Original Research Article

Processing Techniques affects the Vitamin Quality of Edible Insects – Potential for Use in Complementary Foods

ABSTRACT

Aims: To assess the vitamin content of locusts, lake flies, grasshoppers, and termites when fresh, sun-dried, oven-dried, and defatted.
Study design: Whole insect samples were sun-dried and oven-dried. Due to their high-fat content, termites and grasshoppers were subjected to an additional defatting step after the sun-drying and oven-drying.
Place and Duration of Study: The study was carried out in Kenyatta University's food science lab between August 2020 and December 2020.
Methodology: Prepared insect samples were ground and analyzed for vitamins using high-performance liquid chromatography (HPLC). Analysis was done in triplicates and results were expressed in mg/100g of dry insect sample.
Results: Fresh insect samples had high vitamin concentrations in comparison to the processed samples. For ascorbic acid, there was no significant difference between; a) sun-dried and defatted sun-dried termites (p-0.79), b) oven-dried and defatted oven-dried termites ($p=0.51$), c) defatted oven-dried and defatted sun-dried grasshoppers ($p=0.22$) and d) sun-dried, and defatted oven-dried grasshoppers ($p=0.59$). For thiamine, pyridoxine, riboflavin, and α-tocopherol there was a significant difference for all the samples in all the insects ($p<0.0001$). For niacin, fresh, sun-dried, oven-dried, and defatted oven-dried termites showed no significant difference in concentration ($p=0.22$). However, there was a significant difference for the other insects ($p<0.0001$). For beta-carotene, only oven-dried and sun-dried grasshoppers didn't have a significant difference ($p=0.76$). Degradation for water-soluble vitamins was highest in sun-dried samples, while fat-soluble vitamins were highest in oven-dried samples.
Conclusion: Fresh insects contain vitamins that meet the recommended dietary allowance (RDA) values for children up to the age of 36 months, except for beta-carotene. Processing significantly reduces the vitamin levels to below RDA values except for ascorbic acid, thiamine, and alpha-tocopherol in lake flies and termites, which can be used in the formulation of complementary foods to meet 100% of the RDA.

Keywords: Termites, Locusts, Grasshoppers, Lake Flies, Vitamins, Degradation

1. INTRODUCTION

Micronutrients are required in the human body for normal cell function, growth, and development [1]. Micronutrient malnutrition results from a lack of some micronutrients, mainly minerals or vitamins [2]. The leading cause of micronutrient malnutrition is food insecurity due to the high population growth. The current world population is at approximately 7.7 billion and is expected to increase to 8.6 billion persons by the year 2030

[3]. This population growth leads to higher demand for food which is accelerated by a reduction in the fertile land for agricultural purposes [4]. Further, the effects of climate change, pest infestation, insufficient water for irrigation, and high production costs have drastically reduced agricultural and livestock practices [5]. The aftermath is malnutrition which can be in the form of undernutrition, overnutrition, or/and micronutrient deficiencies [6].

Children and young women are the most vulnerable to micronutrient malnutrition due to the increased number of adolescent pregnancies [7] and poor feeding habits of young children [8]. In comparison to older mothers, young mothers have higher risks of being malnourished and end up giving birth to low-weight infants who are more susceptible to malnourishment and diseases. First-born children born by girls under the age of eighteen have a higher risk (33% higher than in normal cases) of having stunted growth due to undernutrition of the young mothers and poor complementary feeding [7]. Short-term effects of micronutrient malnutrition include disability, mortality, and morbidity while long-term effects include stunting, wasting, poor reproductive performance, and metabolic and cardiac illnesses [9].

The problem of micronutrient malnutrition is not something that can be ignored. Though vitamin and mineral supplements are available in the market, they are expensive and most people cannot afford them [10]. Affordable alternative sources of diets rich in all nutrients need to be sought to complement the diet of global populations. One alternative is entomophagy – the use of insects for food [11]. There are over 2000 species of edible insects in the world, being consumed by different local communities. Insects contain substantial levels of minerals, proteins, vitamins, fats and fatty acids, and amino acids all of which would play a critical role in complementing the diets of the global populations and especially in children and adolescents who are at higher risks of malnutrition [12].

Rearing insects requires less land, water, and feed. Also, insects mature on average in six weeks, and hence the production is a fast process in comparison to animals and plants, which take time to grow and develop [13]. On average, a single insect can lay thousands of eggs in a lifetime, and this gives the advantage of multiplying the insects within a short time thereby increasing their availability [14]. For sustainability, it is essential to subject the insects to processing methods that kill microorganisms and extend their shelf life [15]. Reducing moisture content via drying processes is one way of increasing shelf life. However, the processing stability of nutrients in food is affected by processing [16].

Vitamin stability is affected by moisture content, light, temperature, pH, oxygen levels, presence of catalysts such as iron and copper, vitamin-vitamin interactions, and time of exposure to the named factors [17]. Processing methods involving exposure to high levels of oxygen, heat, and light result in more vitamin degradation. Water-soluble vitamins are more unstable when subjected to processing in comparison to fat-soluble vitamins [18]. This study focused on determining vitamin content in grasshoppers, termites, lake flies, and locusts when fresh, sun-dried, oven-dried, and defatted while evaluating their potential for use in complementary foods.

2. MATERIAL AND METHODS

2.1 Insect Samples Preparation

One batch of 2-3 Kg of each insect was collected from Siaya in western Kenya. The samples were put in cool boxes and transported to the International Centre of Insect Physiology and Ecology (ICIPE) for identification. The samples were then taken to Kenyatta University, where they were stored in air-tight bags in a freezer at $-4^{o}C$. For locusts, the samples were divided into three portions. One portion was left fresh, the second portion was sun-dried while the third portion was oven-dried. For grasshoppers and termites, the samples were divided into five portions. The fourth portion was oven-dried then defatted, while the fifth portion was sun-dried and then defatted. For lake flies, the samples were only sun-dried.

Sun-drying was achieved by drying about 1 kg of the insects under direct sunlight for three days. The insect samples were spread on an aluminum foil during the exposure period while minimizing overlap as much as possible to ensure even drying. The exposure temperatures ranged between 24 ^{o}C and 26 ^{o}C while the average time of exposure per day was 8 hours. Oven drying was achieved by drying about 1kg of the insects for 36 hours in an air-dry oven set at 60^{o}C. The samples were spread uniformly on an aluminum foil before insertion into the oven. On drying samples, were stored in air-tight bags before analysis to avoid moisture reabsorption. About 500g of the sun- and oven-dried termite and grasshopper samples were subjected to a defatting process (using a manual pressing machine). The sun-dried/oven-dried samples placed in a muslin bag were put in the manual press and the oil was removed out of the insects by applying pressure. The defatted samples were stored in air-tight bags. Prior to analysis, all the samples were ground using a blender analysis to obtain uniform samples for analysis.

2.2 Chemicals and Reagents

For extraction, analytical grade hydrochloric acid, metaphosphoric acid, pyrogallic acid, acetic acid, sodium acetate, anhydrous sodium sulphate, ether, ethanol, and potassium hydroxide were used. Beta-carotene, α-tocopherol, thiamine, riboflavin, niacin, and pyridoxine analytical standard compounds from Sigma-Aldrich, USA were used for HPLC analysis. HPLC grade water, methanol, acetonitrile, ethyl acetate, and analytical grade phosphoric acid, and potassium acetate were used for chromatographic separation.

2.3 HPLC Instrumentation

Chromatographic separation of the vitamins was done on a SHIMADZU HPLC with the following specifications; a Hyper Clone BDS C18 130A reverse phase column; 250 x 46 mm, a CTO 10AS VP oven, a DGU 20A 5R degasser, an LC 20D pump, a SIL 20AHT autosampler, and an SPD 20A UV detector. The HPLC instrument was running using lab solution software. For B vitamins, a methanol-phosphoric acid mobile phase mixture at a pH of 3.54 under isocratic elution (A/B 33/67; A: MeOH, B: 0.023M H_3PO_4, pH = 3.54) was used. A flow rate of 0.5 mL/min was used. The detector was set at 280nm for vitamin B1, B2, and B3 and at 270nm for vitamin B6 under room temperature conditions. For beta-carotene and vitamin E separation acetonitrile-methanol-ethyl acetate (88: 10: 2) mobile phase under isocratic elution was used. A flow rate of 1ml/min was used. The detector was set at 452nm for beta-carotene and 292nm for vitamin E. For ascorbic acid, potassium acetate (0.1M at PH of 4.9) and acetonitrile-water (50:50) MP at a flow rate of 1ml/min was used.

2.3 Laboratory Procedures

2.3.1 Ash and Moisture Content Determination

Ash and moisture content determinations followed the methods described by the Association of Official Analytical Chemists (AOAC) [19,20].

2.3.1 Determination of Vitamins

B-vitamins and fat-soluble vitamins analysis followed the method described by Sami et. al [21] while ascorbic acid determination followed the method described by [22] with slight modifications. All the procedures were validated through calibration, recovery, and precision following methods described by [23].

2.4 Statistical Analysis

All measurements were done in triplicates and the mean, variance, and standard deviations of the measurements were calculated using Microsoft excel. The results were reported as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to test the level of statistical difference (at $P \leq 0.05$) for the fresh, sun-dried, oven-dried, and defatted insect samples.

3. RESULTS AND DISCUSSION

3.1 Ash and Moisture Content

The percentages (%) for moisture and ash contents of the fresh and processed insects are indicated in Table 1. The highest percentage of moisture content was in fresh samples while the lowest was in oven-dried samples. Fresh grasshoppers had the highest moisture content (55%) while oven-dried locusts had the lowest moisture content of 1.3%. A study conducted in Siaya, Kenya showed that fresh grasshoppers' average moisture content was 66.4% and 71.2% for green and brown grasshoppers respectively [24], higher than the moisture content obtained from this study. Research conducted in Khartoum showed that oven-dried (45^{o}C) migratory locusts contain moisture content in the range of 2.9 to 4.7% [25], which closely agrees with the sun-dried results obtained from this study. Lake flies had a mean moisture content of 15.29% and the findings are contrary to the findings by Okedi [26], who obtained a moisture content of 9%. There was a significant difference between all the different processing methods for all the insects, except for sun-dried and defatted sun-dried (p=0.32) and oven-dried and defatted oven-dried grasshoppers (p=0.37). In comparison to other meat products, insects have lower moisture content [27,28]. The lower moisture content in the insects is a desirable feature since high moisture content contributes to spoilage from mold growth [29,30]. In addition, low moisture content means higher dry matter and thus low moisture content has an economic advantage during processing.

Table 1. Percentage moisture and ash content of the fresh and processed insects

Sample Name	% Moisture	% Ash
Grasshoppers		
Fresh	55.01 ± 2.35^{a}	2.8 ± 0.38^{a}
Sun-dried	7.05 ± 0.05^{b}	3.8 ± 0.13^{b}
Oven-dried	4.00 ± 0.51^{c}	4.01 ± 0.24^{b}
Defatted Oven-dried	3.42 ± 0.88^{c}	2.29 ± 0.09^{c}
Defatted Sun-dried	6.68 ± 0.64^{b}	3.05 ± 0.09^{d}
Termites		
Fresh	40.00 ± 0.12^{a}	2.23 ± 0.32^{a}
Sun-dried	3.70 ± 0.03^{b}	2.96 ± 0.18^{b}
Oven-dried	1.50 ± 0.05^{c}	4.31 ± 0.40^{c}
Defatted Sun-dried	4.02 ± 0.18^{d}	1.35 ± 0.05^{d}
Defatted Oven-dried	3.28 ± 0.14^{e}	0.86 ± 0.08^{e}
Locusts		

Fresh	44.98±0.41^{a}	1.09±0.10^{a}
Sun-dried	2.35±0.26^{b}	1.59±0.06^{b}
Oven-dried	1.38±0.28^{c}	3.35±0.06^{c}
Lake Flies		
Sun-dried	15.29±0.36	5.88±2.89

Results are expressed as mean concentration ± standard deviation of the mean (in mg/100g)
In each column, values with the same superscript are not statistically different at p ≤.05

Sun-dried lake flies had the highest percentage of ash content (5.88%) while the lowest percentage was observed in defatted oven-dried termites (0.86%). Processing by oven-drying increased ash content in the insects, an indication that oven-dried samples would have high mineral content. A study in Siaya Kenya reported that fresh grasshoppers' average ash content was 2.8% and 2.6% for green and brown grasshoppers respectively [24], which is in agreement with these results. The results obtained for termites' ash content are in close agreement with results conducted from a study conducted in Nigeria on fresh termites, which gave results in the range of 3.7 to 4.5 % [31]. The insects have a relatively low ash content when compared to vertebrates since they do not have an internal calcified skeleton that vertebrates have [32]. A comparison of all the processing methods shows a significant difference ($p < 0.001$) for all the insects except for between oven-dried and sun-dried grasshoppers.

3.2 Levels of Vitamins in the Insects

3.2.1 Method Validation

Linearity was determined by regression analysis of the standard calibration curves. The coefficient of determination values ranged from 0.9935 to 0.9998 an implication that over 99.35% of the responses were related to the concentration. A coefficient above 0.99 is used as a criterion of a good linear relationship [33]. Therefore, the calibration curves obtained were confidently used to determine analyte concentrations in the insect samples. The values obtained for the limits of detection (LOD) for B-vitamins were in the range of 0.014 - 0.08 ppm (Table 2) and were comparable to other methods used for the same analysis. Sasaki et. al., [34] reported LODs in the range of 0.03-0.41 ppm while Klejdus et al., [35] obtained LOD values in the range of 0.012 – 0.865 ppm.

Table 2. Method Validation Parameters

Vitamin	Coefficient of Determination	Limit of Detection (LOD)	% Accuracy	% Precision	% Recovery
Thiamine	0.9935	0.0884	99.38	95.91	96.56
Riboflavin	0.9997	0.0198	99.30	99.04	96.95
Niacin	0.9984	0.0434	98.77	98.21	95.89
Pyridoxine	0.9998	0.0144	99.52	99.05	96.65
Ascorbic Acid	0.9962	0.6754	96.56	99.39	99.03
Beta-carotene	0.9983	0.0039	97.45	99.58	99.03
α-tocopherol	0.9987	0.3971	97.41	99.46	97.25

The methods used for HPLC analysis showed good precision and accuracy values (above 95%). The values indicate that the methods had few random and systematic errors [36]. The percentage mean recoveries were all above 95% and were all reproducible to within 15%; an indication that the methods were fit for analytical determinations [33]. For α-tocopherol, the recovery value obtained is comparable to the range obtained by Katsa et al [37] while the range obtained for beta-carotene is comparable to the range obtained by Gebregziabher et al. [38]. Ascorbic acid had a recovery value of 99.03%; comparable to the ranges obtained by Odriozola-serrano et al. and Wall [39,40]. During the determination of thiamine, riboflavin, niacin, pyridoxine, tocopherol, beta-carotene, retinol, lycopene, and folic acid, Melfi et al. [41] obtained average recovery values of 97% ±10%. All the recovery values in our study were comparable to these results.

3.2.2 Water Soluble Vitamins in the Insects

Ascorbic acid was detected in all the insect samples (Table 3). The highest concentration was observed in lake flies, while the lowest concentration was observed in termites. For all the insects, the ascorbic acid concentration is highest in fresh samples. Oven-drying, sun-drying, and defatting processes lower the vitamin concentration in the insect samples with the defatted-sun dried samples having the highest degradation followed by the sun-dried samples, where less than 60% was retained. Ascorbic acid is degraded by exposure to light and heat and hence the losses observed in the processed insect samples [42]. Ascorbic acid concentration for oven-dried termites is in close agreement with a study conducted on oven-dried termites in South Western Nigeria which showed vitamin C concentration of (3.01-3.41) mg/100g [43]. However, the values are lower than those obtained from a de-winged oven-dried edible African termite with an ascorbic acid concentration of 17.76 mg/100g [44]. The levels of ascorbic acid in grasshoppers are much higher than those obtained from the analysis of brown and green longhorn grasshoppers from Siaya Kenya [24]. For lake flies and locusts, not much data is available for vitamin content comparison, however, the levels of ascorbic acid in lake flies are comparable to those obtained for super worms [45].

Statistical analysis showed a significant difference between fresh, sun-dried, oven-dried, defatted sun-dried, and defatted oven-dried concentrations ($p<0.0001$). However, there were no significant differences between; sun-dried and defatted sun-dried termites ($p=0.79$), oven-dried and defatted oven-dried termites ($p=0.51$), and between fresh termites and oven-dried termites ($p=0.05$). For grasshoppers, there was no significant difference between defatted oven-dried and defatted sun-dried grasshoppers ($p=0.22$), sun-dried and defatted oven-dried grasshoppers ($p=0.59$). For locusts, analysis of variance showed a significant difference in ascorbic acid concentrations for fresh, sun-dried, and oven-dried samples ($p<.0001$).

In the human body, ascorbic acid plays a role essential in the formation of blood vessels, muscle cartilage, and bone collagen. It also plays a role in the body's healing process and absorption and storage of Iron [44]. Its' insufficiency is characterized by fatigue, mood changes, and lethargy while its deficiency causes scurvy. The prevalence of ascorbic acid varies in high, medium, and low-income countries with numbers being as high as 79% in low- and middle-income countries such as India [46]. This study confirms that insects can be used to complement diets in such countries. The RDA for ascorbic acid is 2.5, and 3 mg/100g per day for children aged 0 to 6 months and 7 to 36 months respectively [48]. Despite the concentration of ascorbic acid being lowered by processing, the concentrations are still high enough to meet the RDA and are therefore suitable for use in children's complementary feeding. The best source of ascorbic acid would be lake flies, only about 26g of the insect would be needed to make a 1-kg formulation that meets 100% of the daily ascorbic acid RDA. The processed insect can further be used to complement the diets of pregnant and lactating mothers whose RDA is 5.5 and 7.7 mg/100g per day respectively.

Thiamine was detected in all the insect samples, with the highest levels of the vitamin observed in fresh and oven-dried termites followed by fresh and oven-dried grasshoppers (Table 3). For all the insects, vitamin B_1 concentration was highest in fresh samples followed by oven-dried samples, sun-dried samples, defatted oven-dried samples, and lastly defatted sun-dried samples. A high percentage loss of the vitamin was observed (>60%) with the highest loss being in sun-dried samples. The degradation of Vitamin B_1 is dependent on temperature, PH, and exposure to light [18]. Although sun-dried samples were subjected to lower temperatures, they were exposed to other conditions such as visible light, moisture in the atmosphere, as well as long exposure times; these factors attribute to high thiamine loss in sun-dried samples. For oven-dried termites, thiamine concentration is slightly higher than what was obtained from a study conducted on oven-dried termites in South Western Nigeria which gave thiamine concentration in the range of (1.54-1.98) mg/100g [43]. For lake flies, thiamine levels are comparable to that of other insects as reported in previous studies [47]. For fresh grasshoppers, results from this study are slightly higher than 0.47 mg/100g, a value obtained in a study done by [48]. Analysis of variance showed a significant difference between all the different sample processing for termites, locusts, and grasshoppers (p<.0001).

Table 3. Water-soluble vitamins mean concentration in fresh and processed insects

	Ascorbic Acid	Thiamine	Riboflavin	Niacin	Pyridoxine
Termites					
Fresh	4.30 ± 0.05^{a}	4.26 ± 0.00^{a}	ND*	0.026 ± 0.000^{a}	ND*
Sun-dried	2.50 ± 0.03^{b}	0.16 ± 0.00^{b}	ND*	0.027 ± 0.001^{a}	ND*
Oven-dried	3.37 ± 0.27^{ac}	1.00 ± 0.01^{c}	ND*	0.028 ± 0.000^{a}	ND*
DSD*	2.48 ± 0.06^{bd}	0.09 ± 0.00^{d}	ND*	ND*	ND*
DOD*	3.12 ± 0.06^{ce}	0.23 ± 0.00^{e}	ND*	0.028 ± 0.000^{a}	ND*
Grasshoppers					
Fresh	15.25 ± 0.35^{a}	0.69 ± 0.00^{a}	0.77 ± 0.00^{a}	0.079 ± 0.00^{a}	0.058 ± 0.000^{a}
Sun-dried	8.83 ± 0.18^{be}	0.21 ± 0.00^{b}	0.03 ± 0.01^{b}	0.055 ± 0.00^{b}	0.023 ± 0.000^{b}
Oven-dried	11.16 ± 0.14^{c}	0.28 ± 0.02^{c}	0.08 ± 0.00^{c}	0.085 ± 0.00^{c}	0.023 ± 0.000^{c}
DSD*	7.63 ± 0.74^{d}	0.06 ± 0.00^{d}	ND*	ND*	ND*
DOD*	9.17 ± 0.44^{de}	0.11 ± 0.00^{e}	ND*	0.028 ± 0.00^{d}	0.021 ± 0.000^{d}
Locusts					
Fresh	14.34 ± 2.15^{a}	0.189 ± 0.000^{a}	ND*	0.051 ± 0.000^{a}	ND*
Sun-dried	5.07 ± 0.27^{b}	0.036 ± 0.001^{b}	ND*	0.026 ± 0.000^{b}	ND*
Oven-dried	5.64 ± 0.08^{c}	0.054 ± 0.001^{c}	ND*	0.031 ± 0.000^{c}	ND*
Lake flies					
Sun-dried	114.95 ± 2.48	0.25 ± 0.01	ND*	0.033 ± 0.000	0.024 ± 0.000

Results are expressed as mean concentration ± standard deviation of the mean (in mg/100g)
In each column, values with the same superscript are not statistically different at $p < 0.05$
ND* - Not Detected, DSD* - Defatted Sun-dried, DOD* - Defatted Oven-dried

Thiamine deficiency, which causes beriberi, is prevalent in regions that consume diets high in refined and polished cereals and grains and low animal source foods [49]. In such regions, complementary diets need to be sought to ensure that the RDAs are met. The RDA requirements for thiamine are 0.02, 0.03, and 0.05 mg/100g per day for children aged 0-6, 7-12, and 13-36 months old respectively [50]. The insects studied in this work meet the daily RDA and are therefore suitable for use in children's complementary feeding. The best source of thiamine would be oven-dried termites; about 20 to 50 grams of oven-dried termites is what would be required to make a 1-kg formulation that meets 100% of the daily RDA. The processed insect can further be used to complement diets of pregnant and lactating mothers whose thiamine RDA is 0.14 and 0.15 mg/100g per day respectively.

Riboflavin was detected in grasshoppers only. The concentration of riboflavin is highest in the fresh grasshoppers. Riboflavin is highly sensitive to heat and light in the visible and UV region [51]; this attributes to the degradation observed in the oven-dried (only 10.9% retained) and sun-dried samples (only 3.4% retained) in this study. The results for riboflavin are comparable to those obtained from a study conducted on oven-dried grasshoppers in southwest Nigeria. The concentrations were in the range of (0.07-0.08) mg/100g [43]. However, they are lower than what was obtained by [48]. Analysis of variance showed a significant difference between all the fresh, sun-dried, oven-dried, defatted sun-dried, and defatted oven-dried grasshopper samples ($p<0.0001$).

Riboflavin is a flavoprotein involved in the reduction and oxidation of co-enzyme reactions in metabolic pathways and during energy production through the respiratory chain. Its deficiency results in dermatitis, angular stomatitis, migraine prophylaxis, and anemia [52]. Riboflavin deficiency is mostly observed in populations that have a low intake of meat and milk products [53]. The daily RDA for riboflavin is 0.03, 0.04, and 0.05 mg/100g for children aged 0-6, 7-12, and 13-36 months old respectively [50]. Though the sun-dried and oven-dried grasshoppers meet the daily RDA for riboflavin, they would be required in high quantities to meet 100% of the daily RDA; >500g per 1-kg formulation. The insect can be used in lower quantities to meet 50% of the daily RDA for riboflavin.

Niacin was detected in all the insect samples. Grasshoppers showed the highest niacin concentration thus making it the most suitable source of the vitamin among the studied insects. Niacin is more stable in comparison to thiamine and riboflavin. The percentage degradation for niacin was less than 50% for all the insects since niacin is not affected much by light, PH, and temperature. Its loss is mostly due to leaching in cooking water [54]. Oven-drying enhances the extraction of the vitamin in termites and grasshoppers (retention > 100%). Niacin was not detected in the defatted sun-dried samples for both termites and grasshoppers an indication that the specific process completely degraded the vitamin. Atowa et al. [48] obtained niacin levels of 1.35 and 1.10 mg/100g in grasshoppers and termites respectively; these levels are higher in comparison to what was obtained in our study.

Analysis of variance showed no significant difference between fresh termites, sun-dried termites, oven-dried termites, and defatted oven-dried termites ($p=0.22$). However, for grasshoppers and locusts, there was a significant difference between all sample processing ($p<.0001$). Niacin is involved in the transfer of hydride ions in many dehydrogenase-reductase systems in the human body and its deficiency causes dementia, dermatitis, and pellagra [55]. Pellagra is mostly observed in poor regions such as Africa, India, and China [53]. The RDA for niacin is 0.2, 0.4, and 0.6 mg/100g per day for children aged 0-6, 7-12, and 13-36 months respectively [50]. Niacin levels in the studied insects were quite low and they can be used to partially meet (about 10%) the daily RDA for niacin.

Pyridoxine was detected in grasshoppers and lake flies only. Like thiamine and riboflavin, pyridoxine is degraded by heat and light thus attributing to the losses observed in the oven-dried and sun-dried samples [56]. Analysis of variance shows a significant difference between all the sample processing methods ($p<.0001$). On studying pyridoxine levels in de-winged grasshoppers' levels of 25.32 ppm were obtained [48]; this concentration is higher than what has been obtained from this study. Pyridoxine is a coenzyme that aids the metabolism of amino acids, lipids, carbohydrates, and proteins; its deficit results in peripheral neuropathy [57]. Prevalence of pyridoxine deficiency has been reported to be at 10% and 40 % in children living in urban areas and rural areas respectively [49]. RDA for pyridoxine is 0.01, 0.03, and 0.05 mg/100g per day for children aged 0-6, 7-12, and 13-36 months respectively [50]. The insects studied in this work meet the RDA for children aged 0-6 months and can therefore be used for their complementary foods. The insects can be used to meet about 50% of the RDA when used for other ages.

3.2.3 Fat-Soluble Vitamins in the Insects

α-tocopherol was detected in all the insects at relatively high levels. α-tocopherol is not as photosensitive as beta-carotene. Degradation by sun-drying is, therefore, lower in this case. However, degradation increases with an increase in temperature [58]; this explains the lower concentrations in the oven-dried samples. Results also show that defatting enhances the extraction of the vitamin. Defatted samples have higher concentrations than sun-dried and oven-dried samples. Levels of tocopherol in the insects are much higher in comparison to what was obtained from the analysis of brown and green longhorn grasshoppers from Siaya Kenya [24]. Analysis of variance showed a significant difference between fresh, sun-dried, oven-dried, defatted sun-dried, and defatted oven-dried concentrations for all the insects ($p<.0001$).

α-tocopherol acts as an antioxidant in the body and its role is the protection of the cell membrane and lipids (polyunsaturated fatty acids) from injury [59]. The deficiency of α-tocopherol in the body results in complications in the nervous system. α-tocopherol deficiency has been observed in premature low birth weight babies and people with fat absorption complications [60]. Supplementing diets with α-tocopherol that is above the recommended upper limits has no negative effect. Consequently, it leads to improved cell meditated roles in humans and animals [61]. The studied insects meet the α-tocopherol RDA intake of 0.4, 0.5, and 0.6 mg/100g/day for infants aged (0-6), (7-12) and (13-36) months respectively [50]. Grasshoppers and lake flies would serve as the best source of the vitamin. The quantity of ground lake flies and grasshoppers that would be required to make a 1kg α-tocopherol-rich formulation that meets 100% of RDA is very small (<20g) and hence the insects would be very viable for the purpose. The processed insects can further be used to complement the diets of pregnant and lactating mothers whose α-tocopherol RDA is 1.2 and 1.6 mg/100g per day respectively.

Table 4. α-tocopherol mean concentrations in fresh and processed insects

	α-tocopherol	**Beta-carotene**
Termites		
Fresh	17.12±0.40^{a}	ND*
Sun-dried	4.47±0.16^{b}	ND*
Oven-dried	2.37±0.09^{c}	ND*
Defatted Sun-dried	12.25±0.87^{d}	ND*
Defatted Oven-dried	7.37±0.08^{e}	ND*
Grasshoppers		
Fresh	80.92±0.35^{a}	0.0199±0.001^{a}
Sun-dried	43.86±1.08^{b}	0.0040±0.000^{b}
Oven-dried	25.22±0.50^{c}	0.0004±0.000^{c}
Defatted Sun-dried	56.39±0.70^{d}	0.0005±0.000^{d}
Defatted Oven-dried	35.62±0.23^{e}	ND*

Lake fly		
Sun-dried	65.95±3.12	ND*
Locusts		
Fresh	42.00±0.40^{a}	0.101±0.003^{a}
Sun-dried	21.39±0.34^{b}	0.017±0.001^{b}
Oven-dried	19.98±0.33^{c}	0.024±0.001^{c}

Results are expressed as mean concentration ± standard deviation of the mean (in mg/100g)
In each column, values with the same superscript are not statistically different at $p < 0.05$
ND* – Not detected

Insects accumulate their carotenoids from the food they eat [32]. Beta-carotene was detected in locusts and grasshoppers only. This is expected since locusts and grasshoppers consume leaves and plant tissues that contain carotenoids. Lake flies and termites do not consume foods that are rich in carotenoids. Fresh samples were found to have higher levels of the vitamin in comparison to oven-dried, sun-dried, and defatted samples. The vitamin was not detected in defatted samples, an indication that it was completely degraded by the processing. Beta-carotene is susceptible to degradation in presence of UV-light, heat, oxygen, and moisture [62]. The vitamin undergoes oxidation via a free radical process. In the presence of water, the free radical oxidation is reduced. Beta-carotene concentration in grasshoppers is comparable to mealworm values (0.07mg/100g) [45]. Analysis of variance showed a significant difference between all sample processing for termites and locusts. However, there was no significant difference between oven-dried and defatted sun-dried grasshoppers (p=0.76).

Beta-carotene is required in small amounts for the right functioning of the visual system, growth and development, right immune functionality, reproduction, and the maintenance of epithelial cellular integrity [50]. Deficiency of vitamin A in most cases results in an impaired visual system though it may cause other symptoms such as poor reproductive health, high morbidity, and a higher risk of anemia all of which are non-specific symptoms. Sufficient intake of vitamin A for children between 6 months and 6 years reduces their dying risk by an average of 20-30% [50]. Though the studied insects do not meet the RDA of 0.048, 0.06, and 0.036mg/100g/day of beta carotene for infants aged (0-6), (7-12), and (13-36) months respectively, they can be used to meet about 50% of the RDA when used in formulations.

4. CONCLUSION

The study confirms that locusts, grasshoppers, lake flies, and termites contain substantial amounts of water-soluble vitamins. Oven-drying and sun-drying processes lead to water-soluble vitamins degradation, with sun-drying being the most destructive. Defatting further degrades the vitamin content of the sun-dried and oven-dried insect samples. The insects have the potential for use in the formulation of vitamin-rich complementary foods. Lake flies can serve as a source of ascorbic acid, termites as a source of thiamine, and grasshoppers as a source of riboflavin, niacin, and pyridoxine. Processing leads to the degradation of fat-soluble vitamins with oven-drying being the most destructive. The studied insects have high levels of α- tocopherol but low levels of beta-carotene. Whether sun-dried or oven-dried, locusts, grasshoppers, and lake flies can be comfortably used for the formulation of α-tocopherol-rich complementary foods while grasshoppers and locusts can be used to meet part of the daily RDA for beta-carotene.

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