Original Article

Physicochemical and Functional Properties of Edible Dung Beetle Larvae (Scarabaeus satyrus) Flours and Oils and their Significance in Human Nutrition

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ABSTRACT

The potential of edible insects in alleviating the nutritional challenges facing humans has led to an increased interest in their utilization. Edible insects contain substantial amounts of protein and fats, which are very important macronutrients that confer useful functional and physicochemical properties to foods. However, the functional properties of these nutrients have not been given sufficient attention, limiting their use as ingredients in diets and food products. Processing of insects converts them into an ingredient that can be added to various foods, improving their acceptability in terms of flavor, taste, and nutritional content. Popular processing methods for edible insects include frying, boiling, oven drying, roasting, smoking, and toasting. These thermal treatments reduce the insect water content, enabling them to be milled into powders. This study set out to investigate the fatty acid profile, protein digestibility, and physicochemical and functional properties of powders and oils of edible dung beetle (Scarabaeus satyrus). The dung larvae were collected from three counties in Western Kenya. They were cleaned and subjected to drying and milling, then analyzed. Results indicated that protein digestibility ranged between 64.27-70.03%. The dominant unsaturated fatty acid was Oleic (45.71±2.45%), while the main saturated fatty acid was Lauric (45.43 ±0.8%). The saponification value of the oils ranged between 127.94 -130.17 mgKOH/g oil, Acid value (41.96-44.11 mg KOH/g oil), peroxide value (4.23-3.8 Meq.thio / kg sample), refractive index (1.41-1.44/25ºC) and Iodine value (77.89-88.02 g I/100g). Functional properties of the powders showed high lipophilic (332.45±19.73%) and moderate (1.11±0.14 ml/g) hygroscopic tendencies in toasted samples from Bungoma and Siaya Counties, respectively. Emulsifying capacity varied between 80.85%-82.53%, while emulsifying stability ranged from 80.85%-81.33%. These findings show that the edible Scarabaeus satyrus can provide unsaturated fatty acids and can be a good ingredient in foods as an alternative to conventional cuisines.

INTRODUCTION

Insect consumption has long been a common practice among people globally. Due to their high nutrition density, insect foods are recommended to mitigate undernutrition, particularly protein-energy malnutrition (PEM), a phenomenon affecting rural people in Asia, Africa, and Latin America. It is estimated that at least 2 billion people worldwide frequently eat insects. Most of the more than 1900 insect species classified as edible in literature are found in tropical climates (Van Huis, 2015). It is becoming evident that insects are consumed throughout the world; for instance, *Tenebrio molitor* larvae are added to tortillas, a thin flatbread produced from finely crushed maize in Mexico (Pandey & Poonia, 2018), while in Nigeria, the termite *Microtermes bellicosus* (Isoptera: Termitidae: Macrotermitinae) adds nutrients to the low-protein maize (Bukkens & Ardeatina, 1997).

Before incorporating insects into various food applications, it is vital to evaluate the functional properties of insect proteins (Rumpold & Schlüter, 2013). The term 'functionality' has not been extensively defined, leading to various interpretations of this term (Purschke et al., 2018). It refers to 'any property of food or food ingredients, except nutritional, that influences its utilization' such as water and oil absorption capacity, emulsification, gelling, and foaming capacity (Purschke *et al*., 2018). Currently, most insect consumption is a component ingredient of processed foods, and their successful utilization depends on fulfilling one or more functional requirements of good solubility, emulsion/foam capacity and stabilization, and gel formation. (Chalamaiah et al.,, 2012; Garcés-Rimón et al., 2016).

Transforming edible insects into easily identifiable food items can be based on conventional or emerging food processing technologies that enhance product functionality, stability, safety, appearance, and acceptance (Hartmann et al., 2015). High water absorption capacity shows that insect meat can be very useful in confectionery industries that deal with baking processes. The higher oil absorption capacity also shows that insect meat could be useful in cake and pie-making industries since it will easily bind to greater quantities of oil. However, more information needs to be reported on the physicochemical and functional properties of the insect proteins across the various insect species. Furthermore, no work has been reported on the physicochemical properties of processed dung beetle larvae, as well as the digestibility of their proteins.

This study sought to bridge the information gap concerning the physicochemical and functional properties of the dung beetle (*Scarabaeus satyrus*) larvae, as informed by the need to enhance its utilization in diets or as a food ingredient.

MATERIALS AND METHODS

Sample Collection

The study employed a random sampling technique. Edible dung beetle larvae were collected from various farms in three counties of Western Kenya: Siaya, Kakamega, and Bungoma. Seven sites were sampled from each county. The larvae were hand-picked from compost cow dung manure and carried in buckets that contained the decomposed manure from which the larvae had been collected. Each farm site yielded approximately 1-2 kg of the dung larvae, which were mixed to form a composite sample, ready for analysis.

Experimental Design

A completely randomized design (CRD) was used, in which treatments were assigned randomly to experimental materials. It had a factorial arrangement, where two levels (factors) of treatments, that is, geographical regions and processing techniques, comprising Bungoma, Kakamega, and Siaya, and toasting, oven-drying, and roasting, respectively, were considered.

Processing and Milling of the Raw Insect Larvae

A total of nine (9) samples were prepared. Separate samples from Bungoma, Siaya, and Kakamega Counties were subjected to three drying techniques: oven drying, toasting, and roasting. For the oven-dried samples, cleaned and de-gutted raw insects (250 g) were placed in an air-oven dryer (TD-384KN model Thermotec, Tokyo, Japan) and dried at 70 $^\circ$C for 12-24 hrs. The products were removed and left to cool for 30 min, then put in pre-labelled airtight containers. Toasting entailed low-heat frying of the larvae in their own oil on a stainless-steel pan for 10 min until brown, after which they were cooled at room temperature for 30 min before packing in airtight containers. Roasted samples were cleaned similarly, then pricked onto barbeque wires and roasted for 10 minutes, 10 cm above hot charcoal, with occasional turning to avoid charring, after which samples were cooled and stored in airtight containers. Before laboratory analysis, the dried insect larvae from the three processing techniques were milled using a fine mill (Model Bauermeister, Hamburg-Altona, Germany), and the ground samples were put in an airtight zip lock polythene paper before storage at 10 $^\circ$C.

Oil Extraction

Crude oil extraction was done using a modified Soxhlet method (AOAC, 2000). The oil required for physicochemical characterization was extracted from raw (unprocessed) samples, while oil for fatty acid analysis was extracted from processed larvae. 1-2 g samples were placed in a glass stoppered centrifuge tube and denatured at 100 $^\circ$C for three min. 5 ml of water and 20 ml of methanol-chloroform (2:1 v/v) were added, and the mixture was shaken overnight at room temperature. The samples were centrifuged, the supernatant decanted, and the residue re-suspended in 9.5 ml of 2:1:0.8 ratio methanol-chloroform-water mixture, after which the homogenate was centrifuged. 7.5 ml each of chloroform and water were added to the supernatant, the mixture was centrifuged, and the chloroform phase was extracted before being dried in a rotary vacuum evaporator at 40 ^{0}C. Finally, the residue was dried in a desiccator over KOH pellets.

Determination of Fatty Acid Profile

The fatty acid profile of the insect oil was determined using a Gas Chromatograph (Shimadzu GC 9A, Kyoto, Japan) according to the AOAC procedure. The extracted oil was methylated into fatty acid methyl esters (FAME) by placing 2 mg of the sample in a flask and refluxing with 2 ml of 95% methanol-HCl for 1 hr. The methyl esters were extracted with 3 1 ml portions of hexane and then washed with 3 ml distilled water. The resulting hexane layer was dried in a rotary vacuum evaporator, and the residue was re-dissolved in hexane. Afterwards, 0.2 ul of extracted oil was injected into the GC with a Supelco SP^{TM} 2560 capillary column with an internal diameter of 30 m x 0.53 mm; injection/detection temperature, 220 $^\circ$C under a flame ionization detector (FID), with the detector temperature set at 240 °C. Nitrogen gas with a

0.18 m/s flow rate and a split ratio of 1:100 was used as a carrier. Identification and quantification of the fatty acids were conducted by comparing the chromatograms of the samples with the FAME standards (Supelco 37 Component FAME Mix, Supelco Inc., Bellefonte, PA). Identification of the fatty acid methyl esters was performed by comparison of retention times with standards, whereby the area under each peak for each retention time was expressed as a percentage regarding the total area of the peaks for each fatty acid. Fatty acid standards were obtained from Sigma Chemical Co. The polyunsaturated fatty acids/saturated fatty acids ratio (PU/SA) and n-6: n-3 fatty acids ratios were calculated according to Koletzko et al. (2008) .

Determination of Physicochemical Properties of Oils

Measurement of Refractive Index

The specific gravity of the oil was determined using a 25 ml pycnometer at 15.5 ^{0}C, while the refractive index was determined using an Abbe refractometer at 25°C. The specific gravity was calculated by dividing the weight of the sample by the weight of the equal volume of water.

Determination of Peroxide Value

The AOAC method 965.33 (AOAC, 1990) was employed in the assay for Peroxide value. The most commonly used method to determine peroxide value utilizes the ability of peroxides to liberate iodine from potassium iodide (Pearson, 1976). Extracted oils were dissolved in a solvent mixture of acetic acid and carbon tetrachloride warmed with saturated potassium iodide (13 g KI dissolved in 7 ml of hot water). After a complete reaction, the amount of reacted ROOH was determined by measuring the amount of generated iodine. This was done by titration with 0.01 N sodium thiosulfate and a starch indicator (1% starch solution), whereby the amount of sodium thiosulfate required to titrate the reaction is related to the peroxide concentration in the original sample.

Determination of Iodine Value

The iodine value is expressed as the grams of iodine absorbed per 100g of oil according to method 920.158 (AOAC, 2000). The most commonly used method for determining the iodine value of lipids is the "Wijs method" (Pearson, 1976). The oil samples were weighed and dissolved in carbon tetrachloride, to which iodine chloride (ICl) was added. The amount of ICl that reacted was determined by measuring the amount of remaining ICl after a complete reaction. The amount of ICl that remained was determined by adding excess potassium iodide to the solution to liberate iodine, then titrating with a 0.1N sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released.

Determination of Acid Value

Method 936.15 was used (AOAC, 2000). The acid value measures the amount of free fatty acids in a given amount of fat. The oils were extracted from the powder of dung beetle larvae and dissolved in an ethanol solution containing a phenolphthalein indicator. This solution was then titrated with alkali (0.5 N KOH). The acid value was defined by the mass of KOH necessary to neutralize the fatty acids present in 1 g of lipid (Pearson, 1976).

Determination of Saponification Value

The oil was first extracted and then dissolved in an ethanol solution containing a known excess of KOH according to Method 933.08 (AOAC, 2000). This solution was heated so that the reaction could go to completion. The unreacted KOH was determined by adding an indicator and titrating the sample with HCl. The saponification number was calculated from the weight of the sample and the amount of reacted KOH (Pearson, 1976). The saponification value content of the oil was obtained by refluxing the alcoholic potassium hydroxide solution of the oil and then titrated with 0.5 M HCL using the Phenolphthalein indicator (Pearson, 1976).

Determination of In-vitro Protein Digestibility

The In vitro protein digestibility of the complementary foods was determined following the modified pepsin method described by (Mertz et al., 1984) using pepsin enzyme. The method involved the determination of protein content before and after digestion of the samples with pepsin enzyme. Pepsin (1:3000, from HOG Stomach, Loba Chemie) was used to digest the samples.

Total Protein Content

The total protein content (before pepsin digestion) of the dung beetle larvae flours was determined by the Micro-Kjeldahl method (method #979.09) (AOAC, 2000).

Pepsin Digestion

About 0.2 g of the sample was weighed into a centrifuge tube and suspended in 35 ml of a solution of pepsin (1.5 mg/ml) in 0.1 M phosphate buffer (pH 2.0); the mixture was incubated in a water bath shaker (model SHA – C, temp range: RT - 100) with gentle shaking at 37 ℃ for 2 hrs. The tubes were placed in an ice bath for 30 min to attain a temperature of 4 ℃ followed by centrifugation (Type 20 000, Kokusan Corporation, Tokyo, Japan) at 12,000 g for 15 min at 4 ℃. The supernatant was discarded, 10 ml of the buffer solution was added, and then shaking and centrifugation were done again using the same conditions. The supernatant was discarded, and the residue was filtered using a Whatman filter paper no. 3. The residue in the centrifuge tube was washed into the funnel with 5 ml of the phosphate buffer. The filter paper with the residue was oven-dried for 30 min, then rolled and inserted into a Kjeldahl flask. A blank sample was prepared similarly.

Digestible Protein Content

To determine the digestible protein content of the samples, residue digestion, distillation, and titration were conducted according to the semi-micro Kjeldahl method. A mixture of potassium sulphate, 5.5 g copper sulphate, and 15 ml concentrated sulphuric acid was added to the Kjeldahl flask containing the sample and heated under a fume hood until a green-blue colour was formed. The digestate was transferred into a 100-ml volumetric flask and topped up with distilled water. 10 ml of diluted digestate was pipetted into a distillation flask, 15 ml of 40% NaOH was added and then distilled into 4% boric acid. Finally, the distillate was titrated with 0.02 normal HCl. The digested protein of the sample was calculated by subtracting residual protein from the total protein of the sample:

Protein digestibility (%) = (A – B)/A

Where: A = % Protein content in the sample before pepsin digestion & B = % Protein content in the sample after pepsin digestion

$$Protein\ Digestibility\ (\%) = \frac{Digested\ protein}{Total\ Protein\ in\ sample} x100$$

Determination of Functional Properties of *Scarabaeus satyrus* Flours

Determination Of Water and Oil Absorption Capacity

The AACC methods evaluated water holding capacity (WHC). (AACC, 2000). 1 g of the sample was put in a centrifuge tube, followed by 3 ml of water. The samples in the centrifuge tubes were placed in a centrifuge (Beckman CS-6 centrifuge) and centrifuged to 2060 rpm for 10 min. The supernatant was decanted, and the volume of water in the supernatant was determined using.

$$Water\ holding\ capacity = \frac{(volume\ of\ water\ added - volume\ of\ water\ in\ the\ supernatant)}{Weight\ of\ the\ sample.}$$

Fat-absorption capacity (FAC) was determined using the procedure of Lin and Humbert (Lin & Humbert, 1974). The insect flour (0.3 g) was put in a pre-weighed 50 ml centrifuged tube and

mixed with sunflower oil (3 ml) for 1 min. After centrifugation at 2060 rpm for 30 min (Beckman CS-6 centrifuge), the supernatant was discarded, and the tubes re-weighed. The % FAC was expressed using:

$$Fat\ absorption\ capacity\ (\%) = \frac{(weight\ of\ sample\ +\ oil)}{weight\ of\ sample} x100$$

Determination of Emulsifying Activity and Emulsion Stability

Emulsion capacity was determined using a method where 1 g of larvae flour was put in 100 ml of distilled water and homogenized for 10 min. At the fifth minute, sunflower oil was continuously added and stirred. The emulsion was centrifuged at 3000 rpm for 10 min, and the emulsified layer volume was recorded. For Emulsion stability, 1 g of larvae flour was dissolved in 100 ml of distilled water. The suspension was homogenized for 10 min. At the 5th minute, corn oil was continuously added and stirred. The emulsion was heated at 85 °C for 30 minutes and then cooled back to room temperature. The samples were centrifuged at 3000 rpm for 10 min. The emulsified layer volume was recorded, and emulsion stability was calculated as follows:

$$Emulsion\ stability\% = \frac{volume\ of\ emulsion\ layer}{volume\ of\ suspension} x100$$

Determination of Foaming Capacity and Foam Stability

Whipping properties (foaming capacity and stability) were determined according to the method described by Kabirullah and Wills, 1982. 100 ml of 2.5% (w/v) flour suspension was whipped at 'low' speed in a 250 ml Waring blender for 5 min, and foam volume was recorded after 30s. Foam capacity (FC) was expressed as a percent increase in foam volume measured after the 30s and foam stability (FS) was determined by measuring the FC after standing for 5 min. The initial solution volume (V_1) and final volume after mixing (V_2) were recorded. Foaming capacity (FC) was calculated as follows:

$$FC = \frac{V_2 - V_1}{V_1} x100$$

Foaming stability (FS) was determined as the foam volume that persisted after 5 min.

Measurement of pH of the Dung Larvae Flours

The pH of the flours was measured using a pH meter after dispersing the solids in water at 10% w/v. One gram of the powder samples was mixed with 9 ml of distilled water and stirred at 100 rpm for 10 min. The pH value of the mixture was measured at room temperature in triplicate using a pH meter (FE- 20, Mettler-Toledo Instruments Co., Ltd., Switzerland).

Statistical Data Analysis

Analysis of variance (ANOVA) was done using Stata SE version 12 (Stata Corp LP, TX) to determine if there were significant differences between the means. The level of significance was 5 %. Post hoc Tests were done for pairwise comparison of means, using the Bonferroni Test. Shapiro Wilk's Test was used to check for the normality of data. Results were analyzed and expressed as means ± standard deviation of three replicates.

RESULTS

Fatty Acid Profile of Processed *Scarabaeus satyrus*

Table 1 shows the fatty acid composition of processed *Scarabaeus satyrus*. While roasted samples from Bungoma had a comparatively high percentage of this fatty acid. Similar samples from Kakamega and Siaya presented exceptionally low amounts of the Lauric(C12:0) acid (Kakamega-4.59%. Siaya - 0.28) attributable to GC error. Other saturated fatty acids detected in small percentages include Myristic (C14:0), Pentadecanoic (C15:0), Palmitic (C16:0), Heptadecanoic (C17:0), Stearic (C18:0), Arachidic (C20:0) whose amounts ranged between 0% - 10.16%. The major dominant

unsaturated fatty acid was Oleic (C18:3, n3) at 67.62% in roasted samples from Siaya County and 65.99% in roasted samples from Kakamega. Roasted samples from Bungoma had higher levels of Oleic acid (42.65%) compared with oven-dried (39.45%) and toasted samples (41.21%) from the same county.

Linoleic acid (C18:0), another unsaturated fatty acid, ranged between 2.49% in oven-dried samples from Bungoma County to 14.55% in oven-dried samples from Siaya County. Other unsaturated fatty acids that were detected in small amounts were Linolenic (C18:3 n3), EPA (C20:5 n3), and Arachidonic acids (C20:4 n6), with amounts ranging between 0.02% and 3.22%. ANOVA results for Oleic fatty acid showed that percentages of fatty acids in the samples was a function of processing method and sampling county (processing method: F =289.97; df = 2; p <0001; County: F = 16.06; df = 2; p <0.0011). Interaction effects were significant F=61.71, df=2; P< 0.0001).

Level Of Unsaturation, Essential Fatty Acids, and N6/N3 Ratios of Processed *Scarabaeus satyrus* Oils

Table 2 shows the level of unsaturation of the oils from the processed dung beetle larvae. The figures were calculated based on the results presented in *Table 1*. The results show that samples from Kakamega and Siaya had the highest amounts of unsaturated fatty acids. Roasted samples from Siaya had the highest percentage of EFA (17.7%), while roasted samples from Kakamega contained a high of 14.21% EFA. Roasted samples from Bungoma recorded lower percentages of EFA, having a high of 5.56%.

Physicochemical Properties of Raw *Scarabaeus satyrus* Oils

The oil extracted from the raw samples of the dung beetle larvae was assessed for quality properties of Refractive index, Iodine value, Acid Value, Peroxide value and Saponification value (*Table 3*). A one-way analysis of variance showed

significant differences between the oils from the three Counties (P=0.0214).

The oil from Kakamega samples had the lowest Refractive Index (1,41) compared to those from Bungoma (1.42) and Siaya (1.44). Saponification values of the oil showed the effect of county sampling to be significant (P=0.0164). The samples from Siaya had the highest saponification value (130.17 mgKOH/g oil), while those from Bungoma had a relatively lower value of 127.94 mgKOH/g oil.

The Wij's Iodine value was found to be a function of the county of sampling (P=0.0011), with samples from Siaya having the lowest value (77.89 gI/100g oil) while those of Bungoma and Kakamega having values of 88.15 gI/100g oil and 88.02 gI/100g oil respectively. The Acid value ranged between 41.96 and 44.11 mgKOH/g oil for samples from Kakamega and Siaya, respectively, while the oil from Bungoma had an acid value of 42.84 mgKOH/g oil (*Table 3*). The ANOVA showed that county of origin was significant (P=0.0017). Peroxide value was found to be a function of the county of sampling (P=0.0021). The samples from Bungoma, Siaya and Kakamega had Peroxide values of 4.23, 3.8 and 3.5 Meq. thio/kg respectively.

Functional Properties and Protein Digestibility of *Scarabaeus satyrus* Flours

In Vitro Protein Digestibility

The results in *Table 4* show that protein digestibility ranged between 64.27% for roasted samples from Siaya County and 70.03% for oven-dried samples from the same county. No significant differences were observed in the per cent digestibility between oven-dried and toasted samples from Bungoma and Kakamega, which had a digestibility of 69.63% and 69.04%, respectively, for oven-dried and 68.18% and 68.67% for toasted samples (*Table 4*), unlike Siaya County samples, where oven drying and toasting had a significant difference, with digestibility of 70.03% for oven drying and 67.43 % for toasted samples.

Table 1: Fatty acid composition of processed Scarabaeus satyrus flours

	Oven dried			Toasted			Roasted			P
	Bungoma	Siaya	Kakamega	Bungoma	Siaya	Kakamega	Bungoma	Siaya	Kakamega	Value
Capric (C10:0)	0.97 ± 0.17^a	0.47 ± 0.17^{ab}	0.44 ± 0.17^{ab}	0.75 ± 0.17^{ab}	0.79 ± 0.17^{ab}	0.70 ± 0.17^{ab}	0.91 ± 0.17^{ab}	0.96 ± 0.17^a	$0..65\pm o.02^b$	0.038
Lauric (C12:0)	45.11 ± 0.18^a	44.52 ± 0.4^a	42.82 ± 1.41^b	45.43 ± 0.8^a	43.02 ± 1.94^b	43.01 ± 0.92^b	43.56 ± 0.16^b	43.28 ± 0.28^b	43.59 ± 0.2^b	<0.001
Myristic (C14:0)	0.25 ± 0.35^a	0.00^a	0.35 ± 0.34^b	0.32 ± 0.14^a	0.200 ± 0.02^a	0.31 ± 0.01^a	3.05 ± 0.12^b	2.75 ± 0.76^b	nd	<0.001
Myristoleic (C14:1)	nd	nd	0.98 ± 0.18^a	nd	0.53 ± 0.75^a	0.00^a	nd	0.28 ± 0.40^a	nd	0.068
Pentadecanoic (C15:0)	nd	nd	0.05 ± 0.07^{ab}	nd	0.22 ± 0.09^{ab}	3.35 ± 0.19	0.38 ± 0.16^{ab}	0.41 ± 0.10^b	nd	<0.001
Palmitc (C16:0)	0.00^a	0.15 ± 0.01^b	$0.02+0.03^{ab}$	nd	0.07 ± 0.1^{ab}	nd	nd	nd	nd	0.022
Palmitoleic (C16:1	0.00^a	0.31 ± 0.14	0.04 ± 0.06^a	nd	nd	nd	nd	nd	nd	0.003
heptadecanoic (C17:0)	1.42 ± 1.07^a	1.19 ± 0.13^a	0.03 ± 0.04^a	nd	nd	nd	nd	nd	nd	0.167
*cis*10 Heptadecanioc (C17:1)	4.84 ± 0.40	5.99 ± 0.33	7.05 ± 0.34	nd	nd	0.005 ± 0.01^a	nd	nd	nd	<0.001
Stearic (C18:0)	1.40 ± 0.08^a	2.67 ± 0.04^c	3.25 ± 0.66^{ab}	0.9 ± 0.11^a	1.23 ± 1.69^a	1.72 ± 0.30^a	1.5 ± 0.44^a	2.1 ± 0.34^c	1.16 ± 0.47^a	<0.001
Oleic (C18:1)	42.63 ± 3.66^a	30.77 ± 0.08^c	39.01 ± 2.21^b	45.71 ± 2.45^a	38.63 ± 1.37^b	43.13 ± 0.35^a	44.58 ± 1.58^a	36.24 ± 1.13^b	39.58 ± 1.82^b	<0.001
Linoleic (C18:2 n-6)	2.49 ± 0.35^a	14.55 ± 0.49^b	2.72 ± 0.37^a	4.90 ± 0.33^a	13.64 ± 1.10^b	5.55 ± 0.64^a	4.75 ± 0.33^c	12.3 ± 0.57^b	13.38 ± 0.61^b	<0.001
Linolenic (C18:3 n-3)	0.54 ± 0.01^a	3.22 ± 0.39^b	0.66 ± 0.11^a	1.22 ± 0.11^c	1.32 ± 0.02^c	1.48 ± 1.84^c	0.81 ± 0.14^{ab}	0.87 ± 0.29^{ab}	0.83 ± 0.06^{ab}	0.033
Arachidic (C20:0)	0.06 ± 0.07^a	nd	0.16 ± 0.05^a	nd	0.20 ± 0.05^a	0.11 ± 0.15^a	nd	0.68 ± 0.21^b	nd	<0.020
EPA (C20:5 n-3)	0.28 ± 0.08^b	0.15 ± 0.05^{ab}	0.05 ± 0.01^a	nd	0.10 ± 0.09^a	nd	nd	nd	nd	<0.001
Arachidonic (C20:4 n-6)	0.08 ± 0.11^{ab}	0.06 ± 0.03^a	0.02 ± 0.01^a	0.29 ± 0.06^{bc}	0.09 ± 0.00^{ab}	0.23 ± 0.03^{ab}	0.48 ± 0.10^{cd}	0.57 ± 0.03^d	0.83 ± 0.01^d	0.021

Values are Mean ±SD, n=3, Values on the same row with different superscripts are significantly different (P≤0.05); EPA- EPA- Eicosapentaenoic Acid

Table 2: Level of saturation of fatty acids in Scarabaeus satyrus (%) oils, Essential fatty acids and n6/n3 ratios

	Bungoma			Siaya			Kakamega		
	Oven dried	Toasted	Roasted	Oven dried	Toasted	Roasted	Oven dried	Toasted	Roasted
Total UFA	50.79	52.6	50.6	51.00	54.34	50.5	52.86	50.8	54.6
Total SFA	49.21	47.4	49.4	49.00	45.66	49.5	47.14	49.2	45.4
Total MUFA	47.47	45.71	44.58	37.07	39.16	36.52	47.08	43.14	39.58
Total PUFA	3.32	6.89	6.02	13.93	15.18	13.98	5.78	7.66	15.02
PUFA/SFA ratio	0.07	0.15	0.12	0.28	0.33	0.28	0.12	0.16	0.33
Total n6	2.77	5.19	5.23	14.61	13.69	12.87	2.74	5.78	14.21
Total n3	0.82	1.22	0.81	3.37	1.42	0.87	0.71	1.48	0.83
n6/n3 ratio	3.38	4.25	6.46	4.34	9.64	14.79	3.86	3.91	17.12
EFA	3.03	6.12	5.56	17.77	14.96	13.17	3.38	7.03	14.21

Key: UFA-Un saturated fatty acid; SFA- Saturated fatty acid; MUFA- Monounsaturated fatty acid; PUFA= Poly Unsaturated fatty acid; EFA- Essential fatty acids- C18:2+ C18:3). n3- omega 3 fatty acid; n6- Omega 6 fatty acid.

Table 3: Physicochemical properties of oils of Scarabaeus satyrus

	Bungoma	Siaya	Kakamega	P value
Refractive index (25^0 C)	1.42±0.01^{ab}	1.44±0.006^b	1.41±0.01^a	0.0214
Saponification value (mgKOH/g oil)	127.94±1.0^b	130.17± 0.51^a	129.78±0.4^a	0.0164
Wij's Iodine value (g I/100g oil)	88.15±2.96^a	77.89±1.73^b	88.02±0.49^a	0.0011
Acid value (mg KOH/g oil)	42.84±0.35^a	44.11±0.20^b	41.96±0.55^a	0.0017
Peroxide value Meq.$_{thio}$/ kg sample	4.23±0.11^b	3.8±0.16^a	3.5±0.13^a	0.0021

Values are Mean +SD n=3 Values along the same row with the same superscript are not significantly different (P≤0.05).

Table 4: Protein digestibility (%) of processed Scarabaeus satyrus flours

	Protein digestibility (%)		
	Bungoma	**Siaya**	**Kakamega**
Oven dried	69.63±1.23^{ab}	70.03±0.35^b	69.04±0.82^{ab}
Toasted	68.18±1.40^{ab}	67.43±0.79^{ad}	68.67±0.51^{ab}
Roasted	64.90±0.61^{cd}	64.27±0.89^c	64.40 ±0.85^c
P VALUE	< 0.001	< 0.001	< 0.001

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

Functional Properties

The functional properties of *Scarabaeus satyrus* were assessed in order to determine the quality of the flour samples that had been subjected to various processing techniques of oven drying, toasting and roasting. These included water and oil holding properties, whipping properties (foam capacity and stability), gelation, emulsification capacity and stability and pH.

Water Holding Capacity and Oil Absorption Capacity

Table 5 shows the results for water holding capacity and oil absorption capacity. From the analysis of variance for WHC, the main effects of processing and county of sampling were significant (Processing: F=39.51, df=2, P< 0.0001), (County: F=30.13, df=2, P < 0.0001). The water holding capacity of the flours ranged from 0.29% for roasted samples from Bungoma County to 1.11% for toasted samples from Siaya County. In all the processed samples, the roasted samples had the lowest water absorption capacity.

The fat absorption capacity was a function of the processing methods (Processing: F=3.05, df=2, P=0.0405, while the county of sampling and interaction effects were not significant County: F=2.03, df=2, P=0.1599) and Processing*County (F=2.12, df=4, P=0.1198). From the results presented in *Table 5*, fat absorption ranged from 270.47% to 332.14% in roasted and toasted samples from Bungoma County, respectively.

Emulsification Activity and Emulsification Stability

Table 5 shows the results of the emulsification capacity and emulsification stability of flours from *S. satyrus*. The two-way ANOVA of emulsification capacity exhibited the main effects of processing and county of sampling, and the interaction effects were not significant. (Processing; F=3.14, df=2, P=06780, while County: F=1.22, df=2, P=0.3180; Interaction effects (Processing County: F=0.09. df=4, P=0.9839). In the case of Emulsification stability, the main effects of processing and county of sampling, as well as interaction effects, were not significant. (Processing: F=1.17, df=2, P=0.3339; County: F=1.17, df=2, P=0.3339; Processing*County: F=0.67, df=4, P=0.6233). The Emulsification Capacity (EC) ranged from 80.85% for toasted samples from Bungoma and 82.43% for oven-dried samples from Kakamega. There were no significant differences (P=0.3873) in the EC of all the processed samples from the three Counties (*Table 5*). Emulsification stability (ES) of the flours ranged between 80.85% in toasted and roasted samples from Bungoma and 81.82% in oven-dried samples from Kakamega

and Bungoma and toasted samples from Kakamega (*Table 5*). There were no significant differences in ES of all the processed samples (P=0.5252).

Foam Capacity and Foam Stability

The whipping properties (Foam capacity and foam stability) of the flours were assessed. The Two-way ANOVA showed that for foaming capacity (FC), the main effect of processing was not significant (Processing: F=0.9, df=2, P=0.4224), while the county of sampling was significant (County: F=46.06, df=2, P<0.0001). The interaction effects were significant (Processing*County: F=5.65, df=4, P=0.0040). The results of ANOVA for foam stability showed that the main effect of processing was not significant (Processing: F=0.71, df=2, P=0.5040), while the effect of county of sampling was significant (County: F=43.14, df=2, P < 0.0001). The interaction effects were significant (Processing*County: F=5.47, df=4, P=0.0046).

The Foam Capacity ranged from 8.06% in roasted samples from Bungoma and 11.95% in oven-dried samples from Siaya. There were significant differences in the FC across the county of sampling (P<0.0001), as seen in *Table 5*. The Foam stability (FS) of the processed solutions of the insect flours ranged from 8.06% in roasted samples from Bungoma and 11.82% in oven-dried samples from Siaya. The differences in the FS across the Counties were significant (P<0.0001).

pH Values of the flour solutions

Table 5 shows the pH of the flour solutions at 10% concentration. The PH was a function of the main effects of processing and county of sampling as well as the interaction effects, which were all significant (Processing: F=632.42, df=2, P < 0.0001; County: F=111.65, df=2, P < 0.0001, Processing*County: F=108.68, df=4, P < 0.0001). The pH of the flour solutions ranged from 5.88 in toasted samples from Kakamega to 6.76 in toasted and roasted samples from Bungoma.

DISCUSSION

Fatty Acid Profile and Physicochemical Properties of Oils of *Scarabaeus satyrus*

Fatty Acid Profile

From this study, it was seen that the larvae of Scarabaeus satyrus contain high-value oils plus high-value proteins. The quality of the oils was assessed by the presence of unsaturated fatty acids, essential fatty acids and omega 3 and omega 6 fatty acids. Physicochemical properties of the oils, such as peroxide value, acid value, saponification value and refractive index, were assessed in order to find out the quality and functionality of the oils of this edible insect. The quality of proteins was assessed by analyzing the digestibility as well as the techno-functional properties of the larvae flours.

This study showed that the dung beetle larvae contain both saturated and unsaturated fatty acids (*Tables 1* and *2*). Total unsaturated fatty acids ranged between 39.38- 81%, while total saturated fatty acids ranged between 14.75-54-62%. The dominant saturated FA was Lactic acid (C12:0), while the oleic acid was the dominant monounsaturated FA. The essential fatty acids, Linoleic (C18:2n6) and Linolenic (C18:3n3), were in amounts of up to 14.55% and 3.22%, respectively. These results are consistent with what other authors (Jayanegara et al., 2020) found for other insects, in which maggot oil was found to be dominated by C12:0 by more than 40% of total fatty acids. Other fatty acids that were high in maggot oil were C14:0, C16:0 and C18:1n-9. The main fatty acids present in Kroto oil were found to be C18:1n-9 and C16:0. Superworm oil, mealworm oil and cricket oil were similar with regard to their main fatty acid profiles, i.e. C16:0, C18:1n-9 and C18:2n-6, respectively (Jayanegara et al., 2020).

Table 5: Functional properties and PH of Scarabaeus satyrus larvae flours

Processing Techniques	Water holding capacity(mL/g)	Fat absorption capacity (%)	Emulsifying capacity (%)	Emulsifying stability (%)	Foam capacity (%)	Foam stability (%)	pH
Bungoma							
Oven-dried	0.71±0.12[ab]	295.10±15.91[ac]	81.82±0.00[a]	81.82±0.00[a]	9.09±0.00[ac]	9.09±0.00[ab]	5.90±0.05[a]
Toasted	0.68±0.05[ab]	332.45±19.73[b]	80.85±0.84[a]	80.85±0.84[a]	9.09±0.00[ac]	9.09±0.00[ab]	6.76±0.06[c]
roasted	0.29±0.10	270.47±22.96[c]	80.85±0.84[a]	80.85±0.84[a]	8.06±0.90[c]	8.06±0.00[a]	6.76±0.04[c]
Siaya							
Oven-dried	0.90±0.06[c]	323.14±4.31[ab]	82.53±0.97[a]	81.33±0.84[a]	11.95±1.11[b]	11.82±1.20[c]	5.99±0.11[b]
Toasted	1.11± 0.14	323.14±30.73[ab]	81.33±0.84[a]	81.33±0.84[a]	11.64±0.63[b]	11.50±0.55[c]	5.99±0.01[b]
roasted	0.64±0.06[a]	301.97±22.35[abc]	81.33±0.84[a]	81.33±0.84[a]	11.50±0.55[b]	11.38±0.75[c]	6.65±0.03[d]
Kakamega							
Oven-dried	0.83±0.12[bc]	308.58±16.70[ab]	**82.43±2.10[a]**	81.82±0.00[a]	10.00±0.91[a]	9.71±0.75[a]	5.94±0.01[ab]
Toasted	0.74±0.04[ab]	320.17±10.61[ab]	81.82±0.00[a]	81.82±0.00[a]	9.27±0.31[a]	9.14±0.08[ab]	5.88±0.00[a]
roasted	0.60±0.02[a]	321.85±30.04[ab]	81.33±0.84[a]	81.33±0.84[a]	11.52±0.41[b]	11.27±0.24[c]	6.72±0.02[cd]
P Value	<0.001	0.0484	0.3873	0.5252	<0.001	<0.001	<0.001

Values are Mean ±SD, n=3. Valued along the same column with same superscripts are not significantly different at P<0.05

The low saturated fatty acid and high desirable unsaturated fatty acid contents of some of the *S. satyrus* may be considered it an important food component for those who have high blood cholesterol content and are probably at a risk of cardiovascular disease. In this study, the PUFA/SFA ranged between 0.07-1.02. Simopoulos (2000) reported that the PUFA/SFA proportion should be 0.45 for balanced nutrition. From the nutritional point of view, a minimum PUFA/SFA ratio of 0.45 is recommended for the diet as a whole. The oils of the dung beetle larvae fall within the recommended ratio of PUFA/SFA.

Studies have shown that humans can synthesize saturated and monounsaturated fatty acids, but they cannot synthesize the n-3 and n-6 families of PUFA. The parent fatty acids of these families, alpha-linolenic acid (18 carbons, three double bonds with the first double bond in the n-3 position, C18:3n-3, ALA) and linoleic acid (C18:2n-6, LA) are essential fatty acids and must be present in the diet (Koletzko et al., 2008). The fatty acids of these families cannot be interconverted, so both n-3 and n-6 fatty acids are essential.

Physicochemical Properties

The dung beetle larvae oils were found to have high acid, iodine, and saponification values (*Table 3*). Low iodine value could be beneficial since it is always associated with good quality and longer shelf life (Ortega-Nieblas et al., 2001). Falade et al. (2008) reported that low iodine content prevents oxidative deterioration in foods and is also suitable to be used as biodiesel fuel as the oil is not viscous and hence has a high degree of atomization. A high saponification value of oils indicates that the fatty acids present in the oils have a high number of carbon atoms. This means that the oils also, after hydrogenation, could be substituted for some conventional oils in the soap and shampoo industry (Falade et al., 2008).

Generally, oil with a peroxide value of less than 30 mEq peroxide/kg has been considered safe for human consumption (Gotoh & Wada, 2006). It is also noted that the peroxide value of dung beetle larvae oil ranged between 3.5 and 4.23 mEq

peroxide/kg oil, which is well below the level established by the Food and Drug Administration (FDA)/World Health Organisation (WHO) under the Codex Alimentarius Commission. For acid value, the oils had high acid values, which the present of large amount of free fatty acids in the crude oils might attribute. Generally, high acid values above 2 mg KOH/g are correlated with rancidity since the free fatty acids may have off odours or may undergo auto-oxidation to produce off flavours and odours (Ssepuuya et al., 2017). The high saponification value of the oils indicated that the fatty acids present in the oils have a high number of carbon atoms, implying that these dung beetle larvae oils also, after hydrogenation, could be substituted for some conventional oils in the soap and shampoo industry.

Functional Properties and Protein Digestibility of *Scarabaeus satyrus* Flours

Protein digestibility of the flours was assessed. The study established that flours of *Scarabaeus satyrus* had a high digestibility of 69.63%, which is higher than values obtained by Bera et al., (2023) for chickpea, but are in agreement with digestibility values of 67.3, 72.3 and 93.3% for *Hermetia illucens, Tenebrio molitor* and *Musca domestica* (Rodriguez- Rodriguez et al., 2022), indicative of the high digestibility potential of *Scarabaeus satyrus*. Many studies have demonstrated a link between protein physico-chemistry, processing and digestive proteolysis, for instance in legumes, Bera et al., (2023) showed that legume steeping increased digestibility. The high protein digestibility manifest in *Scarabaeus satyrus* implies that less processing is required to ensure protein availability.

CONCLUSION

The analyses of the fatty acid profile of *Scarabaeus satyrus* oils indicate that dung beetle larvae are a source of omega 3 and omega 6 fatty acids and, thus, can complement or replace conventional food sources. Moreover, the presence of oleic acid, which is monounsaturated, confers good quality to the oil; thus, the oil can be used in frying foods owing to its low oxidative

tendencies. Physicochemical characterization revealed that *Scarabaeus satyrus* oil is stable during storage, as evidenced by its Iodine and peroxide values. Functional characterization confirmed the utilization potential of the dung beetle larvae flour, as evidenced by its emulsification stability and capacity, among other properties. Finally, the high protein digestibility of the flour shows that *Scarabaeus satyrus* is an edible insect of great importance in food and nutrition security.

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