. I, Prof. Arnold Onyango our Scientific Editor and our team at JFNSR look forward to receiving more articles for the upcoming issues. Our team welcomes and requires your continued support and offer of valuable ideas for the improvement of future Journal issues, management of the Journal and its sustainability. We look forward to all of us maintaining the tempo and enthusiasm set with this publication for a long time to come. Enjoy!

Prof. Michael N.I. Lokuruka, Ph.D., EBS, MKIFST, AMKNDI
Founding Editor and Editor-in-Chief
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In much of Africa and other developing economies, the problems of food insecurity and malnutrition continue to inflict millions of the resource-disadvantaged communities. In addition, non-communicable diseases such as diabetes, cancer, and cardiovascular diseases are rapidly increasing in prevalence among both affluent and resource-deprived individuals.

The Journal of Food and Nutritional Sciences Research (J. Food Nut. Sci. Res.), published by the Kenya Institute of Food Science and Technology, seeks to publish innovative papers that will contribute knowledge towards improving food security, nutrition, and food-related strategies for reducing the prevalence of non-communicable diseases. The inaugural articles partly portray the mix of topics that are envisaged. For example, Prof. Michael Lokuruka has contributed a succinct review on the anticarcinogenic potential of conjugated linoleic acid. The paper by Aduol and co-workers reports on the production of short chain fatty acids during cowpea fermentation by lactic acid bacteria. Such short chain fatty acids have been documented to reduce the risk of various non-communicable diseases. Kilonzi and co-workers report on the reduction of antinutrients such as tannins and phytates during processing of lablab beans, while Oloo and co-workers have determined the fatty acid and amino acid profiles of different free-range chicken ecotypes in Kenya, showing the differences that may arise from feeding practices.

Henceforth, we look forward to receiving articles in all areas of Food Science and Technology, Postharvest Technology and Nutrition, including both basic and applied research. As we hope to have rapid but rigorous peer review of articles, we request professionals in the various disciplines to generously support the Journal by participating as peer reviewers, and conducting the reviews in a timely fashion.

Arnold N. Onyango, Ph.D
Scientific Editor
**Fatty Acid and Amino Acid Profile of Indigenous Cluster Ecotypes of Kenyan Free-ranging Chicken**

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**ABSTRACT**
An experiment was conducted to determine amino acid and fatty acid profile of indigenous chicken reared in Kenya under a free range feeding system. Five cocks of each ecotype were purchased, slaughtered and transported to JKUAT and Durban University of Technology, within 12 and 24 hours respectively in an insulated box containing ice. Amino acid profile was estimated using Pico Tag by hydrolysis with acid for 24 hours, derivatized and analyzed by reverse phase HPLC. Fatty Acid Methyl Esters (FAME) were synthesized by a direct/one-step extraction transesterification method. FAME were then separated and quantified using gas chromatography. The amino acid and fatty acid profile of indigenous chicken in Kenya was affected by the ecotype as well as the part of the chicken (breast or thigh). The amino acid profile of experimental chicken revealed values higher than the RDA. The ratio of \(\text{ω}-6:\text{ω}-3\) though higher than 4:1, demonstrated that the consumption of indigenous free ranging chicken meat is likely to give favourable health outcomes.

**KEY WORDS:** Fatty acid, amino acid, indigenous chicken, Kenya

**INTRODUCTION**
Poultry in Kenya play an important socio-economic, food and nutrition security roles in most households and especially in rural areas. Kenyan poultry population is estimated at 42.4 million birds (FAO, 2014) out of which 75% are local indigenous chicken. The remaining 22% is broilers while the rest is breeding stock and spent layers. Meat quality and palatability is affected by several factors. A major one is the fatty acid composition in the muscles and adipose tissue (Hoffman et al., 2005). The amino acid profile is also responsible for nutritional and functional quality of chicken meat. The most common rearing method for indigenous chicken is through the free range backyard system. According to (Pavlovski et al., 2013) chicken meat from intensive broiler production differs in quality from native breeds and those in a free range system. Intensification of native/indigenous chicken rearing has been on the increase for the past 20 years. Rearing native chicken under an intensive system in Serbia has been studied by (Bogosavljevic-Boskovic et al., 2010; Milošević et al., 2003; and Blagojević et al., 2009); Pavlovski et al., 2013). Because of their work it is now considered that to develop an intense meat flavour, broilers should be kept under free range production system, a practice that is currently becoming common in Europe and the USA. This concept is also supported by the strong animal welfare groups (Dawkins, 2003). The demand for poultry meat has been estimated to triple in Africa, a trend that is likely to be replicated in other parts of the world by the year 2030 (Zootecnica, 2016; Heinz et al., 2007).

Food nutrition data is important to international
organizations, private individual, food aid programmes, and epidemiologist who often relate patters of disease with the dietary components and nutritional assessment of individual intake and diet counselling (Rand et al., 1991; Almeida et al., 2006). Although the USDA and FAO have published papers with nutrition tables of chicken, these are from the data collected from the chicken reared in the USA and Europe. The composition of total fat, Saturated Fatty Acids (SFA) and Mono Unsaturated Fatty Acids (MUFA) are independent risk factors for all cause cardio-vascular disease (Leosdottir et al, 2005). The American Diabetic Association (2005) advises to limit total daily energy intake from fat to <30%. They also recommend that the SFA and Trans-fatty acids should contribute no more than 10% of total daily energy intake. It has already been demonstrated that replacing red meat with chicken may result in significant decrease in apolipoprotein B and total cholesterol levels in type 2 diabetic patients (Gross et al., 2002). This effect is probably an attribute of the higher PUFA content of chicken meat as compared to beef. At the same time, the beneficial attributes of PUFA is dependent on the ratio of omega 6 fatty acids to that of omega 3. An ideal ratio is often agreed as 4:1 This ratio is often favorable in poultry meat depending on diet (Marangoni et al., 2015a).

With regard to quality of protein food sources, the value of the amino acids measured in terms of the amino acid scores, essential amino acid index and the protein digestibility corrected amino acid scores is important to the understanding of the nutritive value of a given protein in the diet (FAO/WHO, 1985). At the same time, the low levels of collagen in poultry meat is a good indicator of its digestibility and hence biological value (Marangoni et al., 2015). In spite of the apparent benefits of poultry meat to nutrition and especially with regard to provision of essential fatty acids and amino acids, the nutritive composition of these factors in indigenous chicken in Kenya is yet to be reported and documented. This study therefore evaluated the nutritive value of indigenous chicken meat in Kenya by determining the fatty acid and amino acid profiles of three ecotypes.

Materials and Methods

Fat Content Determination

The crude fat content was determined by Soxhlet extraction, using the standard method of the Association of Official Analytical Chemists (AOAC, 2005).

Fatty acid Analysis

Fatty profile acid analysis was carried out according to AOAC (2005) as modified by (Indarti, 2015). Fatty Acid Methyl Esters (FAME) were synthesized by a direct/one-step extraction transesterification method. A tenth of a gram of sample was mixed with 2 ml of a mixture of methanol and sulfuric acid (85:15, v/v) and 2 ml of chloroform. Samples were heated to 100°C for 30 min and cooled to room temperature in a desiccator. Then, 1 ml of distilled water was added to the mixture, followed by vortexing for 1 min. The mixture was allowed to separate and the organic phase (top layer) containing FAME was then transferred and dried with anhydrous Na2SO4. Samples were stored in a freezer (−20°C) while awaiting Gas Chromatography (GC) analysis.

FAME were separated and quantified using a gas chromatography system (Automatic System XL, Perkin Elmer™, Norwalk, Connecticut, USA) equipped with a flame ionization detector and a 30-m x 0.25-mm fused silica capillary column (Omegawax 250, Supelco™, Bellefont, USA). Helium was used as the carrier gas, while hydrogen and compressed air was used for Flame Ionization Detection. The oven temperature was programmed to rise from 50-220°C at a rate of 4°C min⁻¹ and then held at 220°C for 35 min. The injector and detector temperatures were set to 250°C and 260°C, respectively. Individual fatty acids were identified by comparison to known standards (Supelco 37 Component FAME Mix; Supelco) and the areas beneath the identified chromatographic peaks calculated by integration. Saturated Fatty Acids (SFA), Mono- Unsaturated Fatty acids (MUFA) and Poly-Unsaturated Fatty acids (PUFA) and their ratios were calculated from the fatty acid composition results.
Determination of Amino Acid Profile
The total amino acid content of the 17 different amino acids in the sample are reported in duplicate and reported as g/100g sample as is. Total amino acid profile (excluding Tryptophan, Cysteine and Methionine) were determined on lyophilized, ground and homogenous samples by SAGL In-house method 009: as adopted from Pico-Tag™ method as described by (Bidlingmeyer et al., 1984). Samples were hydrolyzed with acid for 24 hours, then derivatized and analyzed by reverse phase HPLC. Cysteine and methionine were analysed simultaneously with SAGL In-house method 015. The samples were oxidized overnight, then hydrolysed, derivatized and analysed with HPLC. Tryptophan was analysed differently with SAGL In-house method 007. The tryptophan was hydrolysed under alkaline conditions and then analysed with HPLC. The samples were first freeze dried before shipping to Central Analytical Facilities at Stellenbosch University, South Africa for the analysis. The Protein digestibility Amino Acid Scores (PDCAAS) was calculated as proposed by FAO/WHO (1991). For practical reasons, the score applies to diets of children older than one year.

RESULTS AND DISCUSSION
Table 1 shows the amino acid profiles (total essential and non-essential amino acids) of the indigenous chicken ecotypes, from three locations and two body parts. Except for the amino acids Histidine and Lysine which were higher in the breasts than in the thigh muscles for all the ecotypes, there were no significant differences on the amino acid profile of the breasts and thighs (P>0.05). The values reported for Methionine (minimum of 6.77 g/100 g), and Lysine (minimum of 7.76 g/100 g) which are limiting amino acids in cereals and legumes were very high in the chicken. This supports the assertion that chicken is a good source of high quality protein and confirms that Kenyan indigenous chicken under free range are equally a good source of the same. Considerable amounts of Lysine, Aspartic acid and Glutamic acid were found in all the three ecotypes—the trend was observed in the breasts and thighs. The high level of Glutamic acid (Figure 1) which has been demonstrated to correspond to the taste is theorized to be a contributor to the claimed better taste of indigenous chicken. This result is in line with the finding of Wattanachant et al. (2004) and Aronal et al. (2012) who reported high values for these three amino acids. They also reported very high values of leucine in the breast and thighs of chicken, a result which is supported by our findings. Protein quality in human nutrition is closely related to the efficiency of protein utilization in the human digestive system. This is usually a factor of protein digestibility, quantity and type of the amino acid.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Chicken parts</th>
<th>Ecotype</th>
<th>FAO/WHO recommendation for adults</th>
<th>FAO/WHO recommendation for children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>Thigh</td>
<td>Kakamega</td>
<td>Naivasha</td>
</tr>
<tr>
<td>His</td>
<td>2.96±0.03a</td>
<td>2.15±0.13b</td>
<td>2.51±0.40</td>
<td>2.46±0.52</td>
</tr>
<tr>
<td>Lys</td>
<td>9.02±0.26a</td>
<td>7.76±0.24b</td>
<td>7.95±0.56</td>
<td>8.43±0.75</td>
</tr>
<tr>
<td>Met</td>
<td>7.96±0.19</td>
<td>6.77±0.89</td>
<td>6.79±0.84</td>
<td>6.90±1.09</td>
</tr>
<tr>
<td>Val</td>
<td>4.33±0.02</td>
<td>3.91±0.22</td>
<td>4.04±0.33</td>
<td>3.99±0.31</td>
</tr>
<tr>
<td>Ile</td>
<td>3.91±0.04</td>
<td>3.62±0.17</td>
<td>3.67±0.29</td>
<td>3.69±0.16</td>
</tr>
<tr>
<td>Leu</td>
<td>7.25±0.10</td>
<td>6.70±0.41</td>
<td>6.81±0.60</td>
<td>6.73±0.35</td>
</tr>
<tr>
<td>Phe</td>
<td>3.42±0.26</td>
<td>3.50±0.27</td>
<td>3.50±0.42</td>
<td>3.25±0.17</td>
</tr>
<tr>
<td>Thr</td>
<td>4.20±0.09</td>
<td>4.04±0.23</td>
<td>4.03±0.35</td>
<td>4.01±0.05</td>
</tr>
<tr>
<td>Total</td>
<td>43.05</td>
<td>38.5</td>
<td>39.3</td>
<td>39.46</td>
</tr>
</tbody>
</table>
Glutamic acid has been reported to have a significant effect on the taste of meat. The higher levels reported in this study and supported by reports from others studies may be one of the factors that is responsible for the better taste already reported in many parts of the world when indigenous chicken and the broiler are compared (Farmer, 1999). Histidine value even though being the lowest was still reported at a value that is more than the recommended value by the WHO/FAO (2007) for both adults and children (Table 1).

**Amino acid-chemical scores**

In the current study, the values of the PDCAAS for all the amino acids from the chicken had a value greater than unity for both the breasts and the thighs. The protein score reflects its amino acid (AA) content in comparison with the ideal protein (Aronal et al., 2012). Normally egg white and milk proteins are considered the ideal proteins due to their high digestibility and hence the rest of the amino acids are compared against these two (FAO/WHO, 1985). However, when there is a need to know the use of an amino acid by the organism, it is necessary to do a correction of the score value by protein digestibility (PDCAAS) (Marangoni et al., 2015a). Poultry meat like that of milk and egg are referred to as high quality proteins, as they have a PDCAAS of 1 or very close to one. This is unlike the legumes and vegetable sources which have PDCAAS of up to 0.5 while wheat and beans are at 0.75 (Marangoni et al., 2015).
Fatty acid profile of Kenyan indigenous chicken

The fatty acid profile of the breast meat of the three ecotypes are presented in Table 2. Poultry meat is a major contributor of triacylglycerols (TAGs), PUFAs, MUFAs, and SFAs in diets. Within the Kakamega ecotype, the palmitic acid composition was highest followed by the oleic and linoleic fatty acid compositions, respectively. The value of Caprilic acid at 0.04 % was the lowest. For Naivasha ecotype, the oleic acid content was highest followed by palmitic and then linoleic acid. The lowest was the composition of Arachidonic acid. For Taita ecotype, oleic acid, acid composition was highest followed by palmitic and then linoleic acid composition. Among the three ecotypes, the composition of the palmitic acid was significantly different in the Naivasha than in the Kakamega and Taita ecotypes. Palmitic acid is a saturated fatty acid and one of the most abundant in nature. The composition of stearic acid was equally significantly higher in the Naivasha than in the Kakamega but not in the Taita ecotypes. Factors affecting fatty acid content of poultry are: animal breed, external and internal fat levels, climate, and rearing methods (Broganolo, 1997. These factors are more often than not determined by the regions and resultant cultural, management and feeding practices of the people.

Table 2: Fatty acid composition (%) of breast meat of three indigenous chicken ecotypes

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Ecotype</th>
<th>Naivasha</th>
<th>Taita</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kakamega</td>
<td>Naivasha</td>
<td>Taita</td>
</tr>
<tr>
<td>Caprylic</td>
<td>0.04±0.02a</td>
<td>0.88±0.51a</td>
<td>0.14±0.07a</td>
</tr>
<tr>
<td>Capric</td>
<td>0.57±0.28a</td>
<td>0.42±0.11a</td>
<td>0.02±0.01a</td>
</tr>
<tr>
<td>Lauric</td>
<td>0.42±0.17a</td>
<td>0.26±0.03a</td>
<td>0.18±0.08a</td>
</tr>
<tr>
<td>Myristic</td>
<td>0.76±0.19a</td>
<td>0.76±0.04a</td>
<td>0.99±0.14a</td>
</tr>
<tr>
<td>Palmitic</td>
<td>20.20±0.71a</td>
<td>17.88±0.59b</td>
<td>20.02±0.64a</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>1.73±0.14a</td>
<td>1.51±0.15a</td>
<td>1.74±0.16a</td>
</tr>
<tr>
<td>Stearic</td>
<td>9.81±0.16b</td>
<td>11.65±0.78a</td>
<td>10.76±0.37ab</td>
</tr>
<tr>
<td>Oleic</td>
<td>19.55±0.60a</td>
<td>20.59±0.67a</td>
<td>20.33±0.59a</td>
</tr>
<tr>
<td>Linoleic</td>
<td>15.06±0.95a</td>
<td>13.12±0.59a</td>
<td>15.75±0.75a</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.89±0.19a</td>
<td>0.84±0.05a</td>
<td>0.58±0.08a</td>
</tr>
<tr>
<td>Arachidic</td>
<td>0.32±0.21a</td>
<td>0.19±0.09a</td>
<td>0.13±0.04a</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>10.22±0.73b</td>
<td>13.11±0.29a</td>
<td>10.13±0.29b</td>
</tr>
<tr>
<td>EPA</td>
<td>0.76±0.14a</td>
<td>0.80±0.10a</td>
<td>1.10±0.05a</td>
</tr>
<tr>
<td>DHA</td>
<td>2.31±0.36a</td>
<td>1.40±0.10b</td>
<td>1.66±0.10b</td>
</tr>
</tbody>
</table>

Legend: Eicosapentanoic acid (EPA) and Decosahexaenoic acid (DHA)

Fat content and fatty acid profiles of TAGs in muscles strongly correlate to meat quality especially tenderness, juiciness and flavor (Wood et al., 2008). Although Wattanachant et al., (2004), reported higher content of saturated fatty acids and lower PUFA in indigenous chicken muscle than in broilers, (Jaturasitha et al., 2008) found the opposite results. In the current study, the fatty acid profile was found to differ by ecotype (p≤0.05) possibly due to the different feeds fed to the different genotypes based on the unique conditions of the farmers. This suggestion was also made by Cherian et al. (2002) who in their study determined muscle fatty acid composition and thiobarbituric acid-reactive substances in broilers fed on different cultivars of sorghum. They noticed significant difference in these values for chicken fed on different cultivars. The fatty acid profile of the thigh meat of the three ecotypes is given in table 3.

For the thigh meat, oleic fatty acid was highest (p≤0.05) in the Kakamega ecotype followed by...
Among the three ecotypes, the values of SFA, MUFA, and PUFA were not significantly different (p≥0.05). With regard to the Naivasha ecotype, oleic acid was highest followed by palmitic and then linoleic acid. The lowest fatty acid was Capric acid at 0.06±0.03%. For the Taita ecotype, oleic acid was the highest followed by linoleic and then palmitic acid and the lowest was capric acid at 0.1±0.05%. Compared across the ecotypes, significant differences (p≤0.05) were noted on Caprylic acid between Naivasha, Taita and Kakamega ecotype. Lauric acid composition was also significantly higher in Kakamega than in the Naivasha and Taita ecotypes. Myristic acid composition was significantly different for Naivasha and Kakamega ecotypes from the Taita ecotype. Finally, the acid docosahexaenoic acid (DHA) composition was significantly highest in Kakamega than in Naivasha and Taita. The DHA fatty acid is part of the long chain omega 3 fatty acids that also include eicosapentanoic acid (EPA), and decosapentaenoic acid (DPA) (Haug et al., 2011).

Table 3: Fatty acid composition (%) of thigh meat of three indigenous chicken ecotypes

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Ecotype</th>
<th>Naivasha</th>
<th>Taita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic</td>
<td>0.27±0.07b</td>
<td>0.81±0.19a</td>
<td>0.44±0.16ab</td>
</tr>
<tr>
<td>Capric</td>
<td>0.18±0.05a</td>
<td>0.06±0.03a</td>
<td>0.10±0.05a</td>
</tr>
<tr>
<td>Lauric</td>
<td>0.23±0.03a</td>
<td>0.10±0.02b</td>
<td>0.13±0.04ab</td>
</tr>
<tr>
<td>Myristic</td>
<td>0.63±0.10ab</td>
<td>0.84±0.06a</td>
<td>1.12±0.14b</td>
</tr>
<tr>
<td>Palmitic</td>
<td>18.34±0.61a</td>
<td>16.58±0.49a</td>
<td>18.47±0.70a</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>2.00±0.15a</td>
<td>2.20±0.18a</td>
<td>2.17±0.25a</td>
</tr>
<tr>
<td>Stearic</td>
<td>12.97±0.33a</td>
<td>13.72±0.53a</td>
<td>12.23±0.48a</td>
</tr>
<tr>
<td>Oleic</td>
<td>19.85±0.63a</td>
<td>23.53±1.31a</td>
<td>22.44±1.35a</td>
</tr>
<tr>
<td>Linoleic</td>
<td>15.73±0.30a</td>
<td>15.98±0.97a</td>
<td>19.18±2.06a</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.59±0.04a</td>
<td>1.51±0.51a</td>
<td>0.78±0.11a</td>
</tr>
<tr>
<td>Arachidic</td>
<td>0.15±0.03a</td>
<td>0.18±0.03a</td>
<td>0.22±0.10a</td>
</tr>
<tr>
<td>Arachidonc</td>
<td>10.64±0.40ab</td>
<td>12.19±0.67a</td>
<td>9.29±0.88b</td>
</tr>
<tr>
<td>EPA</td>
<td>1.18±0.24a</td>
<td>0.60±0.05a</td>
<td>0.91±0.18a</td>
</tr>
<tr>
<td>DHA</td>
<td>2.45±0.21a</td>
<td>0.90±0.10b</td>
<td>1.55±0.24b</td>
</tr>
</tbody>
</table>

Legend: Eicosapantanoic acid (EPA) and Decosahexaenoic acid (DHA)

Among the three ecotypes, the values of SFA, MUFA, and PUFA were not significantly different (p>0.05). However, the value of omega 3 and Omega 6 fatty acids recorded a significant difference (p≤0.05). Kakamega ecotype recorded the highest value of omega 3 as well as omega 6 composition. This was followed by Taita and finally, the Naivasha ecotype. Though many factors may have contributed to this including ecotype (Pavlovska et al., 2013), the other likely factor is that the feeding regime for chicken in Kakamega consists chiefly of free range in which they chicken scavenge on grasses, leaves, and worms. It is reported that the ratio of omega 6 to omega 3 fatty acids is higher in the feeds scavenged by chicken from natural diets than in what they are fed in cereals and legumes (Haug et al., 2011). In the other regions particularly Naivasha, the town influence resulted in farmers relying heavily on food from the hotels in town and some confessed to the use of waste from the dump site. The ratio of omega 6 to omega 3 is predictive of the quality of fat in diets. The current ratio in most western diets ranges between 20:1 and...
30:1, though the ideal is agreed as 4:1 (Marangoni et al., 2015b). The Kakamega ecotype ratio of 9.5:1 is most favourable and is recommended in this regard.

The SFA content of chicken meat was contributed majorly by palmitic, myristic and stearic fatty acids both in breast (Pavlovski et al., 2013) and thigh (Table 2 and 3). Meat in general is associated with the supply of fatty acids especially the SFA which have been associated with the risk of cardiovascular diseases (Milićević et al., 2014). The SFA has been reported to be significantly different between breeds for example naked neck and the broiler (hybro G+ and Cobb 308) (Pavlovski et al., 2013). Fatty acid profiles were also reported to differ significantly (p≤ 0.05) with the part of the body (Pavlovski et al., 2013; Adulyatham et al., 2006). Poultry meat is considered healthier because of a considerably low fat content compared to other meats (Brenes and Roura, 2010). Lipids of animal origin typically contain triglycerides (Abdulla et al., 2015). Similar data expressing the values of the fatty acids on the breast muscles of the three ecotypes is presented in figure 2. There was a significant difference (p≤ 0.05) in the MUFA content of the thighs of the ecotypes (Naivasha and Taita) which had higher values than that of the Kakamega chicken. The Kakamega ecotype had significantly higher value of the Omega 6 fatty acids than the Naivasha and Taita ecotypes.

**Table 4: % amount, ratio of average values of 6:3 fatty acids for ecotype and body part vs. recommended ratio**

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Chicken part</th>
<th>Omega 6</th>
<th>Omega 3</th>
<th>Ratio</th>
<th>Recommended ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kakamega</td>
<td>Breast</td>
<td>12.64±0.81</td>
<td>1.32±0.19</td>
<td>9.5:1</td>
<td>4:1</td>
</tr>
<tr>
<td></td>
<td>Thigh</td>
<td>13.18±0.63</td>
<td>1.41±0.18</td>
<td>9.0:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>12.91±0.51</td>
<td>1.36±0.13</td>
<td>9.5:1</td>
<td></td>
</tr>
<tr>
<td>Naivasha</td>
<td>Breast</td>
<td>13.12±0.32</td>
<td>1.01±0.07</td>
<td>13.0:1</td>
<td>4:1</td>
</tr>
<tr>
<td></td>
<td>Thigh</td>
<td>14.09±0.72</td>
<td>1.00±0.18</td>
<td>14.0:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>13.60±0.40</td>
<td>1.01±0.10</td>
<td>13.5:1</td>
<td></td>
</tr>
<tr>
<td>Taita</td>
<td>Breast</td>
<td>12.94±0.76</td>
<td>1.11±0.09</td>
<td>11.7:1</td>
<td>4:1</td>
</tr>
<tr>
<td></td>
<td>Thigh</td>
<td>14.24±1.57</td>
<td>1.08±0.12</td>
<td>13.2:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>13.59±0.87</td>
<td>1.10±0.08</td>
<td>12.4:1</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2: The fatty acids (%) in breast meat of three Kenyan indigenous cluster ecotypes**
In Brazil, no fatty acid content of local chicken was presented in their tables of food composition and same thing is true for Kenya (Almeida et al., 2006). Though the cholesterol levels of chicken meat have been reported to be more than that of the beef yet there is always reduction of the serum total cholesterol levels from consumption of beef and this could be explained as due to the type of PUFA in diet rather than cholesterol being the most potent regulator of the serum cholesterol. Maturation period of chicken has come down from the 120 days in 1970 to 45 days in the 2000s and currently, less, or as stated by the Centre for Food Integrity, 48 days at 6.2 pounds compared to 112 days and 2.5 pounds in 1925. This rate of growth has been achieved against an increase in high fat content, especially for those fed diets that are rich in omega 6 and protein. Studies also show that palmitic oil in feeds directly contributes to the increase in palmitic acid concentration in chicken (Smink et al., 2008). Pastures provide good sources of omega-3 (Ponte et al., 2008); and usually consumer’s desire for specialty poultry under free range for greater omega-3 and PUFA because these reduce the risk of cardiovascular disease (Tempe, 1996) and inhibits the growth of mammary and prostate gland tumors and cancers (Pandalai et al., 1996). The values and ratios of \( \omega-3 \) and \( \omega-6 \) are presented in Table 4. The lowest ratio was recorded for Kakamega ecotype and it stood at 9:1. The often recommended ratio is 4:1. Phospholipids on the other hand is endowed with a higher composition of PUFA existing as cellular membrane contents (De Smet et al., 2004). Although red meats are high in phospholipids hence higher in PUFA, this characteristic is related to the prediction of possible lipid oxidation (Maragon et al., 2015). The PUFAs undergo rapid oxidation leading to impaired organoleptic quality, short shelf life and if uncontrolled, off flavours. Fatty acid composition or profile could be affected by dietary Ca\(^{2+}\) particularly for broilers (Abdulla et al., 2015). This is because it is believed that they form calcium soaps with fatty acids, resulting in decreased digestion and absorption of fat. Lipid oxidation causes deterioration, affects the colour, flavor, texture and nutritional value of poultry.

![Figure 3: The fatty acids (%) of thigh meat in three indigenous Kenyan cluster ecotypes](image-url)
CONCLUSION

This study revealed significant amounts of amino acid and fatty acid in three ecotypes of indigenous chicken of Kenya. Glutamic acid was the highest amino acid while the lowest value recorded was for Histidine. However, all the reported amino acid values exceeded the recommended intakes by WHO/FAO for adults and children. The high value of Glutamic amino could be related to the good taste proclaimed by consumers of indigenous chicken. There was no significant difference among the values of PUFA, MUFA, and SFA for breast and thigh. The lowest ratio of Omega-6:Omega-3 was 9:1 and was lower than the recommended value of 4:1. The Kakamega ecotype had the lowest ratio among the 3 ecotypes, which points to the superiority of this ecotype with regard to this parameter. Free-range rearing as applied to indigenous chicken results in a favourable Omega-6:Omega-3 ratio, though this was still lower than the recommended ratio.

ACKNOWLEDGEMENT

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Effect of Some Processing Methods on Nutrient Content and Anti-Nutritional Factors of a Variety of Dolichos Lablab (Lablab purpureus L.) Beans Grown in Kenya

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ABSTRACT
This study aimed to determine the effect of different processing methods on the proximate composition and anti-nutritional factors of Dolichos lablab beans (Lablab purpureus) of Kenya. The seeds of KAT/DL–2 variety, sourced from Kenya Agricultural Livestock and Research Organisation, Katumani Dryland Research Station were sorted, then subjected to different processing methods (soaking, cooking and germination). The samples were analysed for proximate composition, tannins, phytates and trypsin inhibitory activity. The results showed a significant increase (2.0%) in crude protein content for germinated lablab beans while carbohydrates content was high in cooked samples. The variety KAT/DL–2 had high levels of phytates; 723.6 mg/100g and tannins 330.3mg/100g and trypsin inhibitor activity 1.3mg/100g. Cooking achieved the highest reduction of anti-nutrients with 88% reduction in TIU. The results revealed that the anti-nutrients in lablab beans can be reduced using different methods of processing. However, there is need to investigate the effect of combined methods on the nutrients and anti-nutrients.

KEY WORDS: Dolichos lablab, proximate composition, processing, anti-nutrients

INTRODUCTION
The United Nations General Assembly declared the year 2016 as the International Year of Pulses (IYP). This was designed to raise public awareness of the nutritional value of legume plants in order to improve food and nutrition security. Dolichos lablab (Lablab purpureus) is a drought tolerant legume crop usually grown for human food, soil conservation and animal fodder. The crop can be incorporated into intercrop systems (Maass et al., 2010). This little known legume (Osman, 2007) is believed to have originated from Asia and was introduced to Africa in the eighth century (Deka & Sarkar, 1990), Australia and America. The legume is known by different names across the world (Mortuza & Tzen, 2009; Murphy & Colucci, 1999). In Kenya it is referred to as Njahe (Kikuyu), Chabi (Meru) and Mbumbu or Nzavi among the Akamba community (Kinyua & Kiplangat, 2012).

Dolichos lablab seeds, like other legumes are a valuable element of a healthy diet due to their content of protein, of high biological value. Additionally, they are a source of other valuable nutrients such as minerals and carbohydrates. They have the potential to contribute towards the alleviation of malnutrition and food insecurity due to adaptability, especially in the current climate inconsistencies (Maass et al., 2010). However, the nutritive value of this legume grain is pegged on nutrient content and the presence or absence of and the varying degrees of anti-nutrients such as tannins, phytates and trypsin inhibitors among others (Egounlety & Aworh, 2003). For proper utilisation of Dolichos lablab, it is necessary to understand their nutritional quality considering anti-nutrients levels. Legumes are normally processed before
consumption. Different methods of processing have been used in enhancing the nutritional quality and mitigating the effects of inherent anti-nutritional factors. Common methods that have been employed for various legumes include thermal processing (Alagbaoso et al., 2016; Wang et al., 2010), fermentation (Egounlety & Aworh, 2003) soaking and germination (Soetan & Oyewole, 2009).

As one of the crops that are categorized as orphan or lost crops of Africa, Dolichos lablab has received minimal research attention and its production has remained at subsistence level for human consumption and as a cover crop in Kenya. In order to enhance production and utilization of Dolichos lablab, research on the effects of different processing methods on nutrients and anti-nutritional factors is required.

**OBJECTIVE**
This study aimed at investigating the effects of soaking, cooking and germination on the nutrients and anti-nutrient content of Dolichos lablab (KAT/DL-2) bean variety commonly utilised in Kenya.

**MATERIALS AND METHODS**

**Sourcing of samples and preparation**
Dolichos lablab bean (*Lablab purpureus*) variety KAT/DL-2 were sourced from Kenya Agricultural Livestock and Research Organisation (KALRO), Katumani Dryland Research Station. The samples were sorted to remove foreign materials and immature seeds. Raw bean samples were ground and placed into plastic bags then stored at 4°C. Other samples were rinsed then placed in perforated plastic bags and cooked in a water bath maintained at 97°C until they were soft (softness was tested by pressing between the fingers). Another set of Dolichos lablab beans (100 g) was soaked in distilled water (1:10 w/v) according to Ramakrishna et al. (2006) at room temperature for 6 hr, 12 hr and 24 hr. Some other samples were soaked for 24 h before being allowed to germinate on paper towels for 48 hr.

**Proximate composition analysis and energy values**
The proximate composition of the Dolichos lablab seeds was determined according to the procedures of AOAC (2000). Crude protein was calculated using the factor of 6.25. The carbohydrates in the samples were estimated by difference, by subtracting the sum of dry matter percentage of crude protein, crude fats and crude ash and moisture from 100. The sample calorific values were estimated [in kcal/g] by multiplying the percentages of crude protein, crude fat and carbohydrates using the Atwater specific factors as adopted by the Food and Agriculture Organisation (WHO/FAO, 2002) for legume grains: proteins (3.47), fats (8.37) and carbohydrates (4.07).

**Determination of phytic acid**
Determination of phytic acid was done according to Clydesdale (1982). About 0.5 g of accurately weighed sample was extracted with 3% H$_2$SO$_4$ and the phytic acid precipitated with ferric chloride, followed by conversion to sodium phytate that was then separated on a C-18 Column of HPLC using a Refractive Index Detector. A standard solution containing 10 mg/ml of sodium phytate (Inositol hexaphosphoric acid) was used for the standard curve work.

**Determination of tannins**
Tannins were extracted from about 0.2 g of accurately weighed sample using acidic methanol as described by Prince and Scoyoc (1978). The resulting extract was made up to 25 mL. One millilitre of the extract was placed in a test tube and 5 mL of freshly prepared vanillin-HCl reagent was added slowly while mixing and the colour developed was read at 500 nm. The results were quantified against a Catechin standard curve. The results were expressed as mg/100 g catechin, dry weight.
**Determination of trypsin inhibitor activity**

The Trypsin inhibitor activity (TIA) was determined according to Kakade et al. (1974) with modifications. Some 50 mL of 0.01 mol/L of NaOH was used to extract one gram of the sample flour for 1.5 hr. Portions (0.6, 1.0, 1.4 and 1.8 mL) of the suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with water. The blank sample tube did not have any NaOH. Then 2mL of trypsin solution (4 mg trypsin in 200 mL 0.001 mol/L HCl) was added to each tube before placing in a water bath maintained at 37 °C. To each tube, 5 mL of N-benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPA) solution was added. The solution (40 mg BAPA in 100 mL water with 1 mL dimethyl sulphoxide) had been previously warmed to 37°C. The reaction was terminated after 10 minutes by adding 1 mL of acetic acid. The absorbance of the solution was measured at 410 nm wavelength against a reagent blank. The trypsin inhibitory activity was calculated using the formula

\[ \text{TIA} = \left( \frac{2.632 \times D \times A1}{S} \right) \text{ mg pure trypsin inhibitor/g sample}, \]

where \( A1 = \text{change in absorbance/mL diluted sample extract}, \) \( D = \text{dilution factor} \) and \( S = \text{weight of sample (g)}. \)

**STATISTICAL ANALYSIS**

The analysis was carried out in three replicates for all determinations. The mean and standard deviation of means were calculated. The data were analyses by one- way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan’s new multiple range test (Duncan, 1955). Significance of the differences was defined as \( P < 0.05 \)

**RESULTS AND DISCUSSION**

Table 1 shows the proximate composition in raw and processed Dolichos lablab beans. The percent raw protein content (24.36%) compared to those obtained for Dolichos beans in Tamil Nadu, India (Kamatchi et al., 2010). However, they were higher than those obtained in other areas (Ragab et al., 2012; Subagio, 2006). There was significant (\( P<0.05 \)) increase in percent protein content in germinated lablab beans. Heat treatment and soaking decreased the protein content.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Proximate composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>Proteins</td>
<td>24.36±0.20</td>
</tr>
<tr>
<td>Ash</td>
<td>4.14±0.27</td>
</tr>
<tr>
<td>Fats</td>
<td>2.59±0.15</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>60.79±0.20</td>
</tr>
<tr>
<td>Energy kcal/g</td>
<td>353.7±0.64</td>
</tr>
</tbody>
</table>

Means with different superscripts within a row indicate significant differences \( P < 0.05 \)
Carbohydrates calculated by difference (100- (crude proteins+ ash +fats))
There was a 2% protein content increase recorded for germinated beans. Similar results were obtained by Borijindakul & Phimolsiripol (2013) who reported an increase of 2.7% after germinating lablab beans, while Ghavidel & Prakash (2007) also reported an increasing pattern of protein content upon germination of other legumes. (Sattar et al., 1989) suggests that this trend may not be an increase in true protein, but elevation of non-protein nitrogen values. However, other studies have attributed this increase to hydrolysis and metabolism of storage proteins, carbohydrates and fats (Rumiayati, James, & Jayasena, 2012). Heat processing and soaking has been reported to decrease protein content in legumes (Haruna & Bichi, 2015), generally. This could be ascribed to leaching of the soluble or the proteineous parts into the soaking and cooking water.

The research recorded ash content of 4.14% for the raw samples (Table 1) similar to (4.48-3.97%) reported by Kamatchi et al. (2010). There was no significant difference (p>0.5) in the ash values between treatments. Thus the treatments had no effect on ash content. Oluwole (2012) reported such relationship for raw, germinated and fermented flour samples. The ash content results are in line with the United States ash content (4.29 per 100 grams) of the lablab beans edible portion tabulated in the National Nutrient Database for Standard Reference, Release 20 (2007).

The results also indicate Dolichos beans have reasonable amounts of fats like other legumes (Pious & Veerabahu, 2013; Megat et al., 2011). The fats content in raw beans ranged between 2.56 and 2.68%. These values are comparable to other research findings on lablab beans (Chau et al., 1998; Kamatchi et al., 2010; Subagio, 2006). The fat decreased significantly upon subjection to cooking, soaking and germination. Contrasting for proteins, germination registered the highest decrease of the fat content. This observation is in agreement with other scientific findings that have observed that processing techniques such as cooking, soaking and germination lower the fat content (D’souza, 2013).

The carbohydrates values were between 60.79-63.68% and those for energy 353.7-361.4 kcal/100 g. These values agree with the findings of Kamatchi et al. (2010). There was a significant increase in carbohydrates content for all cooked and soaked lablab beans. Similar findings were reported for lablab beans (D’souza, 2013; Osman, 2007), and Phaseolus vulgaris (Nakitto et al., 2015). An increase in carbohydrates may not translate to increased kilocalories since protein and fat content have to be considered in the energy calculations.

<table>
<thead>
<tr>
<th>Table 2: Effect of processing on anti-nutritional factors (tannins, TIA and phytates, mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td><strong>Tannins mg/100g</strong></td>
</tr>
<tr>
<td><strong>TIA mg/100g</strong></td>
</tr>
<tr>
<td><strong>Phytates mg/100g</strong></td>
</tr>
</tbody>
</table>

Different superscripts within a row indicate significant differences, P < 0.05.

Raw lablab beans had tannin contents of 330.3±7 mg/100g (Table 2), which significantly reduced 100-fold when the beans were cooked. Soaking the beans resulted in a significant decrease in the tannin contents after 24 hr. However, there was no significant change in the tannin content at 6 h of soaking which could be attributed to low hydration capacity of the beans. These findings are in agreement with other similar works of Nakitto et al. (2015) for common beans, Sharma et al. (2013) for soybeans and in Dolichos beans (Osman, 2007). However, there was significant reduction of phytates and trypsin inhibitory activity (TIU) across all the treatments. Other works have realised such comparable results for Soybean and Cow pea (Egounlety & Aworh, 2003), Jack beans (Doss et al.,
Cooking realised the highest decrease for all anti-nutritional factors. Physical and chemical methods are normally employed to reduce or remove the anti-nutritional factors enhancing the nutritional value of the legume beans (Soetan & Oyewole, 2009). Other methods that have been employed to reduce anti-nutritional factors in legumes include fermentation and enzymatic treatments.

CONCLUSION

Different methods of processing can be used to reduce levels of anti-nutrients without adversely affecting the proximate composition of the beans. Germination was found to increase the protein content, while cooking significantly reduced the anti-nutritional factors. Cooking was therefore found to be the most effective method of eliminating anti-nutritional factors in the beans, thus making the beans safe for consumption. However, there is need to investigate the effect of combined methods and explore the use of enzymatic treatments.

ACKNOWLEDGMENT

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Modification of oligosaccharide and short chain fatty acid content of cowpea milk through fermentation with selected mixed starter cultures

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ABSTRACT
Cowpea milk was fermented with three mixed starter cultures containing (i) Lactobacillus acidophilus, Bifidobacterium sp, and Streptococcus thermophilus (ABT) (ii) Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (DT) or (iii) Lactobacillus rhamnosus GR-1 and Streptococcus thermophilus (GT). Effects of these cultures on flatulence-causing raffinose family oligosaccharides and the production of postbiotic short chain fatty acids were determined. The oligosaccharides and short chain fatty acids were determined by high performance liquid chromatography and gas chromatography, respectively. The stachyose content of raw cowpea (1.388±0.23 g/100 g) was higher than raffinose (0.221±0.06 g/100 g), while verbascose was not detected. Cowpea milk fermentation caused 67-100% reduction in raffinose and 20-70% reduction in stachyose in a culture-dependent manner. All the cultures produced propionic acid, butyric acid and valeric acid in differing concentrations but only GT and ABT produced isovaleric acid. The product fermented with DT attained 2430 ppm of propionic acid, which was four times and ten times higher than the concentrations produced by the ABT and GT cultures, respectively. In conclusion, fermentation of cowpea milk with the three starter cultures reduced flatulence-causing oligosaccharides and produced postbiotic short chain fatty acids that might promote health regardless of the survival of the microorganisms in the gut.

KEY WORDS: Fermentation, Legume milk, Probiotic, Prebiotic

INTRODUCTION
Non-communicable diseases such as diabetes, cardiovascular diseases and cancers are increasing in prevalence throughout the world, and especially in developing countries (Gowshall and Taylor-Robinson, 2018; Somasundaram and Kalupahama, 2016). One of the recommended strategies for reversing this trend is adoption of health-promoting diets (Somasundaram and Kalupahama, 2016). Thus, nutraceuticals, defined as foods or food constituents with health benefits beyond the traditional nutrients, are gaining ground (Schmitt and Ferro, 2018). Probiotic foods are a major class of nutraceuticals; they contain high populations of health-promoting microorganisms (Nagpal et al., 2012). Originally the definition of probiotics was limited to those microorganisms that when ingested, can survive the harsh conditions in the stomach and exert beneficial effects in the intestines, with the concentration of probiotics required to obtain a clinical effect often being quoted as ≥ 10^6 colony forming units in the small intestine and ≥ 10^8 in the colon (Minelli and Benini, 2008). However, recently the beneficial effects of microorganisms in the oral cavity has also attracted attention (Deogade, 2015). Probiotics induce health benefits by multiple mechanisms including inhibiting the growth of pathogenic microorganisms and reducing the production of inflammatory factors by pathogens (Deogade, 2015). Metabolites produced by probiotics
contribute to the suppression of pathogens as well as health benefits not associated with pathogen inhibition. For example, short chain fatty acids such as propionic acid and butyric acid have been found to promote apoptosis of colon cancer cells, and to reduce the risk for obesity, insulin resistance, diabetes, hypercholesterolemia, and cardiovascular dysfunctions (Sivaprakasam et al. 2016; Koh et al., 2016; Canfora et al., 2015; Alvaro et al., 2008; Anderson et al., 1999). Such beneficial metabolites are called postbiotics (Cicenia et al., 2014; Klemashevich et al., 2014).

Legumes including cowpeas have a high potential as nutraceuticals (Trinidad, 2010). Cowpea has been suggested to reduce the risk for gastrointestinal diseases, cardiovascular diseases, diabetes and some types of cancers, and this may be due in part to bioactive peptides and phenolic substances (Jayathilake et al., 2018; Awika et al., 2017). However, cowpea consumption is associated with flatulence and other abdominal discomforts in some individuals (Ndubuaku et al., 1989). The raffinose family oligosaccharides including raffinose, stachyose and verbascose are considered to contribute to flatulence (Granitto et al., 2001). On the other hand, these oligosaccharides are also considered to be beneficial as prebiotics which promote the growth of probiotics (Zartl et al., 2018). Legume milks as alternatives to bovine milk constitute a fast-growing segment of functional foods development, fueled by concerns about cow milk allergy, lactose intolerance, hypercholesterolemia, and high calorie diets as well as the presence of health promoting phytochemicals in the legumes (Sethi et al., 2016). Although not as well balanced in amino acids as cow’s milk, they may provide a cheap alternative to the less economically empowered, especially where milk supply is insufficient (Sethi et al., 2016).

Fermentation of legumes is an effective way of reducing alpha-galactosidic compounds (Granito et al, 2003). The consumption of fermented beans was found to significantly decrease the flatulence problem by 56% in women aged 25 to 40 years (Granito et al., 2005). Fermentation also reduces antinutritional factors such as tannins, phytates, alkaloids, hydrogen cyanide, lectins, and oxalates in legumes (Difo et al., 2014). Thus, fermentation of cowpea milk with probiotic lactic acid bacteria is expected to produce a functional food with reduced antinutritional factors, and enhanced health-promoting effects due to probiotics and postbiotics. Here we fermented cowpea milk with three mixed lactic acid bacteria starter cultures and determined the effect of fermentation on the raffinose family oligosaccharides and the production of postbiotic short chain fatty acids. All the cultures contained Streptococcus thermophilus, and, in addition, one of the cultures contained Lactobacillus delbrueckii subs bulgaricus, the second contained Lactobacillus rhamnosus GR-1 strain, and the third contained Lactobacillus acidophilus and Bifidobacterium sp. All these bacteria have previous claims of probiotic effects (Deogade, 2015; Reid, 2017).

MATERIALS AND METHODS

Cowpeas (white black-eyed variety) were purchased from a local market in Nairobi, Kenya. Three commercial bacterial starter cultures were used for the fermentation of cowpea milk. The cultures containing (i) Lactobacillus acidophilus, Bifidobacterium, Lactobacillus delbrueckii subs. bulgaricus and Streptococcus thermophilus (ABT) and (ii) Lactobacillus delbrueckii subs bulgaricus and Streptococcus thermophilus (DT) were purchased from Prolab, an agent of Chris Hansen, in Nairobi. The culture consisting of Lactobacillus rhamnosus GR-1 strain and S. thermophilus (GT) was obtained from Yoba Foundation. GT, which was in the form of a stable powder was kept at 4 □C, while the other two cultures were kept frozen until use, as per manufacturer’s instructions.

All chemicals and solvents used in this study were of analytical and chromatographic grade. Oligosaccharide (Stachyose, raffinose and verbascose) standards were bought from Sigma-Aldrich, United Kingdom. Butyric, valeric, and isovaleric acid standards were from Sigma-Aldrich, Germany. Propionic acid standard was bought from Fluka Analytical, Germany.
Preparation of cowpea milk
Cowpeas (300g) were soaked for 12 hours in 3 liters of distilled water at room temperature (15-25 °C). After discarding the steeping water, the cowpeas were rinsed with tap water and dehulled manually. Boiling water was used for blending (Von Hotpoint Blender, HB241CW) the cowpeas in the ratio of 1:10 (w/v) for 3 minutes at high speed. The resulting milk was heated to 70 °C and the temperature held for 20 minutes with frequent stirring to prevent the contents from sticking on the equipment. The milk was cooled to an incubation temperature of 45°C and then divided into four portions.

Fermentation of cowpea milk
Three of the four portions of cowpea milk were inoculated with the starter cultures according to the manufacturer’s instructions. No starter culture was added to the control. All the portions were then incubated in an electric incubator at 45°C for 14 hours.

Determination of pH
The pH of the incubated milk was determined after every 2 hours with a digital pH meter (HANNA, H18519N) after calibration.

Determination of oligosaccharides
Oligosaccharides were extracted from cowpea by the method of Borejszo & Khan (1992). Twenty milliliters of ethanol were added to 5 g of sample and heated for 1 hour in a reflux apparatus (model SF-6, Sanshin Industrial Co. Ltd). The samples were then concentrated in vacuo and filtered through 0.45µm pore-size membrane syringe filter. Two microliters of the filtrate were injected into High Performance Liquid Chromatography (HPLC) machine model LC-20 AD/T (Shimadzu Corporation, Kyoto Japan) equipped with a degassing unit (DGU- DGU-20A5R) and an autosampler (SIL-20AHT), coupled with refractive index detector model RID-10A and diode array detector (SPD-M20A). Column used was Hypersil GOLD Amino column (4.6 ×160 mm). The mobile phase consisted of 65% acetonitrile and 35% distilled water and was maintained at a flow rate of 1 mL/min isocratically. Identification of the peaks for raffinose, stachyose and verbascose was based on same retention time as the standards, and quantification was based on the use of standard curves obtained under the same HPLC conditions.

Determination of short chain fatty acids
Extraction of organic acids from cowpea milk samples was carried out according to the procedure by (Shukla et al., 2010). Ten grams of each sample was centrifuged at 10,000 rpm for 30 minutes and then filtered with Whatman filter paper No. 1. Subsequently, 1.2 µl of 2% H₂SO₄ was added to 2.1 ml of the filtrate and filtered through 0.45µm pore-size membrane syringe filter. After this, 2µl of the filtrate was injected into a Gas Chromatography (GC) machine (Model GC-14B, Shimadzu Corporation, Japan) fitted with a flame ionization detector (FID) and Shimadzu C-R6A Chromatopac and 10% SUPELCOWAX capillary column (30m × 0.53mm × 0.5µm). Carrier gas was nitrogen at a flow rate of 150 cm³ per minute. The column temperature was programmed from 150°C (1 min), to 230°C (2 min) at 5°C/min. Injector and detector temperatures were 220°C and 240°C respectively. Short chain fatty acid (propionate, butyrate, valerate, isovalerate) peaks were identified by comparison with retention times of the respective standards, and standard curves were used for quantification.
RESULTS AND DISCUSSION

Change in pH over time during cowpea milk fermentation

During incubation of cowpea milk (control) or cowpea milk inoculated with the different starter cultures, pH was monitored over a 14-hour period as an indicator of the progress of fermentation. As illustrated in Figure 1, the control sample exhibited some, albeit minimal decrease in pH, consistent with a small extent of natural fermentation. On the other hand, samples fermented with the three starter cultures underwent a rapid pH decline from about pH 7 to pH 5 within the first 6 hours, followed by a slower decrease to pH 4.5 (ABT and GT) or 4.8 (DT) between the 6th and 14th hours.

Table 1 shows the content of the oligosaccharides stachyose, raffinose and verbascose in raw cowpea flour, unfermented cowpea milk and cowpea milk fermented with the different starter cultures. Stachyose was detected in all samples, raffinose was detected in all but the ABT and GT-fermented samples, while verbascose was not detected in any of the samples. In all cases, stachyose content was higher than raffinose.

![Acidification curve](image-url)

**Figure 1.** Changes in pH over time during fermentation of cowpea milk

**Effect of fermentation with the different starter cultures on cowpea oligosaccharide content**

The changes in pH over time are illustrated in Figure 1. The control sample showed a minimal decrease in pH, while the samples fermented with the different starter cultures underwent a rapid pH decline from about pH 7 to pH 5 within the first 6 hours, followed by a slower decrease to pH 4.5 (ABT and GT) or 4.8 (DT) between the 6th and 14th hours.
Table 1: Oligosaccharide concentrations in raw cowpea (RC), unfermented cowpea milk (UM) and cowpea milk fermented with different starter cultures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oligosaccharide concentrations (mg/g)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stachyose</td>
<td>Raffinose</td>
<td>Verbascose</td>
<td>Raffinose + Stachyose</td>
</tr>
<tr>
<td>RC</td>
<td>13.9±0.2c</td>
<td>2.2±0.1f</td>
<td>ND</td>
<td>16.1</td>
</tr>
<tr>
<td>UM</td>
<td>8.8±0.1a</td>
<td>0.6±0.0e</td>
<td>ND</td>
<td>9.4</td>
</tr>
<tr>
<td>ABT</td>
<td>3.6±0.0b</td>
<td>0.0±0.0</td>
<td>ND</td>
<td>3.6</td>
</tr>
<tr>
<td>GT</td>
<td>7.0±0.1ab</td>
<td>ND</td>
<td>ND</td>
<td>7.0</td>
</tr>
<tr>
<td>DT</td>
<td>2.6±0.0bd</td>
<td>0.2±0.0e</td>
<td>ND</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Values are mean±standard deviations. Values with different letter superscript in the same column are significantly different at (p<0.05) based on Bonferroni tests. ND = Not Detected

Sreerama et al (2012) found higher contents of the three oligosaccharides than presented in Table 1 for raw cowpea, but a similar trend of stachyose (17.8 mg/g) > raffinose (10.3 mg/g) > verbascose (3.6 mg/g). Unfermented cowpea milk (UM) had lower content of stachyose and raffinose than the raw cowpea (RC) (Table 1). Since the cowpeas were soaked for 12 hours and dehulled prior to milk extraction, the lower oligosaccharide content in the UM is attributable to their leaching into the soaking water and removal with the hulls. Such oligosaccharide-reducing effect of legume soaking and dehulling has been previously reported (Akinyele et al, 1991; Onyenekwe et al, 2000). Fermentation of cowpea milk further reduced the oligosaccharide contents, and the extent of reduction of each oligosaccharide depended on the culture used (Table 1). The GT culture depleted raffinose but only minimally reduced the stachyose content from 8.8 in unfermented cowpea milk to 7 mg/g (20% reduction). On the other hand, DT did not deplete raffinose, but reduced stachyose by 70% reduction. ABT depleted raffinose and reduced stachyose by 59%. In terms of combined stachyose and raffinose reduction, DT was the most effective (70%), followed by ABT (62%) and GT (26%). Thus, DT and ABT might be more effective in reducing oligosaccharide-dependent flatulence than GT. Nevertheless, because soaking and dehulling also contribute to oligosaccharide reduction, cowpea milk fermented with GT has less than half the oligosaccharides in raw cowpea (Table 1). Liu et al (2006) found that L. rhamnosus strains 6013, 6013+DH₁ and 6013 + GH₄ completely metabolized raffinose in soybean within six hours and strain-dependently reduced the stachyose content by between 50 and 72%. Streptococcus thermophilus was found to be more efficient than Lactobacillus acidophilus in the metabolism of these oligosaccharides and combining either of these with Bifidobacterium increased the oligosaccharide reduction (Wang et al, 2003). Thus, the different combinations of bacteria in the starter cultures used in this study should have contributed to differences in the extent of oligosaccharide reductions.
Production of post-biotic short chain fatty acids during cowpea fermentation with different starter cultures

Table 2 shows the effect of cowpea milk fermentation with different starter cultures on the formation of postbiotic short chain fatty acids. Butyric acid, valeric acid and isovaleric acid were not detected in the unfermented, control sample (UM). However, this sample was found to contain a small quantity of propionic acid (50ppm). This can be attributed to natural fermentation, and corresponds to the slight decline in pH of control sample during the 14-hour incubation (Figure 1). The ABT culture produced small quantities of all the short chain fatty acids except isovaleric acid, with valeric acid being the least produced (20 ppm) and propionic acid the most produced (510 ppm). GT culture produced small quantities of all the fatty acids, ranging from 10ppm of valeric acid to 220 ppm of propionic acid. DT culture also produced all the fatty acids, and remarkably high levels of propionic acid (2430 ppm), which was significantly different from the concentrations produced by the other cultures. As mentioned above, propionic acid formation occurred in the control sample, which is attributable to natural fermentation, perhaps by propionic acid bacteria. Such bacteria might also have contributed to propionic acid formation in the treatment samples, since lactic acid bacteria such as *Streptococcus thermophilus* stimulate the growth of propionic acid bacteria, to different extents (Condon et al., 2001). Nevertheless, in other studies, lactic acid bacteria were found to produce a variety of acids including the short chain fatty acids investigated in the present study (Corsetti et al, 1998; Zalan et al, 2010).

Propionic acid is one of the generally recognized as safe (GRAS) food preservatives (Es et al, 2017). It not only inhibits spoilage fungi and bacteria but also pathogenic ones such as salmonella (Haque et al, 2009) and contributes to reducing the risk of various non-communicable diseases (Sivaprakasam et al. 2016; Koh et al., 2016; Canfora et al., 2015; Alvaro et al., 2008; Anderson et al., 1999). Thus, propionic acid may be an important contributor to potential health benefits of cowpea milk fermented with DT culture. Unlike propionic acid, the concentration of butyric acid produced by the three starter cultures was not significantly different. DT culture exhibited higher production of valeric acid than the other starter cultures. GT exhibited the highest production of isovaleric acid, although this was not significantly different from DT, the only other culture which produced this acid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Propionic acid</th>
<th>Butyric acid</th>
<th>Valeric acid</th>
<th>Isovaleric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM</td>
<td>50±6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ABT</td>
<td>510±8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40±1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20±1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>GT</td>
<td>220±17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40±1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10±1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70±1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DT</td>
<td>2430±19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60±1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40±1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean±standard deviations. Values with different letter superscript in the same column are significantly different at (p<0.05) based on Bonferroni tests. ND = Not detected
CONCLUSION AND RECOMMENDATION

Fermentation of cowpea milk with three different mixed starter cultures led to significant reduction in oligosaccharide content and the formation of postbiotic fatty acids. The extent of reduction of specific oligosaccharides or formation of specific short chain fatty acids depended on the starter cultures. Future studies should be undertaken to determine the potential health benefits of such fermented cowpea products.

Conflict of interest
The authors declare that there is no conflict of interest.

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Conjugated Linoleic Acid and Cancer in Humans—Is there a Role or not? A Review of the Scientific Evidence

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ABSTRACT
Conjugated linoleic acids are naturally occurring fatty acids that are found predominantly in ruminant meat, milk and dairy products. They are composed mainly of two isomers: cis-9, trans-11 and trans-10, cis-12 fatty acid. Their synthesis occurs mainly by the action of ruminal bacteria, *Butyrivibrio Fibrisolvens*, and a host of lactic acid bacteria, which isomerize linoleic acid to CLA or by synthesis via α9-desaturase of 11-trans octadecanoic acid, and, through desaturation of free linoleic acid or other unsaturated fatty acids. Although cis-9, trans-11 and trans-9, trans-11 CLA isomers have consistently shown anti-carcinogenicity on animal models and on cancerous human cells, results from clinical trials are inconclusive and conflicting. Despite most of the data on humans being mainly from epidemiological studies, a few clinical studies with breast and colorectal cancer sufferers have shown some promise. Controlled, long-term, racial and gender diverse, geographically spread clinical studies are required to understand the link between CLA intake and incidence of human cancers.

KEY WORDS: conjugated linoleic acid, cancer, link, humans

INTRODUCTION
Conjugated linoleic acids (CLAs) are a family of about 28 positional and geometric isomers of linoleic acid (LA) found mainly in meat, milk and dairy products derived from ruminants (Shokryazdan et al., 2015; Fuke & Nornberg, 2016). CLAs can be either cis, cis or cis, trans or trans, trans-fatty acids, whose double bonds are conjugated and therefore separated by a single bond between them, instead of methylene interruption. They are composed of two main isomers: c9, t11 (cis-9, trans-11 fatty acid), which comprises about 80-90 % of total conjugated linoleic acid (CLA) and t10, c12 (trans-10, cis-12 fatty acid), which comprises about 3-5 % of total CLA of foods that are good sources of the fatty acid (Wang & Lee, 2013). There are also a few other types, but they have rarely been studied (Subbaiah et al., 2010). The discovery of CLA and its health benefits came when Pariza et al. (1979) reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis. The finding of mutagens in grilled beef was confirmatory, but evidence of a mutagenesis inhibitor was a novel discovery that had not been previously reported. Subsequently, Pariza and Hargraves (1985) established that the speculation that the mutagenic activity would inhibit carcinogenesis was indeed the case and Ha et al. (1987) identified the new anti-carcinogen as conjugated linoleic acid. Recognizing CLA’s anti-cancer effects, the National Academy of Science’s publication entitled "Carcinogens and Anti-carcinogens in the Human Diet" stated that "conjugated LA is the only fatty acid that has been shown unequivocally to inhibit carcinogenesis in experimental animals (National Research Council, 1996)." It went on to state that “much of the research to date has been with laboratory animal models, but CLA can reduce new tumor growth and destroy existing tumor cells. CLA has killed existing cancer cells in colon, ovarian and prostate carcinoma, leukemia, melanoma, and breast tumors”. Also, CLA-enriched butter inhibited rat mammary tumor yield by 53%, clearly showing the cis-9, trans-11 isomer was anti-carcinogenic”. Thus
the centrality of CLA in cancer research from then on was established. LA, an omega-6 polyunsaturated, essential fatty acid, provides support, flexibility and integrity to cell membranes (Subbaiah et al., 2010). It is a naturally occurring trans saturated fatty acid that is found mainly in animal products—meat, milk and their derivative products from grass-fed animals (Nuernberg et al., 2005; Churruca, et al., 2009). When animals on pasture eat the omega-3 lipids in grass and other green plants, their gut flora convert the polyunsaturated fatty acids (PUFA) including LA into CLA. In milk, there’s also an additional source—an enzyme in the cow’s mammary gland that converts the fatty acid, vaccenic acid, into CLA (Banni et al., 2011). Although CLA is produced naturally and mainly in the digestive tract of ruminants such as cattle, goats, sheep, buffalo, it is also produced to a lesser degree in pigs, chickens and turkey (s, and the synthesis occurs mainly due to fermentative bacteria, Butyrivibrio Fibrisolvens, which isomerize LA to CLA or by synthesis via α9-desaturase of 11-trans octadecanoic acid by desaturation (Kishino et al., 2002). Other bacteria notably Lactobacillus strains (Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbruechii, Lactobacillus paracasei, Lactobacillus pentosus, Lactobacillus plantarum, and Lactobacillus reuteri), Streptococcus salivarius, Bifidobacterium breve, Bifidobacterium dentium, and rumen bacteria (e.g., Butyrivibrio fibrisolvens) have been shown to have the ability to produce CLA from LA or other unsaturated, free fatty acids (Ogawa et al., 1998; Ogawa et al., 2001; Kishino et al., 2005; Macouzet et al., 2009).

Diet may influence CLA accumulation in the animal body and endogenous CLA production (Dhiman et al., 2005). Although it may be possible to increase CLA concentrations in animal tissues by increasing dietary CLA and other PUFAs in the animal diet, long-term CLA-supplemented feeding, may have an unfavourable cost-to-benefit ratio. As many people eat meat from animals raised in factory farms, our diet is much lower in CLA as has been shown in laboratory analyses of diets in some parts of the World (Ip et al., 1994; Freitsche & Steinhart, 1998; Aro et al., 2000; Rizenthaler et al., 2001), and yet any modern animal feeding practices would benefit human health if they raised the level of CLA in animal food products. In the USA, the average intake was 151mg/day for women and 212 for men (Rizenthaler et al., 2001). The intake was 0.35 mg/day in women and 0.43 mg/day in men in Germany (Freitsche & Steinhart, 1998), 97.5 mg/day in the UK (Mushtaq et al., 2010), and 310 and 90 mg/day in adults with high and low dairy products consumption in Finland, respectively (Salminen, 1998). However, Aro (2000), estimated the intake in adult women to be higher at 132 mg/day in Finland. Generally, these levels are much lower than the current level of 3-3.5 g/day that seems to confer health benefits in experimental animal models. It has been established in clinical trials that the dose of 3 g/day is relatively safe for humans such that the FDA of the US has conferred GRAS status on it (Benjamin et al., 2015). A dose of ≥ 6 g/day, has exhibited undesirable side effects including deposition of fat in the liver (van Wiljen, 2011), increased insulin resistance (Riserus et al., 2002), lowered HDL cholesterol and diarrhoea (Jadszus et al., 2010; van Wiljen, 2011).

CLA has been shown to be anti-carcinogenic (Banni et al., 2011), anti-obesity (Chin et al., 1994), and anti-diabetic (Baumann, 1996), thus implying potential effectiveness in preventing lifestyle diseases. Also, reports suggest that physiological effects of CLA are different between the isomers, for example the 10t,12c isomer promoted mammary tumour growth and therefore raised the risk of breast cancer in overweight individuals with type 2 diabetes (Baumann, 1996), whereas the 9c,11t isomer was shown to be anti-carcinogenic (Banni et al., 1999; Beppu et al., 2007).

In this review, the anti-carcinogenicity of CLA and the possible mechanisms of action are discussed. However, most of the works discussed are on the rat, mice and hamsters. Although human clinical trials are increasingly being conducted, the few trials continue to yield conflicting and inconclusive results (Aro et al., 2000; Chajes et al., 2003; McCann et al., 2004; Chajes et al., 2009). This may in part be due to the small number of experiments, the difficulty experienced in obtaining accurate estimates of dietary CLA intake, studies being carried out in small populations and even if large, for short periods of time; small populations may lack the diversity in food habits (Benjamin et al., 2015). The chemical nature of the pure isomers that are often used in research may not also be in the form and proportions in which they exist in natural foods, where other biological agents may modulate their environment and actions.
CLA: SYNTHESIS, SOURCES AND EFFECTS OF PROCESSING

The fat in beef contains about 1.7 to 10.8 mg CLA/g of fat depending on the nature of the animal’s diet, with 9-cis and 11-trans isomer predominating (Dhiman et al., 2005; Campbell et al., 2008). CLA is transferred naturally to meat, milk and dairy products through the lipids in food (Oliveira et al., 2008). In an experiment, Ogawa et al. (2005) established that due to the diversity of strains of lactic acid bacteria, the CLA produced comprised a mixture of cis-9, trans-11-octadecadienoic acid (18:2) and trans-9, trans-11-18:2. Lactobacillus plantarum AKU 1009a was a potentially good CLA producer, with the CLA production from LA reaching 40 mg/ml under the optimized conditions. The CLA-producing reaction was found to consist of two successive reactions-hydration of LA to 10-hydroxy-12-octadecenoic acid and dehydrating isomerization of the hydroxy fatty acid to CLA. Based on these results, Lactic acid bacteria were found to transform ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid) to CLA (a mixture mainly of cis-9, trans-11-18:2 and trans-9, trans-11-18:2). Castor oil, which is rich in the triacylglycerol form of ricinoleic acid, was found to act as a substrate for CLA production by lactic acid bacteria with the aid of lipase-catalyzed triacylglycerol hydrolysis. L. plantarum AKU 1009a produced conjugated trienoic fatty acids from alpha- and gamma-linolenic acid. The trienoic fatty acids produced from alaphalolinolenic acid were identified as cis-9, trans-11, cis-15-octadecatrienoic acid (18:3) and trans-9, trans-11, cis-15-18:3, while those produced from gammalinolenic were cis-6, cis-9, trans-11-18:3 and cis-6, trans-9, trans-11-18:3.

CLA can be obtained by means of the enzyme a9-desaturase which promotes the desaturation of the 11-trans octadecanoic acid (Grinari et al., 1997). Several different isomers of CLA including 11-trans and 9-cis are the best known because they are found in animal foods (Churruca et al., 2009), but a few others such as trans-9, trans-11 isomer are found predominantly in vegetable oils (Ecker et al., 2010). This later isomer has also been shown to have anti-cancer properties on human colon cancer cells (Beppu et al., 2007). It has also been shown that it is possible to obtain CLA in an industrial form, through the partial hydrogenation of linoleic acid or by thermal treatments, aiming to produce a compound with maximum biological activity and with a defined chemical composition (Blankson et al., 2000). However, the chemical forms which are often used as supplements may not have the proportion of isomers and the effectiveness of the natural CLA as found in foods (Chin et al., 1992; Bissonauth et al., 2006). In healthy humans, CLA and the related conjugated linolenic acid (CLNA) isomers are bioconverted from LA and alpha-linolenic acid, respectively, mainly by Bifidobacterium strains inhabiting the gastrointestinal tract (Nieuwenhove et al., 2007). However, this bioconversion may not occur at any significant level in those suffering from a digestive disease, gluten sensitivity, and/or dysbiosis, due to the reduced gut microbial populations (de Vrese & Schrezenmeir, 2008).

Although food products from ruminants are the richest source of CLA (Fuke & Nornberg, 2016), it is possible to enhance the CLA content of foods from non-ruminants by supplementing CLA with CLA-rich sources in their diets (Aydin, 2005; Bourre, 2005), but the effect ceases on removal of the supplementary feed source, thus underlining the importance of maintaining the natural sources of the fatty acid for optimal human nutrition. Chin et al. (1994) investigated the ability of non-ruminants (rats) to produce CLA. They supplemented the diet with 5% free linoleic acid or 8.6% corn oil (equivalent to 5% free LA as triglyceride) and observed higher tissue CLA concentrations in rats fed free LA than in control animals. These investigators concluded that the intestinal bacterial flora of rats can convert free LA, but not LA esterified in triglycerides, to cis, trans, C18:2 n-9, n-11 and trans, cis C18:2 n-9, n-11. The CLA content in milk, meat, or egg varies greatly from a low of 0.1% or less to a high of 2% or more of the milk, tissue, or egg yolk lipids, with milk lipids from ruminants having the highest concentrations. The CLA content in meat from ruminants is higher than in the meat of non-ruminants (e.g., 1.20% in lamb and 0.12% in pork). In the case of non-ruminants, CLA may originate from dietary sources such as powdered meat and tallow (Freitsche & Steinhart, 1998). A host of factors appear to affect the CLA content in milk, meat, and other food products from various species of animals, which could be broadly classified into diet, animal, and post-harvest related factors (Khanal & Olson, 2004).
Of all these factors, animal diet is the primary one and could be manipulated for enhancing the concentration of CLA in food products, both from ruminants and non-ruminants (Aydin, 2005). While animal-to-animal variation is also of great significance, post-harvest related factors appear to be of minor importance (Khanal & Olson, 2004).

Variability of CLA in Food Sources and Effect of Processing

Food products from grass-fed ruminants are good sources of CLA and contain much more of it than those from grain-fed animals (Nuernberg et al., 2005), though consumers can obtain the same nutritional benefits from consuming high fat, grain-fed food portions of the same products (Daley et al., 2010). It has been demonstrated that meat and dairy products from grass-fed animals can produce more CLA than those of cattle fed different supplementary diet ratios (Dhiman et al., 1999). Eggs from chicken that have been fed CLA and CLA-rich feeds are also rich in CLA (Suksombat et al., 2006). Interestingly, CLA in eggs has been shown to survive the temperatures encountered during frying, but in the presence of antioxidants (Ren et al., 2013), implying that the major route of destruction is likely to be oxidation. The CLA in milk and cheese heated by conventional processing methods is generally stable and may even be enhanced (Herzallah et al., 2005), while that in hard cheese is reduced proportionate with the severity of heating (Herzallah et al., 2006). Some mushrooms, such as the Agaricus bisporus and Agaricus subrufescens, are rare non-animal sources of CLA (Chen et al., 2006).

The average content of CLA found in milk samples from the Azores in Portugal varied from $1.45 \pm 0.21$ mg/g fat in raw milk, $1.44 \pm 0.06$ mg/g fat in thermized milk, and $1.40 \pm 0.11$ mg/g fat in milk samples when pasteurized. The CLA varied between $9.6 \pm 0.5$ mg/g, in pasteurized cheeses and $10.8 \pm 4.2$ mg/g in raw-milk cheeses (Kongo et al., 2014). These values agree with those reported by Pestana et al. (2009) and Regula et al. (2005) who reported that pasteurization causes different changes in the free fatty acid profiles of ewes’ milk, which in general has a higher content of CLA than cows’ milk. However, Shantha et al. (1992) and Garcia-Lopez et al. (1994) reported that the application of heat enhanced the formation of linoleic acid radicals and increased CLA content during the production of natural and processed cheeses, though this may partly be contributed by the concentration of the solids content including lipid fractions as % of serum (by dehydration through pressing and ripening under low relative humidity conditions). Although processing factors such as heating and ripening affect the CLA contents of a dairy product, the major source of its variation among products is the intrinsic amount of CLA present in the raw milk (Kongo et al., 2014), which varies with the livestock species (Pestana et al., 2009; Regula et al., 2005), as well as the nature of feed (Nuernberg et al., 2005). In a study in Argentina, Nieuwenhove et al. (2008) found that CLA averaged 0.85 and 0.96 in milk and 0.76 and 1.04 g/100 g of fatty acids in cheese of cow and goat, respectively, which seemed lower than values reported elsewhere (Ponnampalam et al., 2006). Cis-9, trans-11 was the major isomer present in both animal species in the Argentine study. In bovine raw milk, the CLA values vary from 0.2% to 3.7% of total milk fat, depending on animals’ diet (Dhiman et al., 1999), its physiological state and season (Kelsey et al., 2003), with cheeses generally have much higher values than other dairy products (Shantha et al., 1992). The manner of processing of the cheese also influences the amount (Boylston et al., 1999). Milk from ruminants fed predominantly on pasture is known to be richer in CLA. Ponnampalam et al. (2006) reported higher values of CLA in milk and meat products from Australia and New Zealand than the equivalent products from elsewhere (Nieuwenhove et al., 2008). This was attributed to the greater access to lush pasture, throughout the year by Australasian cattle. Similarly, in the Azores islands, where dairy cows are essentially pastured-fed all year round, Pestana et al. (2009), reported higher contents of CLA in milk as compared to similar milk and dairy products from mainland Portugal. Good fatty acid profiles were also established for cow and goat cheeses, from animals fed on natural pasture during spring and summer in Argentina (Nieuwenhove et al., 2009). The fat content of raw milk and species therefore largely determine the amount of CLA in derivative food products.
Cancer, an affliction that has over 100 different types, which are classified mainly by the type of body cell that is initially affected, is characterized by out-of-control cell growth (National Cancer Institute, 2018). Cancer harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumours (except in leukemia, where cancer prohibits normal blood functioning by abnormal cell division in the blood stream). Tumours can grow and interfere with the digestive, nervous, and circulatory systems, and often release hormones that alter body function (Grundker & Emons, 2017), especially when they metastasize and move round the body.

The WHO estimates that, worldwide, there were 14 million new cancer cases and 9.6 million cancer-related deaths in 2018 (WHO, 2018), with 30% of them from smoking, the probable cause of lung cancer, the leading cause of deaths from cancers worldwide. About 70% of global cancer-deaths occurred in low and middle income, and industrializing countries. The commonest cancer types globally are: anal, bladder, bone, breast, cervical, colon, colorectal, endometrial, kidney, leukemia, liver, lymphoma, ovarian, pancreatic, prostate, stomach, thyroid, oesophageal, vaginal and vulvar cancer, but not in the order of prevalence (WHO, 2018). Currently lung, stomach, liver, colon and breast cancer cause the most cancer-deaths each year globally (MoH, 2013). In the past, cancer has received low priority in healthcare services in Sub-Saharan Africa, the reason partly being the undoubtedly overwhelming burden of communicable diseases. In Kenya, cancer is the 3rd most prevalent cause of death (after infectious diseases and cardiovascular disease) and accounts for an estimated 22,000 deaths annually, out of a reported prevalence rate of 28,000 cases annually, though increasing prevalence is evident (MoH, 2013). The commonest types of cancers in Kenya are oesophageal, prostate and Kaposi in men, while breast, oesophageal and cervical cancer are common in women, in declining order of prevalence in those affected, reported to medical facilities and diagnosed appropriately (MoPHS & MoMS, 2013). Cancer is responsible for 7% of the total national mortality each year in Kenya (MoH, 2013).

Cancer results from internal and external risk factors working together and/or in sequence to trigger the affliction. People may be exposed to risk factors or cancer-causing agents in their environment and/or from their lifestyles.

Cancer Risk Factors

Cancer is ultimately the result of cells that uncontrollably grow and do not die, unlike normal cells in the body which follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form, leading to a mass of abnormal cells that grows out of control.

Cells can experience uncontrolled growth if there are mutations to DNA, and therefore, alterations to the genes involved in cell division. Four key types of genes are responsible for the cell division process: oncogenes tell cells when to divide, tumour suppressor genes tell cells when not to divide, suicide genes control apoptosis and instruct the cell to kill itself if something goes wrong, and DNA-repair genes instruct a cell to repair damaged DNA. Cancer occurs when a cell's gene mutations make the cell unable to correct DNA damage and unable to commit suicide. Also, cancer is a result of mutations often due to carcinogens that inhibit oncogene and tumour suppressor gene function, leading to uncontrollable cell growth.

Carcinogens are directly responsible for damaging DNA, promoting or aiding cancer. Tobacco, asbestos, arsenic, radiation such as gamma and x-rays, exposure to excessive ultraviolet (UV) radiation from the sun, and a host of lab chemicals are all examples of carcinogens (American Cancer Society, 2019). When our bodies are exposed to carcinogens, free radicals affect the body’s ability to function normally.

Cancer seems also to be the result of a genetic predisposition that is inherited from family members (American Cancer Society-ACS, 2019). It is possible to be born with certain genetic mutations or a fault in a gene that makes one statistically more likely to
develop cancer later in life.
As we age, there is an increase in the number of possible cancer-causing mutations in our DNA (WHO, 2018). This potential makes age an important risk factor for cancer. Several viruses have also been linked to cancer including: human papillomavirus (a cause of cervical cancer), hepatitis B and C (causes of liver cancer), and Epstein-Barr virus (a cause of some childhood cancers) (ACS, 2019). Human immunodeficiency virus (HIV)-and anything else that suppresses or weakens the immune system, inhibits the body's ability to fight infections and increases the chance of developing cancer (ACS, 2019), as it is for many other ailments. Other risk factors that predispose one to cancer include: inactivity, race (related to DNA and heredity), pollution (chemical carcinogens), inappropriate diet, chemicals from other sources other than pollution.
The WHO's International Agency for Research on Cancer (IARC) has now placed dirty air in the same category of carcinogens such as tobacco smoke, ultraviolet radiation and plutonium (WHO, 2018). IARC estimates that about 223,000 lung cancer deaths globally, can be blamed on exposure to air pollution (WHO, 2018).
However, experts estimate that at least a third of adult cancer cases are linked to lifestyle (National Cancer Institute, 2018), which is within one’s control. It would therefore follow that with every healthy choice one makes, and every unhealthy habit one drops, cancer risk drops. Eight factors that are regarded as the healthiest habits that one can develop to help prevent cancer include: staying tobacco-free, maintaining an appropriate body mass index, increasing the proportion of plant foods in diets, cessation of alcohol abuse, planned and frequent screening, daily physical activity, shaking off stress, and heredity. In relation to food, certain bioactive compounds including CLA, have been linked mainly in causal studies on animal models to the reduction or at the best, prevention of cancer initiation.

POTENTIAL ROLE OF FOODS IN CANCER PREVENTION

Although several cancers are influenced by lifestyle, delaying or preventing disease onset seems to be considerably influenced by diet (Donaldson, 2004), and more so by diets in which fruits, vegetable foods (Freudenheim et al., 1996) and CLA (Ip et al., 1994) are significant components. However, other studies have not established any positive or strong inverse correlation between fruit, dietary fibre, vegetable intake or the specific vitamins (A, C, E) and/or supplements or food and supplements considered together and the risk of cancer initiation or prevention (Kushi et al., 1996; Fairfield et al., 2001; Botterweck et al., 2000) and yet the encouraging results of the anticancer effects of CLA in animal models (Ip et al., 1994; Ip et al., 1999; Banni et al., 2001), have over the last few years stimulated further investigations through human clinical trials.
Research has generally shown that fruits and vegetables contain a variety of bioactive compounds (Singh et al., 2016). These include insoluble fibre, a variety of polyphenols, organic acids, etc. with the level of antioxidant activity (measured by ABTS- 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and DPPH-2,2-diphenyl-1-picyrylhydrazyl) being indicative of bioactivity. Gallic acid, protocatechuic acid, catechins, caffeic acid, ferulic acid, sinapic acid, quercetin, resveratrol and kaempferol are detectable in different fruits and vegetables, with fruit peels also having high antioxidant activity and therefore promising as valuable sources of minerals and polyphenols (Singh et al., 2016). In a UK study, Proteggente et al. (2002), showed that fruits and vegetables that appeared to be rich in anthocyanins (e.g. strawberry, raspberry and red plum) demonstrated the highest antioxidant activities, followed by those rich in flavonones (e.g. orange and grapefruit) and flavonols (e.g. onion, leek, spinach and green cabbage), while the hydroxycinnamate-rich fruit (e.g. apple, tomato, pear and peach) consistently elicited the lower antioxidant activities. The antioxidant capacities of aqueous/methanolic extracts were comparatively assessed using the TEAC (Trolox Equivalent Antioxidant Capacity), the FRAP (Ferric Reducing Ability of Plasma) and ORAC (Oxygen Radical Absorbance Capacity) assays,
which comprise contributions from polyphenols, simple phenols and the ascorbate component. The TEAC, FRAP and ORAC values for each extract were shown to be relatively similar and well-correlated with the total phenolic and vitamin C contents (Proteggente al., 2002). The same study established that the antioxidant activities (expressed as TEAC) in terms of 100 g fresh weight uncooked portion size were in the order: strawberry > raspberry = red plum >> red cabbage >> grapefruit = orange > spinach > broccoli > green grape approximately/= onion > green cabbage > pea > apple > cauliflower tomato approximately/= peach=leek > banana approximately/= lettuce. In an earlier study, Paganga et al. (1999) showed that the major phenolic antioxidant components of eggplant were chlorogenic acid in the flesh and a delphinidin conjugate in the skin. In the case of apple, the major phenolic antioxidants detected were chlorogenic acid, procyanidins/catechin compounds, rutin and phloridzin, while quercetin glycosides were the major phenolic components of onion. The highly significant correlation between consumption of fats and oils and death rates from leukemia and malignant neoplasia of the breast, ovaries, and rectum among persons over 55 years of age seemed to reflect greater lipid peroxidation (Lea, 1966). Studies on atherosclerosis reveal the probability that the disease may be due to free radical reactions involving diet-derived lipids in the arterial wall and serum to yield peroxides and other substances. These compounds induce endothelial cell injury and produce changes in the arterial walls (Harman, 1992). Brown and Rice-Evans (1998) found that luteolin-rich artichoke extract protected low-density lipoprotein from oxidation in vitro. In two reviews, Devasagayam et al. (2004) and Dillard and German (2000) discuss the beneficial effects of antioxidants and nutraceuticals in disease prevention. Weisburger (1999), concluded that most chronic diseases, including coronary heart disease and many types of cancer depend on the in vivo conversion of cellular macromolecules or of carcinogens to specific reactive, oxidized forms. Therefore, health promoting nutrition recommends the daily intake of five to ten servings of vegetables and fruits, fruit juices, red wine and tea which are rich sources of micronutrients with antioxidant properties, including the antioxidant vitamins C, E and beta-carotene. While tomatoes contain lycopene, a stable, active antioxidant, many vegetables contain quercetin and related polyphenolic compounds. Green tea is a source of epigallocatechin gallate, while black tea is associated with theaflavin and the associated thearubigins (Lin et al., 1998). Red wine contains resveratrol (Das et al., 1999), while ruminant meats, milk and dairy products contain CLA (Boylston et al., 1995). The diverse antioxidants in foods, red wine and tea provide the necessary antioxidant resources for the body to control oxidation reactions in the body, which if not controlled would result in possible adverse health consequences. For example, the oxidation of LDL cholesterol yields a product that damages the vascular system (Harman, 1992). Cancers of the stomach seem to be caused by the consumption of salted, pickled foods yielding direct-acting carcinogens, whose formation is inhibited by the antioxidant vitamins C and E (Glatthaar et al., 1986; Rock et al., 1996). Cancer in the colon, breast, prostate and pancreas may be caused by a new class of carcinogens, the heterocyclic amines (Grieswold et al., 1968; Butler et al., 2003), formed during the broiling or frying of creatinine-containing foods, including fish and meats. Their formation and action was shown to be inhibited by antioxidants in soy, tea, vitamin C and by the synthetic antioxidants BHA and BHT (Chung, 1999; Rock et al., 1996). The growth, proliferation and development of abnormal preneoplastic and neoplastic cells also involves oxidation reactions, including the formation of active oxygen or peroxo compounds (Lea, 1966). Such reactions were found to be inhibited by antioxidants in tea, tomatoes or vegetables (Weisburger, 1999). CLA has been shown to prevent cancer in animal models and cancerous human cell lines (Ip et al., 1991, 1994, 1999; Banni et al., 2001).
Very few clinical studies have been conducted to relate CLA consumption with the incidence of different cancer types, as the data available is mainly from epidemiological studies. However, many researchers have recently focused on human breast and colorectal cancer; in an elaborate follow-up study using Cox proportional hazards models, Larsson et al. (2009) showed that the dietary intake of CLA showed no evidence for a protective role against breast cancer development in women. Also, Chajes et al. (2002) conducted a case-control study among 297 women treated for breast cancer or benign breast disease to evaluate the hypothesis that CLA protects against breast cancer. They could not show a link for the negative association between adipose tissue CLA (predominantly 9-CLA) and the risk of breast cancer. However, high-fat dairy food and CLA intake were examined in 60,708 women of age 40 to 76 (the Swedish Mammography Cohort Study) with 14.8-year follow-up. It was found that women who consumed four or more servings of high-fat dairy foods daily (including whole milk, full-fat cultured milk, cheese, cream, sour cream and butter) showed half the risk of developing colorectal cancer, compared to women who consumed less than two servings per day (Larsson et al., 2005). The study established that the consumption of CLA was associated with an almost 30 percent reduction in the risk of colorectal cancer. In another study (the Western New York Exposures and Breast Cancer Study, WEB), McCann et al. (2004) demonstrated that although there was no clear protective effect of 9-CLA in premenopausal or postmenopausal women with a higher intake, i.e., the number of oestrogen receptor (ER)-negative cells to ER-positive cells, the ratios decreased in the premenopausal women in the higher quartile. The authors concluded that, although CLA intake was not related to overall breast cancer risk, there may be associations with tumor biology at least among premenopausal women. But another epidemiological study (the Netherlands Cohort Study) with 6.4-yr of follow-up evaluated the relationship between the intakes of CLA and other fatty acids. The study failed to confirm the anti-carcinogenic property of CLA in humans with breast cancer incidence (Brown, 2008). A study by Aro et al. (2000) examined the relationship between dietary or serum CLA in women and the risk of breast cancer. The study found an inverse association between dietary and serum CLA and risk of breast cancer in postmenopausal women. But in contrast, the adipose tissue extracts from a population of French patients with invasive breast carcinoma, failed to reveal any positive correlation between adipose tissue CLA and the incidence of breast cancer (Chajes et al., 2003). A study by Hoffmann et al. (2006), examined the polyunsaturated fatty acid profile of healthy renal tissue and cancerous renal parts. Although it revealed differences in CLA content, the design of the experiment could not reveal the role of CLA in renal carcinoma. In conclusion, the available human clinical studies are conflicting and have not convincingly established the anti-cancer property of CLA.

PROBABLE MECHANISMS OF ACTION OF CLA IN CANCER PREVENTION

It seems likely that CLA exerts inhibitory properties in carcinogenesis via one or more pathways with some tissue specificity. A study by Agatha et al. (2004), showed that CLA isomers are converted by the leukemia cells into conjugated diene fatty acids as linoleic acid into non-conjugated PUFAs. The growth inhibitory effects of CLA (with 30-120 microM) on leukemia cells were dependent upon the type and concentration of CLA isomers present. CLA-supplemented cells with low concentrations (<60 microM) were not sufficient to impair cell proliferation. Nevertheless, higher amounts of CLAs (>60 microM) had the CLA type dependent anti-proliferative effects. The authors thus concluded that the 9 cis,11 trans- and the 9 cis,11 cis-CLA isomers regulate cell growth and survival in different leukemia cell types through their existence alone and/or by their inhibitory effects of desaturase activity. A study by Beppu et al. (2006), compared the growth inhibitory effects of pure CLA isomers cis 9, cis 11-CLA, c9, t11-CLA, t9, t11-CLA, and t10, c12-CLA on human colon cancer cell lines (Caco-2, HT-29 and DLD-1). The strongest inhibitory effect was shown by t9, t11-CLA, followed by t10, c12-CLA, c9, c11-CLA and c9, t11-CLA, respectively. The order of the inhibitory effect
of CLA isomer was confirmed in the presence of 1% FBS. CLA isomers supplemented in the culture medium were readily incorporated into the cellular lipids of Caco-2 and changed their fatty acid composition. The CLA contents in cellular lipids were 26.2+/−2.7% for t9, t11-CLA, 35.9+/−0.3% for c9, t11-CLA and 46.3+/−0.8% for t10, c12-CLA, respectively. DNA fragmentation was clearly recognized in Caco-2 cells treated with t9, t11-CLA. This apoptotic effect of t9, t11-CLA was dose- and time-dependent. DNA fragmentation was also induced by 9c,11t-CLA and t10, c12-CLA. However, fragmentation levels with both isomers were much lower than that with t9, t11-CLA. t9, t11-CLA treatment of Caco-2 cells decreased Bcl-2 levels in association with apoptosis, but Bax levels remained unchanged. These results suggest that decreased expression of Bcl-2 by t9, t11-CLA might increase the sensitivity of cells to lipid peroxidation and to programmed cell death, apoptosis. In another study, Huang et al. (2007), investigated the anti-proliferative effects of two isomers of CLA (c9, t11-CLA; t9, t11-CLA) and their mixture on the human colon adenocarcinoma cell line Caco-2, incubated in serum-free medium. The anti-proliferative effects of different concentrations (0, 25, 50, 100, 200 micromol/L) of linoleic acid, c9, t11-CLA, t9, t11-CLA (the purity of LA and CLA was 96%) and a mixture of c9, t11-CLA and t9, t11-CLA (1:1 v/v) on caco-2 in various action time (1d, 2d, 3d, 4d) were tested in the study. The anti-proliferative effects of the four substances in the same concentration and with the same action time were compared. All substances tested could inhibit Caco-2 cell proliferation. The higher anti-proliferative activity in the four materials was: the mixture of CLA, then t9, t11-CLA, c9, t11-CLA, and linoleic acid, respectively. The activity was closely related to treatment time and concentration. The authors concluded that the isomer t9, t11-CLA has considerable anti-proliferative activity. Mechanisms of inhibition of carcinogenesis therefore include reduction of cell proliferation, alterations in the components of the cell cycle and induction of apoptosis (Belury et al., 2002; Miller et al., 2003). In addition, CLA modulates markers of immunity and eicosanoid formation as well as lipid metabolism and gene expression (Yamasaki et al., 2000; O’Shea et al., 2004).

SUMMARY AND CONCLUSION

CLA, a naturally occurring mixture of positional and geometric isomers is found mainly in animal food products from grass-fed animals. It is synthesized mainly by ruminal bacteria, although an enzyme in the mammary gland also synthesizes it. The isomers, cis-9, trans-11 and trans-10, cis-12 CLA, are the main components; it has been shown in most studies that the cis-9, trans-11 and trans-9, trans-11 isomers are anti-carcinogenic, but others such as linoleic acid, and a mixture of the isomers have also shown marked bioactivity in cancerous tissue studies. The mechanism of action which continues to evolve, seems to be a combination of anti-proliferation, apoptosis, immune modulation, and DNA fragmentation, with different target cells and mechanism of action for different CLA isomers. As most research work to the present has been done with animal models, and whatever work has been done on humans has mainly been of epidemiological nature, more clinical trials on humans need to be undertaken to establish the benefits of CLA in cancer prevention, as the few clinical trials have given conflicting and therefore inconclusive results. It is thus imperative that controlled studies be carried out with large populations of different gender and racial groups, of diverse dietary habits and in different geographical locations. Future research should also seek to establish methods for controlling the amount of CLA and CLA isomers in animal products and to determine the CLA amount that must be consumed to improve human health.
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Socio-economic Factors Influencing Nile Tilapia Aquaculture in Kenya

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ABSTRACT
This study aimed to unravel the factors that may be contributing to the declining aquaculture output in Kenya. The project obtained data using a semi-structured questionnaire, expert interviews and observations in Bomet, Kericho and Nakuru counties of Kenya. Eighteen farms, rearing Nile tilapia by semi-intensive method, with fifteen using on-farm formulated feeds, were purposively selected. Three of them, one per County, and all using commercial feed, served as the control. The study investigated on-farm feed formulation practices, ingredient and feed storage methods, production costs and training service provision. Nakuru County recorded the highest cost per gram of protein, while Bomet County recorded the lowest unit cost (p<0.05). Fifty five percent of farmers used the Pearson Square Method for fish feed formulation, with most of them being from Nakuru County and the least coming from Kericho County. Sixty percent of participating farmers were appropriately trained. The study recommends that the State Department of Fisheries and Blue Economy and Counties make aquaculture a major operational and results area. Additionally, credit to farmers and subsidy of cost of inputs should be provided alongside appropriate extension services.

KEY WORDS: Socio-economic factors, Aquaculture, Nile Tilapia, Kenya

INTRODUCTION
The increasing world population has led to a global focus on food security and sustainable strategies of food production. Aquaculture tops the list of the sectors most governments are currently giving attention to for food security and poverty reduction. Aquaculture has been recognised as one of the fastest-growing food-producing sectors in the world (FAO, 2015). This is attributed to the increase in fish consumption and production. In 2013, fish represented 16 percent of all animal protein consumed by humans globally (FAO, 2015). Kenya’s capture fisheries and aquaculture sectors contribute approximately 0.54 percent to the country’s Gross Domestic Product (FAO, 2013). The total fishery and aquaculture production in 2016 amounted to 147,916 and 14,952 metric tons, respectively (GoK, 2016). The contribution from aquaculture, however, declined in the year under review, from 18,656 metric tons in 2015 (GoK, 2015). As the development of aquaculture remains a priority to the Kenya Government, various initiatives have been undertaken to encourage farming and consumption of fish and to attract investments in the sector. These ideas gained momentum in 2009-2010 through a government initiative dubbed “Economic Stimulus Program”, whose aim was economic...
development and poverty alleviation (GoK, 2013). Despite the success stories in the sector, aquaculture in Kenya still faces challenges which have resulted in stagnation of the industry, and a sudden decline which was experienced from 2014 upwards (Figure 1).

Some of the challenges are lack of readily available and affordable quality fish seed and feed, poor adoption of recommended fish husbandry techniques by some farmers and inadequate market information. These were some of the constraints highlighted in the Annual Fishery Statistical Report by the Ministry of Agriculture, Livestock and Fisheries (2015). The problem of inadequate quality and affordable fish feeds tops the list of these challenges. Furthermore, it has been widely reported by researchers and experienced by most fish farmers in the country. This problem stands out for the reason that fish feed accounts for more than 40 percent of total fish production cost (Abowei et al., 2012). With the small number of certified commercial fish feed producers in the country and the increased fish feed demand due to the increasing number of farmers in the sector, the result is inadequate feeds in the market. Producers are also scattered all over the country and for this reason, farmers travel long distances to obtain the feeds which results in high travel costs and eventually high costs of production (Liti et al., 2005).

The exorbitant prices of commercial fish feeds, has slowed down the growth of aquaculture in many parts of Kenya. To counter this problem, farmers have opted to use locally available ingredients to formulate feeds on-farm at lower costs. Limited information is currently available on the quality of feeds formulated by small scale farmers in most parts of the country, especially for pond and tank culture. There is therefore need for the compositional analysis of on-farm formulated feeds, how they are formulated and the technical knowhow of farmers on various management practises, to ensure that they meet the Kenyan fish feed standards for Nile Tilapia farming. This study therefore aimed at assessing the aforementioned aspects of aquaculture production of Nile Tilapia by the semi-intensive method. However, the compositional analysis of on-farm formulated feeds for quality is described elsewhere and will therefore not be part of the current discussion. Estimates of costs incurred in the formulation of the on-farm made feeds, was also assessed, as well as the feed management practises being used by the small scale fish farmers.

**Figure 1:** Aquaculture production in Kenya in metric tons (2006-2016). Source: (Kenya National Bureau of Statistics-KNBS, 2017).
MATERIALS AND METHODS

Study area

The study was carried out in 2016-2017 on selected fish farms in Nakuru, Kericho and Bomet Counties of the Rift Valley Region of Kenya. The data collection sites are shown in Figure 2. This region is of interest because in recent years, fish farming has become a point of attention (Ngugi, 2009). This has been attributed to the desire by farmers to increase farm production per unit area, as land area for food production continues to decline (State Department of Fisheries and Blue Economy, 2016)

The main crops cultivated in these counties include; maize, wheat, tea, vegetables and fruits (Lukuyu et al., 2011) These crops are a potential source of local fish feed ingredients, thus making the region potentially suitable for sustainable and cost-effective aquaculture. Moreover, the temperature range of 10–28°C in this region is favourable for Nile Tilapia culture (Bowman et al., 2007). Fish production in the Rift Valley Region is largely semi-intensive, with more than 3,000,000 m² of culture area established (Ngugi, 2009).

Criteria Applied at Farm Selection

Farms were selected from the three counties, based on the selection criteria given below:

i. Small scale farms using semi-intensive method of farming Nile Tilapia with at least one pond to a maximum of 4 ponds.

ii. Farms that used on-farm formulated feeds, with single or mixed ingredients.

A pilot survey was conducted before the start of the study to determine the farms to work with based on the above selection criteria. Thirty five farms were selected randomly, with forty percent of them being sampled, giving a total of fourteen farms. One farm using commercial fish feed was picked from each county for comparison. This made a sample total of eighteen farms rearing Nile Tilapia by semi-intensive culture. Data on training provision for farmers and the applicable feed management practices was obtained through observations and survey using a semi-structured questionnaire.

Figure 2: A map of Kenya showing the counties in the Rift Valley Region and fish farms where the study was undertaken.
Feed Cost Analysis

The unit market prices of individual ingredients used were obtained, then the best-buy technique was used to compare the ingredients with one another on the basis of cost per unit of protein or lipids, depending on what major nutrient the ingredients/feeds are supposed to provide.

Cost per unit of protein = cost per gram of feed / amount of protein per gram of feed

N.B: Best-buy formula (Bhosale et al., 2010)

DATA ANALYSIS

One-way ANOVA test was performed to compare differences in means of nutrient content of commercial and on-farm formulated feeds and the Official Standards; the mean costs of on-farm formulations and commercial feeds between and among counties were also compared.

RESULTS AND DISCUSSION

Method of Feed Formulation

Guided by the main objective of this study, it was important to assess the results of the methods used by the fish farmers to formulate fish feeds on-farm. This was done through the use of structured interviews and observations. The results of this aspect of fish production are shown in Figures 3a and 3b. More than half of the farmers interviewed (55%) used the Pearson’s Square Method to formulate fish feeds on-farm. The rest of the farmers used trial and error methods, in which the various ingredients were mixed without measuring their proportions unlike in the Pearson’s Square Method. It was evident that Pearson’s Square Method was used across the three counties (Figure 3a) but most widely in Nakuru County, where 35% of the farms used the method (Figure 3b).

Figure 3a and 3b: Methods of feed formulation in the three counties

Fish Feed Costs

The cost per gram of on-farm formulated feed was determined through interviews and market price surveys, which was then divided by the protein content in a gram of the feed (Bhosale et al., 2010). Protein was used here because of its importance in the growth of fish and because it is usually the most expensive ingredient in fish feeds. Nakuru farm 1 and 3 recorded the highest value in terms of the cost per gram of protein. On the other hand, Kericho farm 3, 4 and Nakuru farm 5 and 6 recorded the lowest values (Figure 5). The high cost
of feeds was because the manufacturers and a few farms imported the ingredients to make feeds. These included shrimps, fish meal, cotton seed cake and sunflower oil. The farms with lower feed costs, mostly utilized local ingredients. Examples of these ingredients included: *Leucena Trichandra* leaves, avocado fruit, kales, poultry and kitchen wastes, which were obtained from the farm and in the homestead. In terms of counties, Nakuru County recorded the highest cost per gram of feed. Most farms in Kericho and Bomet recorded the lowest values (p<0.05). These results are given in Figures 4 and 5.

In this study, it was found that fish feeds containing locally available ingredients were cheaper than those containing imported ingredients. This agrees with Musiba et al. (2014) who established that locally made fish feeds can be used cost-effectively to produce fish feeds. The difference is mainly due to the high transportation costs incurred in sourcing the ingredients from distant places. It can also be attributed to the fact that ingredients such as fish meal and shrimps have continually reduced in supply due to dwindling stocks in inland fisheries and the high competition faced as these ingredients may also be used in poultry and other animal feeds manufacture. Seasonality of ingredients such as *Rastrineobola argentea* or “omena” also contributes to the high prices, especially during low seasons. Kwikiriza et al. (2016) showed that some farmers spent a lot of resources on ingredients that only provide low levels of protein in fish feeds. This situation can be countered by using locally available ingredients which are of relatively low costs, and which contribute the same or higher levels of protein in fish feeds. *Leucaena trichandra* leaves for example, which can be obtained at extremely low cost are more cost-effective to use than shrimp meal, although in terms of protein content, shrimps contain twice as much as *Leucaena trichandra* leaves (Franzel, 2014). The high costs incurred in fish feed formulations on-farm have been reported to contribute significantly to the stagnation of aquaculture (FAO, 2015).

![Figure 4: The mean cost per gram of protein in feeds in the three counties.](image-url)
Knowledge of the farmer on nutrient requirements of Nile tilapia

Knowledge of the farmer on nutrient requirements of Nile Tilapia was an aspect of interest in this study. This information indicated if the farmer was trained or not. It was also important because the way the farmer applied the information learnt would affect the quality of the on-farm formulated feed. Figure 6a shows that 35% of the farmers had been trained in Bomet County. The group of farmers who admitted to have had no training on fish farming, used trial and error method in fish feed formulation. Those who had the moderate to high knowledge on nutrient requirements of Nile tilapia had attended training at least twice over one year and yet applied Pearson’s Square Method, whenever they formulated fish feed on-farm.

Sixty percent of the farmers who took part in the study, were appropriately trained. Three quarters of them were from Nakuru County (Figure 6b). Proper management practises are important in aquaculture, as they determine the level of production (Pillay, 1990). Management practises range from the daily, weekly and yearly activities that enhance operations of a fish farm. Ngwili (2014) noted that the right experience, proper information and sufficient knowledge are the key pillars to proper management of fish farming. Inadequate outreach programmes and inefficiency in dissemination of technology to farmers, has been shown to be the major reason for the slow development of the aquaculture sector (Kiptot, 2012; Shitote, 2012; Ngwili, 2014). In this study, more than 30% of the farmers had no training on fish farm management. Higher results were reported by Shitote (2012), who recorded 95% of farmers in Western Kenya, faced challenges in managing their fish farms due to lack of appropriate training. The high percentage of untrained fish farmers has been attributed to challenges facing the organizations responsible for providing extension services in the aquaculture sector. From the results of this study, fish farming extension services are mainly provided by the Government through the State Department of Fisheries, Aquaculture and the Blue
Economy, through collaboration with affiliate organizations. Apart from the State Department of Fisheries and Aquaculture, (Ngwili (2014) suggested that Non-Governmental Organizations, Radio Stations and Social Media could be used to sufficiently disseminate practical information to farmers. These suggestions arose as a result of the major challenges facing extension services offered by the National-level of Government. The reasons for the poor performance of the training organizations include: low funding of the sub-sector, understaffing and lack of expertise by the extension service providers. These challenges were also echoed by Shitote (2012). On the other hand, lack of entrepreneurial skills among farmers, further amplifies the problem. The formation of farmers’ cooperative societies in Bangladesh proved to be effective in developing entrepreneurial skills among farmers who shared vital management information among themselves during meetings (Saha, 1985) and thus enhancing the dissemination of information. The results of this study agree with the findings of Saha (1985). In this study, there were more cooperative societies in Nakuru County, with more than 40% of trained farmers who used the Pearson Square Method in fish feed formulation, and who attained more than 25% crude protein in their feeds, coming from the county. Halver (2002), emphasised that proper feed formulation is based on sufficient knowledge on specific nutrient requirements of the fish and knowledge on specific nutrient constituents of feed ingredients. As fish farming is a scientific practice, the experience of the farmer alone cannot achieve proper skills, as was shown in this study. Proper aquaculture management can be achieved through proper training of farmers combined with such practises as on-farm demonstrations by extension workers and the formation of farmer cooperative societies to enhance entrepreneurial skills.

**Figure 6a and 6b: Extent of Farmers’ Application of Knowledge at Feed Formulation and Fish Production**

**Feed Management Practices**

This study made observations of how the on-farm formulated fish feeds were handled in terms of storage and supply to the fish. These two aspects were assumed to have effects on the proximate composition of the feed and on the quality of pond water, respectively. The two main feed storage containers that were used in the enterprises studied were gunny bags and plastic
bags. Storage in plastic bags was observed in equal proportion 5% (Figure 7) in the three counties. It also emerged that 15% of farmers who stored their fish feeds in plastic bags did not have appropriately structured storage rooms with wooden racks, as they placed the feeds directly on cement and earth floors. Most of those who placed the gunny bags containing feed on floors came from Bomet County. However, Seventy three percent of the farmers in the three counties stored the feeds in gunny bags on raised wooden platforms.

Fish feed represents the major production cost for fish farmers, and therefore great care is necessary in its handling. Prior to use, the farmers often stored their feed for a week or even months, in large buckets at the pond or cage sites. Although these buckets are usually covered by lids, excessive heat can negatively affect the nutrient composition of the feeds (Bhujel, 2013), as the containers were kept outdoors. This study established that farmers using on-farm formulations, produced feeds just enough to be used for a day to two weeks, while those using commercial feeds bought them in bulk during low price season to last for more than a month in order to keep costs down through economies of scale. Russo (2010), found out that storage of feed for more than two weeks leads to chemical deterioration. The deterioration is greater when storage temperatures are above 27°C. Oxidation of lipids occurs in feeds under storage leading to rancidity of the feed at temperatures of more than 20°C when room ventilation was taken into consideration (Watanabe, 1982). The ambient outdoor temperatures in the three counties was above 20°C for most of the year. Some farmers fermented the on-farm feed so as to increase the shelf-life of the feed. However, these feeds contained high moisture contents of 20% and above which promoted rotting of the ingredients during storage, according to our observations. Fermentation has been found to increase the shelf-life of feeds, but proper drying of the feed after fermentation and grinding is highly recommended (Samaddar et al., 2015).

![Figure 7: Feed Storage Practices in the three Counties](image)

**CONCLUSIONS AND RECOMMENDATIONS**

**Conclusions**

It was apparent from the study that the more training sessions the farmers had attended, the greater the use of the Pearson Square Method for on-farm feed formulation. The extent to which farmers were trained also influenced the level of the application of appropriate management practices in Nile Tilapia culture.
Recommendations

The study recommends that both national and county governments ensure farmers are properly trained on the use of the scientifically-tested Pearson Square Method to formulate on-farm made fish feeds. The application of good fish management practices be adhered to by farmers for good results. In addition, credit facilities to farmers or subsidy of fertilizer, seed, feeds, etc. be provided alongside appropriate extension services.

For a wider understanding of the sub-sector, we recommend that similar studies be carried out in other areas of Kenya.

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Quantitative Changes of Ascorbic acid and Beta carotene in African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) due to traditional cooking methods used in western Kenya

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ABSTRACT

African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) are among African leafy vegetables (ALVs) that are consumed in Kenya. Studies were conducted to establish the traditional cooking methods for ALVs and to determine quantitative changes in ascorbic acid and beta carotene on cooking the two ALVs. Results revealed that the cooking methods had distinct steps. The amount of time and water for cooking were unspecified. Ascorbic acid decreased from 28.2mg/100g to 1.8mg/100g in Spider plant (93.6% loss) and from 19.5mg/100g to 5.8mg/100g in African nightshade (70% loss). Beta carotene decreased from 2.1mg/100g to 0.1mg/100g in Spider plant (94.4% loss) and from 1.8mg/100g to 0.9mg/100g (50.6% loss) in African nightshade. All results were significant (P˂ 0.001). The study concludes that there are existing methods of cooking ALVs. For the two ALVs, cooking led to drastic losses of ascorbic acid and beta carotene. Losses from the African nightshade were generally lower than from the Spider plant for the same nutrient, under similar processing conditions. The study recommends procedural changes in processing methods so as to conserve the two nutrients.

KEYWORDS: ALVs, cooking, Beta carotene, Vitamin C

INTRODUCTION

Cooking is one of the ways in which food is made tender, palatable and safe for consumption. Moreover, cooking enhances digestion and preserves body energy since it takes place outside the body using outside sources of energy (Pollan, 2013). Most of the foods we eat are cooked prior to consumption. This makes it imperative to study cooking, which is both an art and a science. Teherani-Kroenner (2017) argues that in most cases, we do not eat raw food materials. Studies that have been conducted on cooking have removed the foods from the kitchen context and have not taken in to account the methods that are used by the local consumers. African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) are among African leafy vegetables (ALVs) that are commonly consumed in Kakamega County, western Kenya. Some of the
benefits of these ALVs are enhancing food security, nutrition and health and being part of the meal culture of the local people (Abukutsa-Onyango, 2010; Brückner, & Aswani, 2017). Despite these benefits, the preparation and cooking of the ALVs in a nutritious way has remained a challenge. Most recently, preparation and cooking of the ALVs has been described by modern consumers as a tedious process (Musotsi et. al., 2018). This has been attributed to the time consuming requirements of plucking (de-stalking) of the leaves from the stem and boiling them, prior to frying and fermenting. Research on documentation of recipes in western Kenya revealed that most ALVs are cooked by boiling and steaming in unspecified amount of water, or some form of wet heating (Musotsi et. al., 2005; Musotsi et. al., 2017). Recipes developed using Spider plant and African nightshade boil them for 40-45 minutes. It is argued that the long duration of cooking helps to reduce the bitter taste associated with the two varieties of vegetables (Musotsi et. al., 2005). Cooking, therefore, contributes to making a palatable meal for a majority of people. However, nutrient values of vegetables are affected by cooking methods, among other factors (Shackleton et. al., 2009). This presents a very delicate situation where balance has to be made during cooking to retain vitamins and minerals and to eliminate some phytochemicals that cause bitterness in ALVs.

According to Miglio et. al. (2008) both positive and negative effects of cooking vegetables have been reported. These effects depend upon differences in processing conditions, and morphological and nutritional characteristics of vegetables. Physical properties of vegetables are also greatly affected by heat treatments. Texture and color are important parameters in the cooking of vegetables and they may strongly influence consumer purchases of these food items. Changes in texture are often dramatic because of the membrane disruption and the associated loss of turgor. In addition, cooked vegetables exhibit poor color quality in comparison with fresh ones. Yuan et al. (2009) established that all cooking treatments, except steaming, caused a dramatic loss of vitamin C in broccoli. Steaming did not cause any significant loss of vitamin C, compared with the raw sample (Yuan et al., 2009). Further, Rodrigues-Amaya and Kimura (2004) reported that home preparation of vegetables generally increases losses in the following order: microwaving, steaming, boiling, and sautéing. Deep frying, prolonged cooking, combination of several preparation and cooking methods, baking and pickling all result in substantial loss of carotenoids. Whatever the preparation method, carotenoid retention decreases with longer processing time, higher processing temperature, and cutting or pureeing the food.

On the other hand, cooking processes that involve heating also make certain nutrients more available for the body to use. For example, the amount of total carotenoids content in carrots and other vegetable-based dishes was found to be higher in boiled versions (Miglio et al., 2008). Hence, we can conclude that cooking of vegetables is a delicate matter and that a suitable method of cooking should be employed in order to conserve nutrients. Nutrient values of ALVs could be improved by selecting species and varieties high in nutrient content. In addition, nutrient losses can be minimized by improved post-harvest handling and modification of current food practices such as reduced time of thermal treatment, improved drying processes, avoiding the chopping of vegetables before washing and adding vegetables to boiling water instead of cold water for cooking. Also, rapid processing at high temperature is a good alternative than slow, prolonged cooking.

**OBJECTIVES**

The objectives of this study were to:

1. Establish the traditional cooking methods for African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) in Kakamega County.

2. Determine the effect of the cooking methods on levels of Ascorbic acid and Beta carotene in African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*).
MATERIALS AND METHODS
The study applied both qualitative and quantitative research methods. Qualitative research was conducted using exploratory case studies. These established the cooking methods for African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) in Kakamega County. Participatory observation, focus group discussions and key informants were used to generate data. The sample size for case studies applied the methodological principle of “saturation”. Saturation refers to the criterion for judging when to stop sampling the different groups pertinent to a category. It means that no additional data are being found for development of a category for analysis. The sample size for this study comprised thirty-three participant observation interviews, twenty two key informant interviews and fifteen focus group discussions.

Secondly, an analytical laboratory based experimental design was used. Laboratory experiments were conducted to determine the nutritive value of the two vegetables. Raw ALVs of Spider plant and African nightshade were analyzed to establish quantities of the ascorbic acid and beta carotene. Further tests were done to determine the quantity of nutrients in cooked ALVs according to the various recipes collected from Kakamega County. The nutritive values were compared with values for raw vegetables to determine the changes.

**Determination of Vitamin C**

Vitamin C was analyzed using a reversed-phase HPLC method by Ekinci and Kadakal (2005). To 5 g sample was added 20 ml distilled water and the mixture homogenized at medium speed for 1 minute. The homogenized samples were centrifuged for 10 min at 14 × 10 g. Vitamins were then separated using ODS C-18 size 250mm*4.6mm* 0.5 ul column. They were eluted with 10 ml methanol. The elute was concentrated by Rotary Vacuum evaporator and vitamins re-dissolved in the mobile phase and 20 ul injected into the HPLC. A HPLC (Shimadzu 20A series, Tokyo, Japan) with Photodiode array detector (PDA). Absorance readings were taken at the wavelength of 266 nm.

**Determination of Beta carotene**

Beta carotene content was analyzed using the method described by Rodriguez-Amaya and Kimura (2004) where column chromatography and a UV Spectrophotometer; acetone and petroleum and ether were used for extraction. Approximately 2 grams of fresh sample was weighed, chopped finely and placed in a mortar with about 10 mL of acetone. This was thoroughly ground and the acetone extract transferred into a-100 mL volumetric flask. The residue was again extracted with 10 mL acetone and the extract was added to the contents of the volumetric flask. The extraction with acetone was continued until the residue no longer gave colour. The combined extract was made to a volume of 100 mL with acetone. Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator. The residue was dissolved with 10 mL petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The Beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance was read at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV – Vis 1800 PC, Kyoto, Japan). Beta carotene standard was prepared for the construction of a calibration curve. All chemicals used were of analytical grade. All the measurements were done in triplicates.
RESULTS AND DISCUSSION
Traditional cooking methods for Spider plant and African nightshade in Kakamega County

Results from the qualitative studies showed that African nightshade (*Solanum nigrum*) and spider plant (*Cleome gynandra*) were the ALVs that were eaten regularly. They were eaten after cooking as accompaniments to starchy staples. There were systematic methods (recipes) for preparing and cooking the ALVs. Most of the recipes used were complemented with vegetables. African nightshade (*Solanum nigrum*) and Spider plant (*Cleome gynandra*) were each complemented with Ethiopian kale and/or amaranth. The steps in cooking were: de-stalkling, washing, boiling, wet-frying and sometimes fermentation. Usually, the amount of water for cooking was unspecified. Fermentation according to the respondents, was a method of preservation where addition of fresh milk to cooked ALVs was done while re-heating on a daily basis. This method could be used to preserve ALVs for up to 7 days. Besides, fermentation was also used to enhance the taste of the ALVs. From observation, the water for boiling was often excess and had to be drained off and discarded before the cooked vegetables were fried. Further, most respondents did not specify the cooking time. Table 1 shows the typical recipes used to prepare and cook the two ALVs.

Table 1: Typical recipes of Spider plant and African nightshade in Kakamega County

<table>
<thead>
<tr>
<th>Type of ALV</th>
<th>Requirements</th>
<th>Preparation method</th>
<th>Changes observed</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spider plant</td>
<td>250-300 g spider plant 75-100 g amaranth Cooking oil Tomatoes Onions Salt to taste Fresh Milk or cream</td>
<td>Pluck the vegetables Wash vegetables several times with plenty of water Place vegetables in a pan. Add ab. ½ litre of water Cover with banana leaves and a plate Boil for 1 hour Fry onion in the oil until brown, add tomatoes. Add salt Fry the tomatoes until they become very soft. Add the vegetables and mix well. Add milk or cream and cook for 10 minutes. Vegetables are ready to serve</td>
<td>The color of washing water turned green, and this intensified with each wash. As vegetables began to boil, there was a characteristic smell. This changed to a savory smell. The color also changed from bright green of the raw leaf to brown color after cooking</td>
<td>Plucking- ab. 30 minutes depending on amount Washing- ab.15 minutes Boiling-1½- 2 hours Simmering- 10 -15 minutes Total duration of preparation and cooking- 2½- 2¾ hours</td>
</tr>
<tr>
<td>African nightshade</td>
<td>250-300 g nightshade 75-100 g amaranthus Cooking oil Tomatoes Onions Salt to taste Fresh Milk or cream</td>
<td>Pluck the vegetables Wash vegetables several times with plenty of water Place vegetables in a pan. Add ab. ½ litre of water Cover with banana leaves and a plate Boil for about 1 hour Fry onion in the oil until brown Add tomatoes. Add salt Fry the tomatoes until they become very soft. Add the vegetables and mix well. Add milk or cream and cook for 10 minutes. Vegetables are ready to serve</td>
<td>As above, except that the washing water had more color intensity</td>
<td>Plucking- ab. 30 minutes Washing- ab. 15 minutes Boiling- ab. 1 hour Simmering-10 minutes Total duration of preparation and cooking- approx. 2 hours</td>
</tr>
</tbody>
</table>
By outlining the steps and explaining reasons for each step, the respondents demonstrated that they had knowledge on cooking of the ALVs. The study also found that this knowledge was passed from mothers to their children by word of mouth. This reveals the cooking of Spider plant and the African nightshade in Kakamega County still employs indigenous knowledge. It reveals the importance of such knowledge in ensuring food and nutrition security for the local communities. On the other hand, knowledge on the preparation and cooking of ALVs is also evolving. From the study, there were modifications to the traditional recipes by modern consumers. Frying is one such modification. Because of frying, addition of milk is now optional as opposed to the past when it was almost the only additive. Although the modification of frying may not be perceived as adding value for some consumers whose impression of cooking oil was negative, it is the common practice today. This trend suggests that there is room for change in the traditional recipes, a positive thing that could support the adoption of new recipes based on laboratory analyses.

The time taken to accomplish the steps in preparation, cooking and fermentation is significant with regard to the amount of nutrients retained. De-stalking took up to 30 minutes to accomplish, meaning long exposure times of the ALVs to oxidation. There is rapid loss of ascorbic acid when vegetables are exposed to air and light. Moraes et al. (2010) also showed that there are significant losses of vitamin C during improper storage, preparation and transportation. Other nutrients that may be lost considerably include beta carotene, total antioxidants and minerals: calcium, iron and zinc. This reveals that to minimize losses in the preparation of ALVs, the processes need to be done fast.

Washing of ALVs is important as it enhances food safety and palatability. Washing several times is a way of ensuring that the food is safe for consumption by removing soil and sometimes microorganisms such as *Escherichia coli*, which are often found on the surface of the vegetables (Mahan & Raymond, 2017). In the process of washing, it was noted that the water turned color to green as the number of washings increased. The green color is a symbol of chlorophyll which is evidence that some materials, which may include nutrients and phytochemicals, are dissolved or leached into the water. Chlorophyll contains vitamins A, C, E and K as well as beta carotene. It is also rich in antioxidants and minerals such as iron and calcium (Masfuzal et al., 1997; Ifemeje 2015; Maseko et al., 2017). When these are leached during washing it further reduces the quality of the ALVs and makes them less nutritive. This is in agreement with Kirigia et al. (2017) who showed that post-harvest processing of ALVs can also lead to damage through leaf tearing, crushing and other physical damage. This eventually leads to loss of nutrients through oxidation, leaching and senescence. The duration of washing is also significant. The more the time spent on washing, the longer the exposure of the ALVs to oxidation. This could lead to loss of sensitive nutrients (Kirigia et al., 2017).

The time spent on boiling/steaming was between 1-2 hours. Further, the water added for cooking was estimated to about two litres of water for cooking approximately two Kilogrammes of vegetables. This water was left in the ALVs after cooking and was discarded during frying. While boiling and steaming are considered safe methods of cooking that allow softening of food, for vegetables the vitamin losses are higher due to the high temperatures used. Traore et al. (2017) found that boiling of the African nightshade for 30 minutes reduced the amount of beta carotene significantly, from 16.40mg/100g to 5.37mg/100g. A study done by Agbmalfe et al. (2012) also showed that with increased boiling time, there was drastic loss of beta carotene and vitamin C in green vegetables consumed in Ghana. The losses were attributed to oxidation and isomerization (Traore et al. 2017). While vegetables are generally an important source of vitamins and minerals, a considerable amount of them is lost when vegetables are cooked in water. Therefore, vegetables are best cooking using rapid methods such as stir frying and perhaps steaming for those that need moist method.

Wet frying and addition of tomatoes and onions is expected to improve taste and palatability of the ALVs. Adding oil can also protect and enhance absorption of the fat soluble vitamins (Mahan & Raymond, 2017). On the other hand, oil has been associated with increased energy density and excessive consumption of oil is known to result in increased risk to lifestyle diseases and poor health. In
addition, simmering after frying increases the cooking time for the ALVs thus exposing the heat sensitive nutrients to further loss. Fermentation could have both positive and negative effects in the recipes. Since there is addition of fresh milk on a daily basis, fermentation could enhance availability of proteins, zinc and calcium. The preservative role can enhance food security in the household since the vegetables were said to last up to one week when fermented. Enhancement of taste and flavor can also help to increase intake as consumers will be encouraged to eat more of the ALVs. On the other hand, the prolonged methods of reheating every morning may lead to loss of heat sensitive nutrients. These results reveal the importance of time in preparation and cooking methods for Spider plant and the African nightshade. There have been some arguments about cooking of Spider plant and the African nightshade. Some consumers argue that they need to be cooked for long to reduce bitterness (associated with high levels of phytochemicals) while others prefer to cook them for a short time to preserve vitamins that are susceptible to leaching and breakdown due to prolonged cooking and the use of large amounts of water. The balance between the two arguments is what many studies have failed to capture, with most of them shying away from recommending the optimal cooking time for the two ALVs. Thus, the above recipes were subjected to laboratory analyses in order to determine the levels of ascorbic acid and beta carotene.

Retention of Vitamin C and Beta carotene after cooking ALVs using traditional recipes

**Vitamin C**

Results showed a decrease in the amount of Ascorbic acid after cooking as compared to the raw leaf. There was a decrease of the Ascorbic acid (A.A) in Spider plant from 28.2 mg/100g in the raw leaf to 5.6 mg/100g in the boiled leaf, representing 80% loss. In the fried recipe, Ascorbic acid reduced to 3.0 mg/100g (89% loss), while in the fermented recipe, Ascorbic acid content reduced to 1.8 mg/100g (93.6% loss). In the African nightshade, the change was from 19.5 mg/100g of raw leaf to 10.2 mg/100g in the boiled leaf, representing a 47.6% loss. The fried recipe contained 8.0 mg/100g of Ascorbic acid, which was equivalent to 58.9% loss. The fermented and reheated product contained 5.8 mg/100g of Ascorbic acid (70% loss). The losses were statistically significant at p˂0.001. These losses are mainly attributed to repeated washing in plenty of water and the prolonged duration of cooking.

![Figure 1: Amount of Vitamin C in raw and processed Spider plant and African nightshade](image)

Data are means ± standard deviations of replicates of the sample
The results of Vitamin C analysis revealed that preparation, cooking, and cooking the fermented vegetables led to drastic losses of vitamin C in both vegetables. Vitamin C is abundant in most green leafy vegetables but it is easily lost during cooking and processing due to its unstable nature in the presence of oxygen, heat and water. The results were consistent with the findings by Nwozo et al. (2015), Agbemafle et. al. (2012), and Muthiani (2004), who, in their studies, found that there were significant reductions in Ascorbic acid of African green leafy vegetables during processing. Hossain et. al. (2017) and Singh (2016) also found similar results when investigating Vitamin C content of different Asian leafy vegetables subjected to different processes. In the studies for both African and Asian vegetables, it was found that boiling, as a method of cooking contributed to the greatest loss of Ascorbic acid. Thus, boiling may not be a suitable method of cooking leafy vegetables when there is need to conserve Vitamin C.

Vitamin C is essential for growth and repair of body tissues and enhances absorption of iron from food, among several other functions in the human body. Further, its powerful anti-oxidative ability makes it useful in protecting body tissues from oxidation (Mahan & Raymond 2017). The human body cannot make vitamin C, and, therefore, food (especially fruits, vegetables and organ meat) is the main source of the vitamin. Considerable amounts of the vitamin are required in the diet on a daily basis because it cannot be stored by the body. The requirement for daily intake of Vitamin C is 60 mg/day for non-smoking men and women (Mahan & Raymond 2017). The results above attest to losses where 93.6% occurred in fermented and reheated Spider plant and 70% occurred in fermented and reheated African nightshade. This implies that people consuming 100 g of fermented Spider plant dish would obtain less than 10 mg of Vitamin C, while those consuming fermented African nightshade would obtain much less than one third of what is contained in the raw leaf. Thus, the amount of vitamin C available in Spider plant and African nightshade cooked using the traditional methods in western Kenya may not meet the RDA if they consume a serving of 100 g. These ALVs, hence require cooking methods that are more preservative to vitamin C such as steaming and stir-frying. Additionally, extreme care, including protection from extreme light and air, minimizing cooking time and water need to be taken to conserve this vitamin during preparation and cooking to prevent the dramatic losses.

**Beta Carotene**

The study revealed a decrease in the level of Beta carotene in the cooked Spider plant and African nightshade as compared to the raw ones. The level of the nutrient also decreased in the fermented vegetable but was higher than in the cooked vegetable. In Spider plant, the amount of beta carotene dropped from 2.1 mg/100g in raw leaf to 0.3 mg/100g in boiled ones, which was an 87.6% loss. In the fried recipe, Beta carotene declined to 0.1 mg/100g representing 94.4% loss. In the fermented recipes, the amount of Beta carotene was 1.0 mg/100g which was a 52% loss compared to the content in the raw leaf. In the African nightshade, the values were 1.8 mg/100g in raw leaf, which then dropped to 0.6 mg/100g in the boiled leaf (65% loss). After frying, the amount of Beta carotene was 0.1 mg/100g, which was a 93% loss and but was higher in fermented vegetable at 0.9 mg/100g. This represented a 50.6% loss compared to the raw leaf. These changes are represented in Figure 2. Losses in both Spider plant and African nightshade were statistically significant at p<0.001.
The losses could be attributed to prolonged boiling (over 30 minutes) during cooking. While Beta carotene is known to be stable to heat, prolonged cooking could destroy it due to degradation and polymerization. The increase after fermentation may be as a result of addition of milk. According to Ullah et. al. (2017) and Strusinka et. al. (2010), cow milk is a good source of Beta carotene. This is dependent partly on the cows’ feed. The natural yellow color of cow’s milk comes mainly from Beta carotene. This is found in milk (FAO/Government of Kenya, 2018) showed that the amount of Beta carotene found in milk is 153 mcg/100g. When added to ALVs on a daily basis as in the case of fermented product, the level of Beta carotene could be increased.

The findings on cooking and fermentation of Beta carotene agree with Agbemafle et. al. (2012) and Muthiani (2004), who in their studies found reduction in Beta carotene in cooked green vegetables as compared to their raw counterparts. However, some studies have shown that Beta carotene is stable during heating. Mosha et. al. (1997) and Mduma (2009) showed that carotenoids were retained at higher levels as compared to the raw leaf when green vegetables including cowpea leaves and amaranth are cooked for a shorter time (10-15 minutes). This could be attributed to the breakdown of cell walls of plants during cooking, making carotenoids more available. Also, Chang et. al. (2013) found similar results although these were not related to green leafy vegetables but other vegetables such as cabbage and red and white spinach. This shows that Beta carotene may be affected depending on the type of vegetables cooked or processed and the cooking time.

Beta carotene, a member of the carotenoids, is a provitamin A carotenoid which is absorbed and converted into vitamin A (retinol) in the body. The vitamin A activity of Beta carotene is calculated as 6 µg (0.006mg) being equivalent to 1 µg (0.001mg) of retinol (Webster-Gandy et al., 2006). It is abundantly found in fruits and vegetables and some studies have shown that it is also found in milk (Ullah et. al., 2017, Strusinka et. al., 2010, Swensson & Lindmark-Mansson 2007). Among the major functions of Beta carotene are promoting cognitive functions, maintaining skin health, promoting visual health and preventing cancer. The recommended daily intake of vitamin A (RDA) for men and women is 1 mg and 0.8 mg retinol, respectively (Mahan & Raymond, 2017). From the data above, a person consuming 100 g of cooked Spider plant and African nightshade would not meet their RDA since it would provide a maximum of 1 mg and 0.9 mg respectively of Beta carotene, whereas the contribution towards meeting the RDA would be 6 mg for men and 5.4 mg for women, when 100 g of the two ALVs are consumed.

Lack of vitamin A is an issue of public health concern in Kenya. Consequently, the Government of Kenya, has put in place public health interventions that make it a requirement that all children under five
years be supplemented with vitamin A. Spider plant and African nightshade are rich sources of Beta carotene. However, most of it is lost during cooking and processing as seen from the above results and people eating these vegetables may not meet their RDA. In order to prevent vitamin A deficiency (VAD), we not only need to eat a diversity of foods rich in Beta carotene, but also ensure the foods are cooked using nutrient-sensitive methods in order to retain Beta carotene.

Loss of Ascorbic acid and Beta carotene by leaching during cooking

The water drained after boiling of Spider plant and African nightshade were analyzed to determine the presence of the two vitamins. This water is usually discarded in traditional recipes. Results showed that Ascorbic acid and Beta carotene were not detected. The lowest detection level of UV/vis for beta carotene was 0.01 µg/ml while that of HPLC Shimadzu 20 series used for vitamin C was 0.001 µg/ml, according to the manufacturers. Kirigia et. al. (2017) reported that leaching can be occasioned by damage to the plant cells during processing. Washing after destalking and cooking of ALVs are processes that can lead to leaching. Washing and cooking are especially significant to leaching because nutrients and bioactive compounds are released into the water. Overall, results on cooking water revealed the importance of taking into account the amount of water used in preparation and cooking Spider plant and African nightshade. If too much water is used, leaching of bioactive compounds occurs. This has negative effects on the nutritional quality and health benefits of the ALVs in most cases. There is need to balance the amount of water used versus the bioactive compounds desired to be retained.

Modified recipes for Spider plant and African nightshade for conservation of bioactive compounds

Based on the findings, the researchers suggested modified recipes as shown in table 2.
Table 2: Modified recipes for retention of bioactive compounds in Spider plant and the African nightshade

<table>
<thead>
<tr>
<th>TYPE OF ALV</th>
<th>REQUIREMENTS</th>
<th>PREPARATION METHOD</th>
<th>DURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spider plant</td>
<td>250-300g Spider plant 75-100g amaranth Cooking oil Tomatoes Onions Fresh Milk or cream</td>
<td>Wash vegetables 2-3 times Pluck the vegetables Place vegetables in a pan/pot. Add about 200-250ml of water Cover with a lid Steam Fry onion in the oil until brown, add tomatoes. Fry the tomatoes until they become very soft. Add the vegetables and mix well. Add milk or cream and cook for 10 minutes. Vegetables are ready to serve</td>
<td>5 minutes 15-20 minutes (depending on quantity) Steaming-15 minutes Simmering-5 minutes Total duration of preparation and cooking- 40-45 minutes</td>
</tr>
<tr>
<td>African nightshade</td>
<td>50-300g African nightshade 75-100g amaranth Cooking oil Tomatoes Onions Fresh Milk or cream</td>
<td>Wash vegetables three times Pluck the vegetables Place vegetables in a pan. Add 200-250ml of water Cover with a lid Steam Fry onion in the oil until brown Add tomatoes. Fry the tomatoes until they become very soft. Add the vegetables and mix well. Add milk or cream and simmer Vegetables are ready to serve</td>
<td>5 minutes 15-20 minutes (depending on quantity) Steaming-15 minutes Simmering 5 minutes Total duration of preparation and cooking- 40-45 minutes</td>
</tr>
</tbody>
</table>

The purpose of washing before de-stalking is to prevent excessive leaching of water soluble nutrients during washing. Plucking injures the plant, creating points at which nutrients can escape. This can be avoided if the ALVs are washed before plucking. The time for plucking has also been reduced and this will prevent prolonged oxidation. The recipes in Table 3 have limited amount of water for cooking (200-250ml). This also is to prevent excessive leaching of water soluble nutrients. Further, the overall time for preparation and cooking has been reduced from 2-2½ to 40-45 minutes. This is to help reduce excessive breakdown of cell structures of the ALVs during cooking, which can lead to leaching and oxidation. The preferred method of cooking is steaming and not boiling. Boiling utilizes a large amount of water and for a long duration of time. On the contrary, steaming utilizes minimal water and is done for a shorter time thus conserving bioactive compounds.
CONCLUSION
The methods of preparation and cooking of Spider plant and the African nightshade in Kakamega County have already been established. Some changes in these methods such as the addition of oil on cooking have been accommodated over time by some people in the community. Boiling the chopped vegetables and heating the fermented vegetables before consumption resulted in drastic losses of Ascorbic acid and Beta carotene.

RECOMMENDATIONS
The study recommends the following:

- That preparation of Spider plant and the African nightshade, including processes such as de-stalking and washing be done just before cooking and as fast as possible. This is so as to prevent the loss of vitamin C which is sensitive to oxidation.
- Washing of the ALVs should be done before de-stalking in order to prevent damage of cells and leaching of the nutrients into the wash-water.
- Cooking should be done with minimal amount of water in order to avoid left over water in the boiled/steamed ALVs, which is normally discarded.
- Cooking time should be reduced to at least 15 minutes of boiling/steaming and 5 minutes of simmering. Studies have shown that over this time, anti-nutrients are broken down and nutrient loss is minimized.
- Sensitization should be conducted in the local community for them to accept new cooking methods in order to maximize nutrient retention from the cooking of the two ALVs.

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