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Nutritional composition and microbial quality of Processed edible dung beetle larvae (*Scarabaeus satyrus)*

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Abstract

The larva of the African dung beetle (Scarabaeus satyrus) is a delicacy in human diets. This insect is presumed to be nutritious, while its safety aspects are in doubt. The objective of this study was to evaluate the nutritional composition and microbial quality of the processed larvae using standard methods of analysis. The larvae were collected from farms in three regions of Bungoma, Kakamega and Siaya using random sampling. The samples were subjected to toasting, oven drying and roasting. A factorial arrangement was used in the study and data analysed using generalized linear models. The study focused on proximate composition of Moisture, protein, fat, fibre, and ash, elemental Mineral analysis as well as microbial content of total viable counts (TVC), Salmonella, Staphylococcus sp., Coliforms and yeasts and moulds. Results showed that S. satyrus larvae contained crude protein of 59.65 - 66.05 g/100g, crude fat (15.18-16.87 g/100g) and crude ash (4.45-4.67g/100g) on dry weight basis. The mineral Iron was the most abundant trace element with a value of 19.19mg/100g, while phosphorus was the most plentiful macro mineral with 331.42mg/100g. Salmonella sp. was not detected in any of the samples. Total viable counts, Staphylococcus aureus, Escherichia coli, yeasts and moulds were present with raw samples from Bungoma County containing highest amounts of total viable counts (6.20±0.06 Log cfu/g). The roasting technique had the greatest effect of reduction of viable counts by over two log cycles to 4.15±0.05 Log cfu/g. The study showed that heat processing is effective in lowering the microbial load to levels that are safe for human consumption. These findings indicate that S. satyrus is a rich source of macro nutrients and minerals, and is recommended as an alternative protein source.

Keywords: *Nutritional composition; microbial quality; Mineral, Scarabaeus satyrus*

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Introduction

Food and nutrition security has become a major concern more so due to the increasing world population, yet food choices are limited to the conventional and familiar options. Human diets for the vulnerable and resource poor are bereft of proteins and vital minerals. This has led to incidences of protein-energy malnutrition as well as micronutrient deficiencies commonly known as hidden hunger. Insects could provide a valuable source of minerals and vitamins (Finke, 2013) essential for human development (Ajai *et al.*, 2013). Studies have reported that insects are a

vital and essential source of protein, fat, minerals and vitamins (Durst et al., 2010) in Africa and other developing countries as well as among various cultures scattered throughout the world. Furthermore, it is estimated that insects commonly contain a higher amount of the micronutrients iron, zinc, and calcium when compared to the rest of the diet of developing countries (Christensen *et al*., 2006).

People throughout the world have been eating insects as a regular part of their diets for many years. As people in rural areas suffer from under nutrition, especially protein-energy malnutrition (PEM) in Africa, Latin America and Asia, alternative nutritional food sources, that can be sustainably produced, are required. Insects present a perfect food since they require less water and land to produce, and their foot prints on the environment is negligible because they produce less greenhouse gases.

The most commonly eaten insect groups are beetles, caterpillars, bees, wasps, ants, grasshoppers, locusts, crickets, cicadas, leaf and plant hoppers, scale insects and true bugs, termites, dragonflies and flies (Jongema, 2015). It is estimated that entomophagy is practised by at least 2 billion people worldwide. More than 1900 insect species have been documented in literature as edible, most of them in tropical countries (Van Huis, et al., 2013). In Eastern Africa, the insects commonly consumed are termites, crickets, grasshoppers, black ant, and beetles, among others. In Kenya, insect species such as lake flies, 'agoro' termites, black ants, crickets, and grasshoppers, form part of traditionally consumed meals in the western part of the country (Ayieko et al., 2012).

The larvae of dung beetle (*Scarabaeus satyrus*) are mostly consumed by communities inhabiting the western region of Kenya. It is commonly consumed after toasting, roasted or fried and served as snacks or taken with carbohydrate foods. In spite of the dung beetle larvae being a delicacy in the human diets, its habitat predisposes it to contamination with food borne pathogens and microbes such as aerobic bacteria, coliforms, *Staphylococcus sp*., and yeasts and moulds. Many challenges have hindered it from being widely consumed or utilized in the diets. Traditional processing methods, pre- cooking preparation and cooking methods have an influence on the amounts of nutrients that would eventually be available at consumption and absorption by the body. It is against this background that this study set out to investigate the nutritional and safety aspects of the processed larval stage of the dung beetle.

The larvae of the *Scarabaeus satyrus* was collected from three regions of Western Kenya, namely Bungoma, Kakamega and Siaya. These regions were chosen for sampling of the study materials because the inhabitants are familiar with this edible insect. In Bungoma and Kakamega, this insect is a delicacy and has been traditionally consumed in diets as well as fed to poultry over many decades. In Siaya County, while the inhabitants have not yet embraced it in their diet, it has been reported that poultry normally rummage in the composite manure searching for the insect larvae and swallowing them after shredding them into small pieces using their beaks.

It is envisaged that this study will provide valuable information that will lead to the in depth understanding of the edible dung beetle. This information will add to the body of knowledge about this insect, as well as contribute to its enhanced utilization for food and feed and help solve the nutritional challenges that face vulnerable populations in most parts of the world.

Materials and Methods

Experimental design

A completely randomize design was employed in the study, where treatments were allocated at random to experimental materials. A factorial arrangement was used, where two levels (factors) of treatments were considered, that is geographical regions and processing techniques. The three regions were: Bungoma, Kakamega and Siaya, and three processing techniques were: toasting, oven-drying and roasting. Some samples for microbial analysis were blanched before thermal processing. Each of the experimental analysis was replicated three times. The nutritional and safety variables were the dependent variables while processing techniques and regions of sampling were the independent

variables. It was assumed that the experimental materials were fairly homogenous.

Sample collection

Purposive sampling was employed in the study, whereby 2 kg of edible dung beetle larvae were collected from seven identified farms each, in Siaya, Kakamega and Bungoma counties, making 21 samples in total. The samples were hand-picked from composted cow dung manure and transported in buckets *in situ* to the University Food Science Laboratories to await evisceration and analysis. The seven larvae samples from each county were then mixed to form three separate composite samples, each weighing 14 kg.

Sample preparation

Processing and milling of the insect larvae into flour before experimental analysis

3 kg each of the raw composite samples of the larvae were cleaned before processing. Cleaning involved first washing the larvae with running tap water to remove the external debris, followed by de-gutting and rinsing with distilled water. The de-gutting was done carefully by removal of only the dung waste at the lower end of the abdomen via evisceration using a sharp knife. After cleaning, 3 kg samples each from Bungoma, Siaya and Kakamega regions were subjected to three drying techniques of oven drying, toasting and roasting. For the oven-dried samples, 300 g of cleaned and de-gutted raw insects were dried for 24 hours until brittle in an air-oven dryer (TD-384KN model Thermotec, Tokyo, Japan), maintained at 70^{0}C. After drying the products were cooled to 25^{0}C for 30 minutes, after which they were put in pre labeled 500g zip lock bags.

To evaluate the effect of blanching on microbial load, 300 g of eviscerated larvae samples from each of the three counties were subjected to blanching at 60^{0}C prior to toasting, while another batch was not blanched before toasting. During toasting, 300 g of the cleaned and de-gutted raw larvae were heated on a stainless-steel shallow pan at 300^{0}C. The larvae were occasionally turned for ten minutes until brown, after which they were cooled at room at 25^{0}C for 30 minutes then packed in 500g zip lock bags. Roasted samples were pricked onto barbeque wires and heated 10 cm above hot charcoal embers at 500^{0}C with the larvae being occasionally turned to avoid charring, until the larvae were sufficiently dry and brittle after which they were cooled and stored in airtight 500g zip lock bags at 25^{o}C. Before analysis, all the processed samples were ground into flour using a fine mill (Model Bauermeister, Hamburg-Altona, Germany) after which the flour samples were put in air tight zip lock bags and kept in a refrigerator (10^{0}C) until nutritional and physicochemical analysis. Samples for microbial analysis were kept in a deep freezer at -10^{0}C.

Data collection

Determination of Proximate composition

Moisture content was assayed by the drying method, crude fat by Soxhlet extraction method and crude protein by semi-micro-Kjeldahl method (AOAC, 2000). Nitrogen values obtained were converted to crude protein using a factor of 6.25. Crude ash was determined by incinerating 5 g of processed larval powder sample in a muffle furnace at 550^{0}C. Dietary fibre was determined by enzymatic gravimetric method (AOAC, 2000). Available carbohydrate value was calculated as the difference between 100 and the sum of the percentages of protein, fat, ash and dietary fibre.

Analysis of specific minerals

The processed samples were analysed for elemental mineral content of Ca, Zn, Fe, Mg and Cu, using a Shimadzu model Atomic Absorption Flame Emission Spectrophotometer (Shimadzu Corp., Kyoto Japan, Model AA-6200) using the respective cathode lamps. Total Phosphorus (P) was determined by the Ascorbic acid method using UV-Visible spectrophotometer with absorption wavelength of 420nm. Sodium (Na) and Potassium (K) were analysed using the Wagtech Flame Photometer. Preparation of samples for analysis was as follows: 2 g of each of the samples were dry- ashed in a muffle furnace at 550 ^{0}C for about ten hours. The ash was dissolved in 1 % HCl acid in a conical flask and made up to 100ml mark using a standard volumetric flask. The individual mineral element composition was calculated from the AAS or U-Visible spectrometer, or Flame Photometer using the formula generated from the Standard curves. Analyses were done in triplicate.

Calculation of total Energy (Kcal/g)

The energy content of the processed larvae of edible *Scarabaeus satyrus* was calculated using the Atwater general factor system:

Energy = (CHO x 4) + (Crude Protein x 4) + (Crude Fat x 9) Kcal/g.

Evaluation of microbial content of Scarabaeus satyrus

Sample preparation

Samples were prepared for analysis according to Khanom, Shammi, & Kabir, 2016 protocol**.** 10 g of each raw or processed sample was homogenized with 90.0 ml of sterile normal saline to prepare stock solution. Stocks were serially diluted (1:10) to 10^{-5} by adding 1ml of stock solution to 9 ml normal saline in test tubes. One milliliter of diluted sample was inoculated on Nutrient agar (NA), Baird Parker agar (BPA) and Potato Dextrose agar (PDA) media following pour plate method and incubated at 37^0C for 18-24 hours except for PDA which was incubated at 25^0C for 48-72 hours. All media were prepared according to manufacturer's instructions.

Experimental Design

A CRD in a Factorial arrangement was used. Two factors (region of sample collection and processing techniques) were used. 12 samples were used in each experiment. 3 composite samples, 3 replicates for each and 4 processing techniques were employed in the study.

Data Collection

Determination of total aerobic bacterial count

Nutrient agar (NA) media was used to determinate the total bacterial count (Khanom et al., 2016). NA plates were labelled for appropriate dilutions to be used for dilution and pour plate method. Plates were inoculated and incubated at 37 ◦C for 18-24 hours. Total number of bacteria cfu/g of sample was calculated and recorded for interpretation of the results.

Determination of counts of Staphylococcus aureus

Baird Parker agar (BPA) was used to determine the counts of *Staphylococcus aureus* (Birgen, Njue, Kaindi, Ogutu, & Owade, 2020). Plates were inoculated and incubated at 37 ◦C for 18-24 hours. Observation ensued for typical colonies appearing black or grey, shining and convex, and 1-1.5mm in diameter after 24 hours and 1.5-2.5mm after 48 hours of incubation, surrounded by a clear zone but partially opaque zone. Typical *S. aureus* colonies were counted to calculate colony forming units per gram of sample.

Determination of coliform bacteria

Coliform Count Plate agar was used for measurement of coliform counts and the cells were incubated at 35 ± 1^0C for 24 ± 2 hours. The number of colonies forming bubbles around the red colonies was counted by selecting the plates that produced 30 to 300 colonies (Khanom et al., 2016)

Enumeration of yeasts and moulds

Diluted samples were inoculated onto Potato Dextrose Agar medium supplemented with chloramphenicol (40mg/L) by spread plate method. The plates were incubated at 25◦C for 48-72 hours. Visible colonies were counted and calculated as the total yeast and mould and recorded as cfu/g.

Calculation of cfu/g

$$N = \frac{\sum C}{(N1 + 0.1N2)D}$$

∑C – Is the sum of colonies counted on all the plates retained

N1- number of plates retained in the first dilution

N2 – numbers of plates retained in the second dilution

D- The number of dilution factor corresponding to first dilution factor

Statistical data analysis

Both the nutritional and microbial data was analysed using Stata SE version 12 (Stata Corp LP, TX). Descriptive statistics of mean and

standard deviation of the values were obtained. The microbial counts were transformed into log CFU. The Analysis of Variance (ANOVA)

test was used to test for statistical difference in the nutritional content and microbial counts with statistically different means separated using Bonferroni test. Statistical significance was tested at $p \leq 0.05$.

Results

Proximate composition and energy of raw and processed larvae of Scarabaeus satyrus

The results of proximate composition and energy of the raw and processed larvae of dung beetle (*Scarabaeus satyrus*) are presented in Table 1. Moisture content ranged between 71.4 and 73.26

The protein quantity in processed samples varied considerably ($P \leq 0.05$) from one another as compared with the raw samples. These protein levels were found to be either higher or lower than raw samples, and ranged between 50.49 g/100g for roasted samples from Kakamega to 68.31 g/100g for oven dried samples from Siaya. The protein content was significantly lower ($P \leq 0.05$) in toasted samples from Bungoma and Kakamega by 1.27g/100g and 11.56g/100g respectively, translating to a 2.06% and 18.6% reduction, respectively, when compared to raw samples%. Based on these figures, the dry matter of the larvae ranged between 26.74-28.6 g/100g. Protein content (Table 1) was 61.67, 59.65, and 62.05 g/100g (all on dry weight basis for raw samples from Bungoma, Siaya and Kakamega regions respectively. Samples from Siaya had lower protein content followed by Bungoma samples, with Kakamega having the highest amount.

Table 1

Proximate Composition and Energy Content of Raw and Processed Larvae of Scarabaeus. Satyrus

County	Processing technique	Proximate composition						
		Moisture (%)	**Crude Fat (g/100g)**	**crude fibre (g/100g)**	**Ash (g/100g)**	**Protein (g/100g)**	**CHO (g/100g)**	**Energy (Kcal/100g)**
Bungoma	Raw (un-processed)	71.14±0.36^{f}	16.87±0.55^{a}	8.4±0.04^{abcd}	4.49±0.54^{bcd}	61.67±1.66^{a}	8.57±1.58^{d}	432.79
Siaya		73.05±0.63^{c}	15.78±0.27^{acd}	8.28±0.12^{adf}	4.45±0.51^{bcd}	59.65±0.64^{ab}	11.84±0.32^{a}	427.98
Kakamega		73.26±0.12^{c}	16.75±0.73^{a}	7.54±0.06^{ef}	4.67±0.42^{cde}	62.05±1.40^{a}	8.98±0.89^{d}	434.87
Bungoma	Oven dried	7.63±0.12^{a}	16.14±0.19^{ad}	8.51±0.13^{abcd}	3.53±0.07^{a}	66.55±0.98^{d}	5.27±0.99^{b}	432.54
Siaya		7.65±0.17^{a}	14.63±0.08^{bce}	8.38±0.32^{abd}	5.41±0.09^{e}	68.31±0.25^{d}	3.27±0.46^{b}	417.99
Kakamega		7.91±0.09^{a}	16.44±0.33^{a}	7.77±0.52^{def}	4.11±0.39^{abc}	58.89±0.33^{b}	12.78±1.07^{a}	434.64
Bungoma	Toasted	11.49±0.15^{e}	13.48±0.32^{e}	8.67±0.07^{abc}	3.67±0.08^{ab}	60.40±0.64^{ab}	13.77±0.88^{a}	418.04
Siaya		8.18±0.10^{ab}	14.40±0.41^{be}	8.57±0.26^{abc}	5.29±0.06^{de}	67.48±0.16^{d}	4.26±0.42^{b}	416.56
Kakamega		8.22±0.06^{ab}	16.41±0.50^{a}	9.14±0.09^{c}	3.97±0.14^{abc}	50.49±0.45c	19.99±0.84^{c}	429.61
Bungoma	Roasted	6.15±0.08^{d}	15.00±0.11^{bcd}	9.08+0.44^{bc}	3.55±0.05^{a}	51.25±0.65^{c}	21.11±0.37^{c}	424.44
Siaya		7.54±0.20^{a}	15.01±0.33^{bcd}	8.52±0.08^{abcd}	3.92±0.04^{abc}	60.58±0.15^{ab}	11.97±0.38^{a}	425.29
Kakamega		8.76±0.18^{b}	15.13±0.41^{bcd}	7.42±0.08^{e}	3.77±0.08^{ab}	51.25±0.65^{c}	22.43±0.92^{c}	430.89
P VALUE		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Notes

All the values except moisture are presented on dry weight basis
Values are mean, SD, and n=3. Values with the same superscript in the same column are not statistically different at the $P \leq 0.05$ level

The fat content of *Scarabaeus satyrus* was found to be 16.87g/100, 15.78g/100g and 16.75g/100 for raw samples from Bungoma, Siaya and Kakamega region respectively. On the other hand, processed samples differed significantly ($P \leq 0.05$) in amounts of crude fat depending on processing technique and County of sampling, and ranged from a low of 13.48g/100g for toasted samples from Bungoma to a high of 16.44g/100g for oven dried samples from Kakamega ($P \leq 0.05$). In the current study, processing decreased fat content by a range of 0.43g/100g to 3.39 g/100g (Table 1). The lowest decrease was in oven dried samples from Kakamega while the highest decrease was in toasted samples from Bungoma County. Oven drying, toasting, and roasting all reduced the fat contents of Bungoma samples by 4.33%, 20.10%, and 11.08%, respectively.

Crude fibre content of raw samples was less than 10g/100g with Bungoma, Siaya and Kakamega sampling sites having amounts of 8.4, 8.28 and 7.54 g/100g respectively (Table 1). Processed samples had amounts of fibre ranging from a low of 7.42g/100g for smoked samples from Kakamega and a high of 9.08g/100g for oven dried samples from Bungoma.

Mineral content of processed Scarabaeus satyrus larvae

Table 2 illustrates the findings on the mineral content of the *Scarabaeus satyrus*. All the samples collected from the three regions of Bungoma, Siaya and Kakamega contained substantial amounts of Iron, Zinc, Calcium, Copper, Magnesium, Sodium, Phosphorus and Potassium. The amounts of micro mineral Iron in the oven dried, toasted and roasted samples were found to be a function of processing method and County of sampling.

The amount of fat, protein, fiber, and ash was subtracted from one hundred to determine the nitrogen free extract (soluble carbohydrate). As a result, the amount of carbohydrates in both raw and processed samples varied depending on the nutrients' dry matter content. Raw samples had carbohydrate (CHO) amounts of 8.57 g/100g, 11.54g/100, and 8.98g/100 dry weight basis for Bungoma, Siaya and Kakamega respectively. On the other hand, the CHO content ranged from a low of 3.27 g/100g for oven dried samples from Siaya to high of 22.43g/100g for roasted samples from Kakamega (Table 1).

The energy content in *Scarabaeus satyrus* larvae was found to range between 416.56 to 434.87 Kcal/100g on dry weight basis for both raw and processed insect. Raw samples from Kakamega contained the highest amount of energy (434.87 Kcal/100g d.w), while toasted samples contained relatively lower energy amounts when compared to the other samples (416.56 Kcal/100g d.w). For people who can only consume 2000 Kcal/day, this insect may be able to provide 20.83% to 21.74% of energy for every 100g of the insect consumed on dry weight basis.

Table 2

Mineral Composition of Processed Larvae of Scarabaeus satyrus (mg/100g (Dry Weight Basis))

County	Processing technique	Minerals (mg/100g d.w)							
		Calcium	Iron	Zinc	Magnesium	Copper	Sodium	Phosphorus	Potassium
Bungoma	Oven dried	16.19±0.5^{a}	14.38±0.86^{d}	6.04±0.25^{a}	142.48±6.29^{e}	1.21±0.14^{ac}	54.33±4.93^{a}	234.61±59.49^{a}	151.67±37.53^{bcd}
Siaya		17.61±0.6^{a}	17.61±0.64^{ab}	6.35±0.72^{ab}	166.78±0.60^{cd}	1.49±0.08^{abd}	63.00±2.65^{ab}	230.21±3.30^{a}	183.33±20.21^{abcd}
Kakamega		24.18±1.6^{b}	17.77±0.14^{ab}	6.04±0.23^{a}	157.58±1.13^{a}	1.39±0.1^{ab}	60.33±4.51^{ab}	267.99±9.98^{abc}	140.00±18.03^{bc}
Bungoma	Toasted	17.49±0.50^{a}	16.96±0.56^{ac}	6.03±0.34^{a}	150.76±1.27^{b}	1.69±0.59^{d}	58.67±5.51^{ab}	268.74±5.81^{abc}	129.33±4.51^{b}
Siaya		17.80±0.39^{a}	19.19±0.62^{b}	6.33±0.14^{ab}	167.41±0.83^{d}	1.52±0.13^{bd}	63.67±5.51^{ab}	244.55±12.4^{a}	216.67±15.28^{a}
Kakamega		22.43±0.98^{b}	19.09±0.15^{b}	6.34±0.11^{ab}	159.04±0.35^{a}	1.08±0.08^{c}	68.33±4.16^{b}	331.42±8.00^{cd}	223.33±12.58^{a}
Bungoma	Roasted	16.38±1.95^{a}	15.33±0.15^{cd}	6.52±0.18^{b}	149.63±0.72^{b}	1.42±0.12^{abd}	63.00±1.00^{ab}	278.43±11.18^{d}	195.00±18.03^{acd}
Siaya		15.28±0.23^{a}	18.08±1.10^{ab}	6.48±0.10^{b}	160.48±0.79^{ac}	1.35±0.08^{abc}	60.33±4.04^{ab}	253.07±11.43	206.67±2.89^{ad}
Kakamega		23.79±1.18^{b}	16.54±0.55^{ac}	6.20±0.11^{ab}	154.99±0.29^{ab}	1.35±0.04^{abc}	66.33±1.15^{ab}	309.82±10.27^{bcd}	163.33±23.09^{abcd}
P value		<0.001	<0.001	0.022	< 0.001	<0.001	0.02	< 0.001	< 0.001

Notes

Values are Mean ±SD, n=3, Values on the same column with different superscripts are significantly different (P≤ 0.05)

The effect of processing was not significant in the case of copper; however, the effect of County of sampling was ($P \leq 0.05$). According to Table 2's values, the quantities of zinc in roasted samples from Bungoma ranged between 6.03 mg/100 g and 6.52 mg/100 g. The Bungoma samples that were roasted and oven dried did not significantly differ from one another ($P \geq 0.05$). Likewise, no significant differences were observed between oven dried and toasted samples from Siaya. Toasted and smoked samples from Kakamega did not have significant differences ($P \leq 0.05$). The amounts of Copper ranged between 1.08-1.69mg/100g on dry weight basis for toasted samples from Kakamega and toasted samples from Bungoma.

According to Table 2, the calcium concentrations ranged from 15.28 mg/100 g in Siaya's roasted samples to 24.18 mg/100 g in Kakamega's oven-dried samples. In all of the samples from Bungoma and Siaya, the quantities of calcium did not significantly differ ($P \leq 0.05$). Samples from Kakamega had the highest calcium content as compared to the other regions. The processing methods had no effect on the levels of sodium (Na) in the samples of *S. satyrus*. The amounts of Sodium ranged between 54.33 mg/100g for oven dried samples from Bungoma and 66.3 mg/100g in roasted samples from Kakamega. The amounts of Potassium (K) ranged between 151.67mg/100g for oven dried samples from Bungoma and 223.33mg/100g for toasted samples from Kakamega. Oven dried samples from Siaya and Kakamega had relatively lower amounts of Potassium as compared to toasted and roasted samples (Table 2).

The results show that Magnesium content ranged from 142.48 mg/100g in oven dried samples from Bungoma and 167.41 mg/100g in toasted samples from Siaya (Table 3). Oven drying and toasting was not significant for samples from Kakamega. Likewise toasted and roasted samples from Bungoma region did not have significant differences in their amounts of Magnesium ($P \leq 0.05$). Phosphorus was the dominant macro mineral in all the processed samples of *S. satyrus*. Amounts of the Phosphorus in the samples ranged between 234.61 mg/100g in oven dried samples from Bungoma and 331.42 mg/100g in toasted samples from Kakamega. The amount of phosphorus in the samples was not influenced by oven drying or toasting for samples from Siaya ($P \leq 0.05$).

Microbial content in raw and processed Scarabaeus satyrus

Table 3 displays the findings of the investigation of the microbiological content of raw and processed *Scarabaeus satyrus*. Raw and processed (oven dried, toasted and blanched, toasted and roasted) samples of *S. satyrus* were assessed for microbial load of total viable counts (TVC), *Staphylococcus aureus, Escherichia coli*, *Salmonella*, as well as moulds and yeasts, with representative samples presented in Figures 1 to 4.

Figure 1

Moulds and Yeasts in Scarabaeus satyrus samples

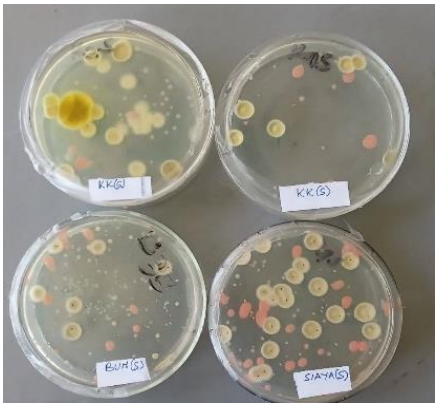


Figure 2

Staphylococcus sp in Scarabaeus satyrus samples

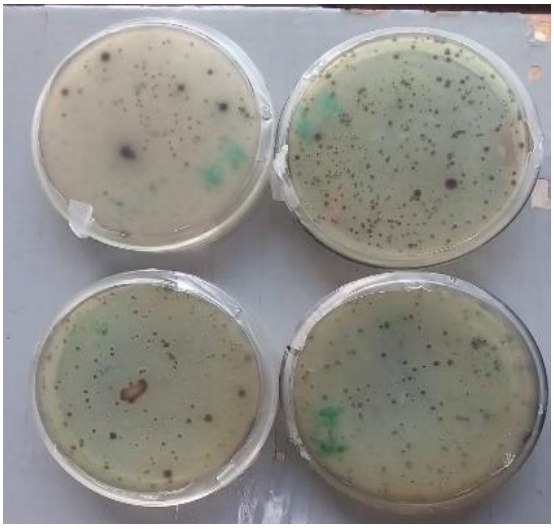
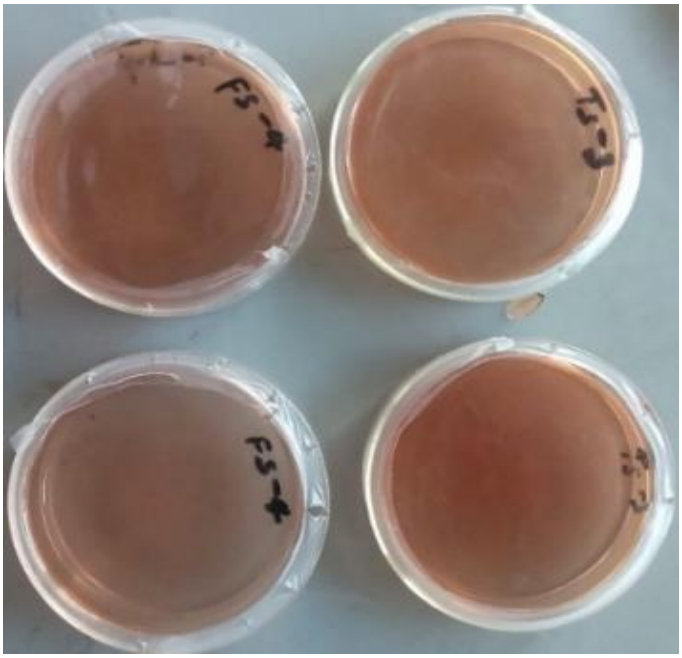


Figure 3

Coliforms /Lactose fermenters colonies in Scarabaeus satyrus samples

Figure 4

Salmonella colonies (not detected) in Scarabaeus satyrus samples



From the results, it can be seen that analysed samples contained the analysed microbes, except s*almonella* which was not detected in any of them. The total viable counts (TVC) were highest in raw samples (6.20 Log CFU/g) from Bungoma County and lowest in smoked samples (3.38 Log CFU/g) from Kakamega County. Oven drying had the least reduction in the TVC, while thermal treatment by roasting contributed to the highest reduction in the TVC for all the samples from the three regions. Raw samples from Kakamega contained 6.03 Log CFU/g of TVC, and on exposing them to thermal processing, the TVC reduced to 5.41Log CFU/g, 4.23 Log CFU/g, 4.65 Log CFU/g and 3.38 Log CFU/g for oven dried, blanched and toasted, toasted without blanching and smoked samples respectively, representing a reduction in the microbes by 0.62 Log CFU/g, 1.8 Log CFU/g, 1.38 Log CFU/g, and 2.65 Log CFU/g for oven drying, blanched & toasted, toasted without blanching and roasting respectfully.

The reduction in the TVC by processing was in the order: roasting > blanched and toasted > not blanched and toasted > oven died. A two-way analysis of Variance for total viable counts suggested that the main effect of processing was significant, while the County of sampling was not significant Coliforms in the raw and processed samples were also assessed, and results indicated that only the processing methods were significant in affecting the levels of microbes in the samples. The coliforms (*E. coli*) in raw samples from Bungoma, Siaya and Kakamega were 5.25 Log CFU/g, 5.22 Log CFU/g, and 5.18 Log CFU/g respectively. On subjecting them to the processing methods of oven drying, toasting with blanching, toasting without blanching, and smoking, the reduction in the amounts of coliforms was significant ($P < 0.001$).

Table 3

Microbial Content of Raw and Processed Scarabaeus satyrus (Log CFU/G)

County	Processing technique	Microbial counts per region (Log cfu/g)				
		Total viable counts	***E. coli***	***S. aureus***	**Moulds & Yeasts**	***Salmonella***
Bungoma	Raw	6.20±0.06^{h}	5.25±0.51^{a}	5.38±0.07^{a}	3.32±0.36^{i}	0.00
	Oven dried	5.24±0.09^{cde}	4.82±0.08^{ab}	4.22±0.07^{ae}	2.71±0.01ab^{gh}	0.00
	Blanched &toasted	5.04±0.04^{bcd}	4.48±0.01^{bc}	3.62±0.02^{cd}	2.30±0.02ab^{cdef}	0.00
	Not blanched &toasted	5.13±0.04^{bcde}	4.61±0.01^{ab}	3.89±0.01^{ac}	2.58±0.04^{abfg}	0.00
	Roasted	4.15±0.05^{af}	3.12±0.05^{d}	3.23±0.03^{b}	1.95±0.05^{cde}	0.00
Siaya	Raw	5.75±0.51^{egh}	5.22±0.53^{a}	4.44±0.04^{eg}	3.24±0.47^{hi}	0.00
	Oven dried	5.33±0.03^{de}	4.91±0.01^{ab}	4.19±0.01^{ae}	2.88±0.06^{bghi}	0.00
	Blanched & toasted	4.56±0.02^{ab}	3.82±0.01^{ce}	3.41±0.02^{bd}	2.02±0.07^{cdef}	0.00
	Not blanched& toasted	4.70±0.01^{abc}	3.90±0.01^{c}	3.91±0.01^{ac}	2.40±0.01^{abdef}	0.00
	Roasted	3.89±0.01^{fi}	3.08±0.04^{d}	2.83±0.02^{f}	1.78±0.08^{c}	0.00
Kakamega	Raw	6.03±0.62^{gh}	5.18±0.48^{a}	4.62±0.46^{g}	3.17±0.44^{ghi}	0.00
	Oven dried	5.41±0.03^{deg}	4.82±0.10^{ab}	3.92±0.01^{ac}	2.78±0.12^{abghi}	0.00
	Blanched & toasted	4.23±0.03^{af}	4.30±0.02^{bc}	3.21±0.03^{b}	2.20±0.03^{acdef}	0.00
	Not blanched & toasted	4.65±0.02^{abc}	4.79±0.01^{ab}	3.51±0.02^{bd}	2.49±0.01^{abef}	0.00
	Roasted	3.38±0.03^{i}	3.19±0.04^{de}	2.51±0.02^{e}	1.85±0.13cd	0.00
	Max. counts*	5	<1	<1	2	Absent
	P value	< 0.001	<0.001	<0.001	<0.001	<0.001

Notes Values are Mean ±SD, n=3 Values in the same column with the same letters are not significantly different at 0.1%

The results in Table 3 depict a scenario where all the analysed samples contained *Staphylococcus aureus*. Raw samples from Bungoma, Siaya and Kakamega were found to contain 5.36 Log CFU/g, 4.44 Log CFU/g and 4.62 Log CFU/g of this microbe respectively. Upon subjecting these samples to processing, the *Staphylococcus aureus* in samples from Bungoma reduced to 4.22 Log CFU/g, 3.62 Log CFU/g, 3.89 Log CFU/g and 3.23 Log CFU/g for oven dried, toasted with blanching, toasted without blanching and roasting respectively. In all of the samples that underwent the various processing methods, the quantities of microorganisms varied significantly ($P \leq 0.05$). Oven drying had the least reduction in the *Staphylococcus aureus* while roasting had the highest reduction. This reduction was by 1.16 Log CFU/g, 1.76 Log CFU/g, 1.49 Log CFU/g and 2.15 Log CFU/g respectively for oven dried, blanched and toasted, toasted without blanching and roasted samples. Samples that were blanched before toasting had lower amounts of the *Staphylococcus aureus* as compared to the un-blanched ones.

Depending on the processing method, the reduction in the microbes followed the order roasting >toasted with blanching>toasted without blanching>oven dried. Processing and County of Sampling had the most notable and significant effects. The reduction in the content of *Staphylococcus aureus* through processing followed the same pattern for the samples from Siaya and Kakamega regions. Moulds and yeasts were found to be present in the analysed samples (Table 3) though in relatively lower amounts compared to TVC, *Escherichia coli* and *Staphylococcus aureus*. Raw samples from Bungoma had 3.32 Log CFU/g, Siaya 3.24 Log CFU/g of moulds and yeasts. Oven drying reduced amounts of moulds and yeasts to 2.71 Log CFU/g, 2.30 Log CFU/g, 2.58 Log CFU/g, and 1.95 Log CFU/g for samples from Bungoma, Siaya and Kakamega respectively relative to the raw samples. This represented a reduction by 0.62 Log CFU/g for Bungoma samples, 0.41 Log CFU/g for Siaya samples and 0.39 Log CFU/g for Kakamega samples. When oven drying was compared to roasting, it was found that there was a reduction by 1.37 Log CFU/g for Bungoma samples, 1.46 Log CFU/g for Siaya samples and 1.32 Log CFU/g for samples from Kakamega region. This shows that roasting was a more effective processing method in reducing the amounts of moulds and yeasts in the samples. Samples that were subjected to toasting after blanching had a higher reduction in moulds and yeasts as compared to un-blanched samples. Results for moulds and yeasts indicated that the amount of these microbes was dependent upon processing techniques. The effects of processing and County of sampling were significant ($P \leq 0.05$)

Discussion

Proximate composition and energy of raw and processed larvae of Scarabaeus satyrus

Larvae from Bungoma had the lowest moisture content of 71.4% as compared to Siaya which had 73.05% while those from Kakamega County had the highest moisture of 73.26%. These figures are higher than the 61.85% for the beetle *Rhynchophorus phoenicis* (F) found in Nigeria (Ekpo & Onigbinde, 2005). The high moisture content of the larvae predisposes them to low dry matter content (26.74-28.6%). Additionally, the high moisture increases the perishability of the insect after the process of degutting and cleaning. Dehydration of the insect would increase the shelf life while concentrating the nutrients. In the case of processed samples, moisture content ranged between 6.15% for smoked samples from Bungoma and 8.76% for smoked samples from Kakamega. This variation was not significant ($P \geq 0.05$).

It was also observed that variations in moisture for raw samples from the three Counties was not significant ($P \geq 0.05$). Studies on larva of dung beetle (*Aphodius rufipes*), in Nigeria reported a moisture content of 3.25 ± 0.1 % (Paiko, Dauda, Salau, & Jacob, 2012), which was low compared to the dung beetle in our study. In edible ants and larva of *Cirina forda*, moisture content as low as 8.6 % and 10.85 % respectively, has been found (Omotoso and Adedire, 2007). In other studies Omotoso and Adedire (2007) reported a moisture content of 8.40% in the late larva stage of palm weevil (*Rhynchophorus phoenicis*), an insect that belongs to the order Coleoptera. Low moisture content is a desirable quality in food processing industries since low moisture reduces food

spoilage and prolongs shelf life, while reducing the bulk of the food product

The results in the current study showed a very high protein content in the dung beetle larvae (50.49%- 68.31%) as presented in Table 1. The diverse agro-ecological zones from which the samples were obtained were the source of the variation in quantity of proteins in the raw samples. This amount is higher than what has been reported for most edible insects. The larva of *Rhynchophorus phoenicis* (F) was found to contain 22.06% protein on dry weight basis (Ekpo & Onigbinde, 2005). Numerous studies have been conducted on the protein composition of various insect species. Protein content was calculated in all analyses as total nitrogen (N) using a conversion factor of 6.25 (Bukkens and Ardeatina, 1997). With the exception of the witchetty grub, which had a protein value of 22 g/100 g dry weight, another study indicated that caterpillars have a high protein content of about 50–60 g/100 g dry weight.

The most significant edible insect among the beetles, the palm weevil, was found to have a protein level that ranged from 23g/100g to 36g/100g dry weight (Bukkens and Ardeatina, 1997). This amount of protein was much lower than what was found for *Scarabaeus satyrus* larvae in the present study. Generally, insects have protein contents that are comparable to those of traditional meats (such as beef and pork), which typically have protein contents that vary from 40 to 75 g/100 g dry weight (Bukkens and Ardeatina, 1997). Proteins play a significant role in human nutrition. The recommended daily allowance (RDA) for protein for adults is 0.8g/kg of body weight (Wolfe et al., 2017). The adult RDA is described as the average daily level of intake that is adequate to satisfy the nutrient needs of healthy individuals. The amount of protein in *S. Satyrus* has been found to be substantially greater than that of chicken and red meats (Clarkson et al., 2018)

The loss of fat contents per processing technique followed the order toasting (20.10%) > smoked (11.08%) > oven dried (4.33%). The same pattern of decrease in fat content per processing method followed those samples from Kakamega and Siaya. This implies that toasting resulted in higher loss of fat from the samples. Oil is a substance that is primarily abundant in insects. It is well known that animals may use oil as a source of energy. Similar to protein, the amount of oil in insects varies greatly depending on the species, stage of development, and substrate used for rearing (Sánchez-Muros et al., 2014); (Nowak et al., 2016).

Data on insect fibre content has been provided by several authors. Insects with a hard exoskeleton, such as raw termites, raw crickets, and raw grasshoppers, have fibre values of 4.9, 12.1, and 6.4 g/100 g dry weight, respectively. This is comparable to the fibre content of caterpillars, which has a soft exoskeleton and ranges from 6.5 to 11.4 g/100 g dry weight. (Bukkens and Ardeatina, 1997). Based on these data, insects have a fibre content that is unquestionably higher than that of other animal products and is comparable to that of grains. (Bukkens and Ardeatina, 1997).

The quantities of CHO for the different processing methods varied significantly ($P \leq 0.05$), with oven dried samples having the lowest amounts while roasted samples had the highest amounts of CHO for all the three Counties. The average amount of carbohydrates in edible insects range from 6.71% (stink bug) to 15.98%. (cicada) (Mlcek, Rop, Borkovcova, & Bednarova, 2014).

Mineral content of processed Scarabaeus satyrus larvae

Minerals play a significant role in the human body. More than 300 metabolic processes in the body require magnesium. It promotes healthy immunological function, stabilizes cardiac rhythm, supports proper muscle and neuron function, and controls blood sugar levels (ls-Erik L. Saris , Eero Mervaala & Jahangir A. Khawaja, 2000). As a result, edible insects can provide the nutrients that are required for human body functioning. Additionally, to further supplement the diet of these insects, they can be eaten along with other foods and animals that are rich in other critical minerals. The *Scarabaeus satyrus* was found to contain good amounts of the mineral Magnesium.

Microbial content of Scarabaeus satyrus

In this study, samples of the larvae of the dung beetle were analysed for total viable counts (TVC), yeasts and moulds, *Staphylococcus sp., Salmonella,* and coliforms. The samples contained no traces of *salmonella.* Raw samples were found to harbour higher amounts of the microbes as compared to the samples that had been exposed to thermal treatment of oven drying, toasting or smoking (Table 3). It was expected to find the bacteria in the raw larvae because the cow dung from which they were collected is a potential contaminant. The source of contamination could be the environment or humans at the time of heaping the dung while preparing the compost manure. The presence of coliforms could be attributed to faecal matter that would find its way to the cow dung manure through flood waters. This is possible because of open defecation by some families who don't have toilets. Yeasts and moulds are mostly dispersed by air.

All the three processing methods of oven drying, toasting and roasting had a positive effect in reducing the levels of these microbes. Roasting was found to be most effective in decontaminating of the samples compared to oven drying and toasting. It should be noted that blanched samples had slightly lower microbial counts than the un-blanched ones. We can therefore infer that thermal processing before consumption can decontaminate the food to improve its quality and safety. Despite the vegetative cells being destroyed by cooking but easily reintroduced during handling, *Staphylococcus aureus* was present in both the raw and processed samples of *Scarabaeus satyrus*, which was relevant because of its capacity to produce enterotoxins. (Mutungi *et al*., 2019). The acceptable levels of *S. aureus* are 4 Log CFU/g (Stannard, 1997), though raw samples had higher levels while processed samples had lower levels than 4 Log CFU or slightly higher. Thermal treatment of food is a good step towards reducing or eliminating food pathogens. Most foods are cooked before eating using methods such as frying, toasting, roasting, boiling or sun drying. The current study only looked at three methods of toasting, roasting and oven drying. In all these methods, the reduction in the microbes was not absolute. This would call for a combination of methods, like wet frying after roasting or oven drying to completely eliminate the microbes that could still linger after dry heat treatment. Generally, thermal processes serve to reduce food borne pathogens. (Nyangena et al., 2020). This was also supported by research aiming at quantifying the microbial load of edible insects found in Belgium (i.e., fresh mealworms and house crickets from European farms and smoked termites and caterpillars from a traditional Congolese market) *(*Megido *et al*., 2017).

Food standards regarding the minimum levels of microbial counts have been developed by individual countries. A total plate count (TPC) of fewer than 10^5 Log CFU/g is recommended by the Canadian standards for the microbiological quality of ready-to-eat foods that require no additional preparation before consumption in order for the product to continue to be considered acceptable. The state of New South Wales has a similar regulation that authorizes the same value for fully cooked foods intended for immediate sale or consumption.(Ssepuuya *et al.,* 2017) (Ssepuuya *et al.,* 2017). The maximum permissible total viable count in edible foods is 7 Log CFU/g. Total viable counts for all of the dung beetle larvae samples, both raw and processed, were less than 7 Log CFU/g. Under the direction of the Standards Projects Committee and in compliance with Kenya Bureau of Standards processes, the Nutrition and Foods for Special Dietary Uses Technical Committee created the Kenya Standards on edible insects' products. The standards concentrate on parameters that are directly related to product quality and safety, such as moisture content, which affects how well products keep their quality, and heavy metal and microbiological contaminations because insects are vulnerable to contamination. They also provide recommendations for packaging and labelling. For instance, the maximum allowable counts for aerobic bacteria are 10^5 CFU/g, *Salmonella* should be absent, while for *Escherichia coli,* the maximum allowable counts are less than 10 CFU/g, *Staphylococcus sp*. should be less than 10 and yeasts and moulds not more than 10^2 CFU/g (KEBS, 2020).

Conclusion

The beetle larvae were found to contain relatively high amounts of protein, fat and ash, which implies that this insect could contribute to recommended daily allowance of nutrients and minerals, as well as energy, and can also be used in food fortification owing to the content of minerals. The microbial content of the processed larvae was above the acceptable maximum limits therefore, the insect larvae require higher temperatures and durations in processing before utilization in human diets. Moreover, the safety of the edible insect larvae can be ensured by proper preparation. In comparison to oven drying and toasting, thermal treatment by roasting was shown to be the most efficient in decontaminating the samples. The bacteria count of blanched samples were marginally lower than those of un-blanched samples.

From this study, it is recommended that further research may be done in all stages of the production of the edible dung beetle (*Scarabaeus satyrus*), particularly postharvest processing. More processing techniques should be incorporated under controlled temperature, to establish the optimal conditions that have less effect on the nutritional content of this edible insect. Optimization should be involved to ensure absolute decontamination of the samples. The dung beetle larvae should be analysed for vitamins, anti-nutritional factors as well as amino acids. Anti - microbial peptides could be analysed to ascertain their presence in both the larvae and adult beetles

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Ethical Approval

This research was conducted with ethical approval by Jaramogi Oginga Odinga University of Science and Technology

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