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Techno-functional Properties of Armoured Cricket *(Acanthoplus discoidalis)* Flour

Mugova A. K[1*], Zvidzai C. J[1], Musundire R[2]

[1]Department of Food Science and Technology, Chinhoyi University of Technology, Off Magamba Way, Private Bag 7724, Chinhoyi, Zimbabwe
[2]Department of Crop Science and Postharvest Technology, Chinhoyi University of Technology, Off Magamba Way, Private Bag 7724, Chinhoyi, Zimbabwe

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***Corresponding author:** Mugova A. K

Abstract | **Original Research Article**

Acanthoplus discoidalis is an insect that has potential use in new product development because of its nutritional quality. This study focused on function properties of *A. discoidalis* flour. Protein solubility, bulk density, water and oil holding capacity and foaming and emulsifying properties were determined. Protein solubility values of *A. discoidalis* protein extract at different temperatures were minimum at pH 5. After sieving of the ground flour, the highest retention yields for both the oven dried and sun dried insect flour were observed at mesh size 150µm thus, 47 % and 45 % respectively. Bulk density ranged from 52.33 to 82.67g/mL. Water holding capacity values ranged from 236 to 183.73g/g. A comparison between the water holding capacity with relation to mesh size revealed that the finer the flour, the lower the water binding capacity. No significant difference ($p < 0.05$) was observed between water holding capacity of the protein isolate and oven dried *A. discoidalis* flour ground to 500µm. Oil holding capacity of *A. discoidalis* flour ranged from 110.57 to 179.67g/g. Significant differences ($p < 0.05$) for oil holding capacity were observed between air dried and sun dried flour samples. Foaming capacity values of *A. discoidalis* flour ranged from 4 to 33.83%. Foaming capacity decreased with an increase in mesh size for both the oven dried and sun dried flour samples. No significant difference ($p < 0.05$) was observed between foaming stability of the protein isolate and that of sun dried flour ground to 300 and 500µm. Emulsion capacity and emulsion stability values ranged from 43.33 to 84.07% and 24.73 to 41.83% respectively. The obtained results could be valuable to industries that would want to take up *A. discoidalis* in their formulation of improved foods and feeds. Insect flours are rich in protein, good extenders, good thickeners and good gelling agents.

Keywords: Edible insect, insect protein, functional properties.

INTRODUCTION

The use of marginalsed protein rich sources as food has increased significantly. Insects are protein rich sources that are currently being integrated into conventional food matrices. In recent researches, insects were proposed as one of the most promising alternative source of protein to solve the global issue of protein shortage [1-3]. The environmental advantages of insect use over other protein sources are well documented [4-6]. Nutritionally, insects are readily available sources of protein, lipids, carbohydrates and certain vitamins [7-10]. They are also rich in micronutrients such as zinc, iron, calcium, manganese and copper [3, 11-13]. A major obstacle to the use of insects as food is their acceptance.

In order to overcome acceptance challenges of insect-based foods, extent of perceptibility should be minimised thus increasing the acceptability of edible insects [14, 15]. It is considered a good way to serve insects in a masked form as insect flour or protein extract as suggested by Hartmann and Siegrist [16]. Literature suggests that processing of insects from visible serving forms to invisible serving forms has the potential of promoting wider acceptance of edible insects [16, 17]. The milling of edible insects produces high protein flour and at the same time improves palatability [18] but however, information on how factors such as processing, chemical and heat treatments affect functionality of these flours is scanty. An in depth knowledge base on how processing, chemical and heat treatments affect the variations in functional properties

could allow food processors to fully exploit this underutilised protein resource.

In this study, Acathoplus discoidalis (Orthoptera: Tettigoniidae) was used as the study insect. The insect species is abundant and consumed in some parts of Southern Africa. No commercial value chain exists for the insect's use as a food or feed. It is of importance also to note that A. discoidalis is a serious pest to crops. Significant economic losses have been reported during outbreaks [19, 20]. DeFoliart [21] suggested sustainable exploitation by local people of edible insects to reduce the use of pesticides in agriculture by developing efficient methods of harvesting pest species that are also traditional foods. The objective of the study was to determine function properties of A. discoidalis flour. Bulk density, protein solubility, water and oil holding capacity, and foaming and emulsifying properties were determined.

MATERIALS AND METHODS

Raw materials and processing

Armoured crickets were randomly collected in Mbire district. Five wards were randomly drawn using the simple random sampling technique. Random selection was used to select armoured crickets from the selected wards. From each ward, insects were randomly collected/ harvested by hand picking into a 20 L bucket. The crickets were then inactivated in lukewarm water and prepared in the same way as they are prepared by locals [22] thus removal of the head and thorax, degutting and boiling. The prepared crickets from the five wards were then mixed together to produce a composite sample from which laboratory size samples (2kg) were then either freeze dried, sun dried or oven dried. Figure-1 shows the experimental design.

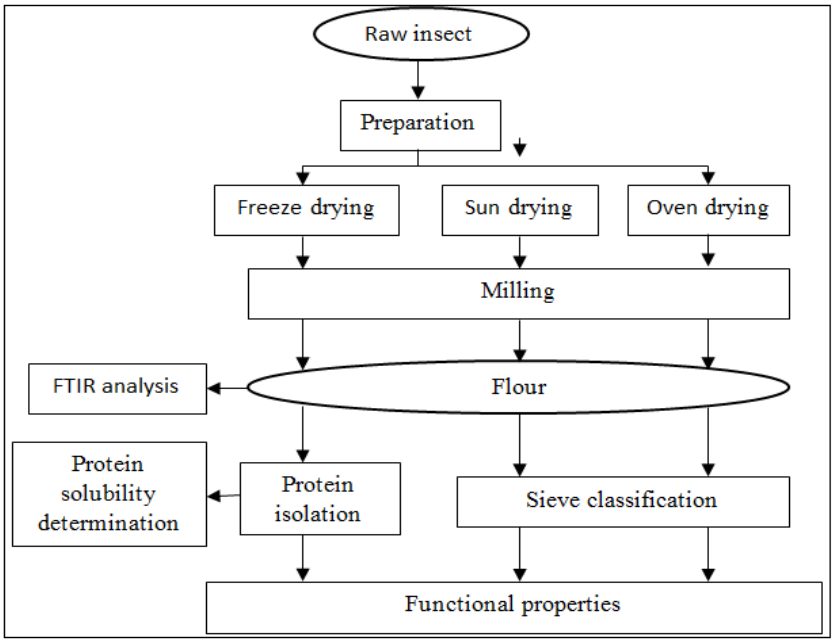

Fig-1: Experimental Design

For the freeze dried sample, freeze-drying was done using a benchtop lab scale freeze-dryer. The freeze dryer was loaded with 2kg of insect material and drying to constant mass done at 0.2 mbar over a period of 48h. After drying, the insects were polyethylene bagged and stored at room temperature for further use. The freeze dried samples were ground and the flour used for protein isolation and FTIR analysis. The protein isolate obtained was used to determine the effects of temperature and pH on insect protein solubility. For the oven dried sample, 2kg of prepared insects were dried to constant mass for a period of 24h at 60°C in a convection oven. After drying, the insects were cooled to room temperature, sealed in polyethylene bags and stored at room temperature for further use. For the sun dried sample, 2kg of insect material were placed on and dried in the open sun to constant mass over a period of 3 days. The dried insects were subsequently sealed in polyethylene bags and stored at room temperature for further use. The sun dried and oven dried samples were ground and the flour sieved using three mesh sizes. These different flours together with the protein isolate were used for the determination of functional properties. Experiments were done in triplicate over 2 seasons, 2019 and 2020.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

Insect powder samples were dried before analysis to obtain constant weight. The IR Affinity-1 Spectrometer with an Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan) was used to determine the molecules and bonds that constitute the structure of A. discoidalis insect

powder. Six sample replicates were analysed in the region of 4000-500 cm^{-1} at a resolution of 4 cm^{-1} for 32 scans separately.

Particle size distribution

Insect powder samples weighing 100 g were subjected to granulometry determination using a method by Betancur-Ancona et al., [23] as reported by Gupta and Premavalli [24] using a sieve shaker equipped with 150, 300 and 500 µm mesh sieves. Each sample either sun dried or oven dried was placed on a series of sieves with the largest mesh size on top. The sieve shaker was subsequently shaken for 20 min. The retained material on each sieve was weighed and expressed as a percentage of the original weight of the initial 100 g sample.

Protein extraction

Proteins were isolated according to Girón-Calle et al., [25]. Freeze dried insect flour was stirred for 1h with 0.2% NaOH at a ratio of 1:10 (w/v), pH 11, at room temperature. After centrifugation at 8 000g, precipitation of proteins was carried out at the isoelectric point pH 5 and room temperature. Precipitated proteins were centrifuged at 4 °C for 20 min at 8 000g and washed with distilled water. Afterwards, the protein preparations were lyophilised and kept at −18 °C and used for further analysis.

Protein solubility (PS)

PS was determined using the method by Morr et al., [26] with minor adjustments. Approximately 0.5g/L of the dried protein extract was weighed into separate 0.1L beakers and aliquots of 5.85g/L NaCl solution were added followed by stirring to form a smooth paste. An additional 5.85g/L NaCl was added in order to bring the total volume of the dispersion to 0.04L. The mixture was transferred to holding beakers connected to a thermostatic water bath with temperatures varied from 30°C to 60°C. The pH values varied from 2 to 10 and maintained by adding 1M HCl or 1M NaOH when it was necessary. The samples were agitated for 1 h, the resulting dispersion was transferred to a 0.05L volumetric balloon and the volume completed with 5.85g/L NaCl. The solution was centrifuged at 14 000rpm for 30min at 40°C and the supernatant was filtered in Whatman paper no. 2. Aliquots of 0.002L were taken and their soluble protein content was determined using the micro Kjeldahl method [27]. The percentage soluble protein was calculated by:

$PS = [(A \times 50)/ ((W \times S)/100)] \times 100$

Where,
A – supernatant protein concentration (g/L)
W – sample mass (g)
S – the sample protein concentration (g/100 g).

Functional Properties

Bulk density (BD)

BD was determined according to the modified method of [28]. Two grams of insect flours was put into a 10ml measuring cylinder on a vortex vibrator for 1min to obtain a constant volume of the sample. The volume of the sample was recorded against the scale on the cylinder. The BD values were calculated as the ratio of mass of flour and the volume occupied in the cylinder. BD was calculated using the formula below.

$BD = W_S/V$

Where,
W_S – Sample weight
V – volume occupied.

Water holding capacity (WHC)

The WHC of the insect fractions was determined according to a method described by Honikel and Hamm [29]. Firstly, 1g of the insect flour was weighed, put into a centrifuge tubes and 10ml of distilled water was added. The contents in the centrifuge tube were vortexed for proper mixing followed by centrifugation at 5000rpm for 30min and the tubes were weighed after decanting the supernatant. WHC was calculated using the formula below.

$WHC = (W_2-W_1)/W_2$

Where,
W_2 – final sample weight
W_1 – initial weight of the sample.

Oil Holding Capacity (OHC)

The OHC was determined according to the method by [30]. A sample of 0.5g flour was added to 10ml of sunflower oil and mixed for 30s in a vortex mixer. Subsequently, the dispersion was centrifuged at 8,000g for 15min. The precipitate was weighed and the difference in the weight calculated. The results were presented as gram of oil absorbed per gram of the sample. OHC was calculated using the formula below.

$OHC = (W_1-W_2)/W_1$

Where,
W_1 – initial weight of the sample
W_2 – final sample weight

Emulsion Properties

The method of Wu et al., [31] was used for the determination of emulsion properties. The sample was dispersed in 1% w/v distilled water. Subsequently, 15ml of the dispersion was homogenised together with 15ml of vegetable oil at 20 000rpm for 1min. The sample was then centrifuged at 3000g for 5min and the volume of the resulting layers were read off. Emulsion stability (ES) was determined by heating the emulsion for 30min at 80°C. The sample was then centrifuged at 3000 g for 5min. Emulsion activity (EA) and emulsion stability were calculated by:

$ES (\%) = (V_{30}/V_e) \times 100$ $EA (\%) = (V_e/V) \times 100$

Where: V – total volume of tube contents, V_e – volume of the emulsified layer and V_{30} – volume of the emulsified layer after heating.

Foaming Properties

The method by Guo et al., [32] was used for the determination of foaming capacity (FC) and foaming stability (FS). Twenty milliliters of a 1% sample was homogenised at 16 000rpm for 2min. The homogenised sample into a cylinder. The total volume was then read off at time zero and after 30min of homogenising. The FC and FS were calculated by:

$FC\ (\%) = ((V_0 - V)/V) \times 100$ $FS\ (\%) = (V_{30}/V_0) \times 100$

Where: V – volume before homogenising, V_0 – volume after homogenizing and V_{30} – volume after homogenising and standing for 30min.

RESULTS AND DISCUSSION

FTIR analysis

Analysis showed different absorption bands at specific wavenumbers. Figure-2 and figure 3 show the absorbance spectrum for different functional groups and amide bands observed for armoured cricket insect powder.

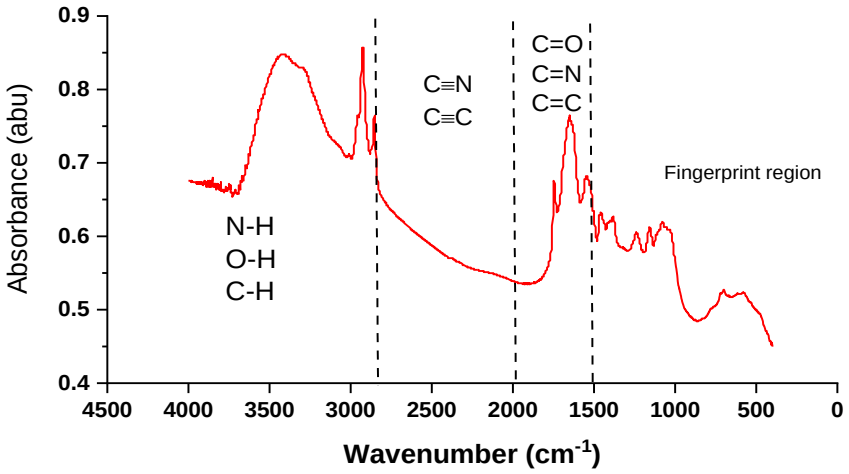

Fig-2: Functional groups identified for A. discoidalis flour

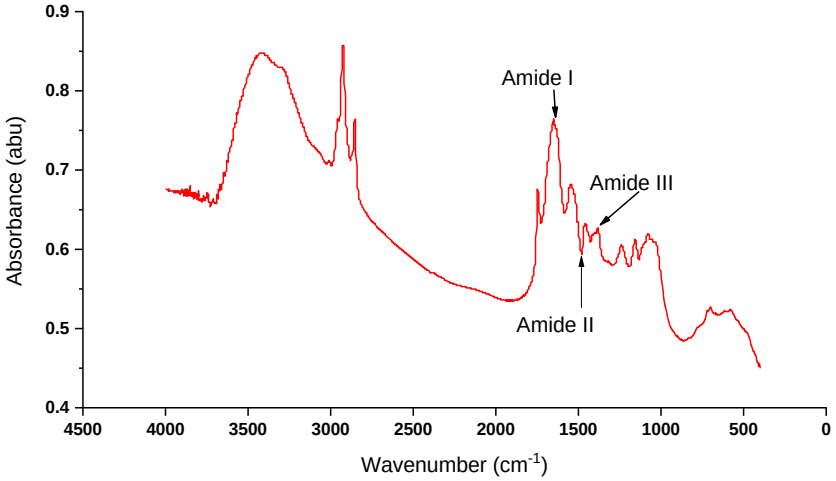

Fig-3: FTIR spectrum of A. discoidalis flour showing amide I, amide II and amide III bands.

Table-1 summarises some of the functional groups that are in high amounts in A. discoidalis flour while Table-2 shows the amide band assignments.

Table-1: Bond interactions in A. discoidalis edible insect flour

Bond Interaction	Wavenumber (cm^{-1})
C-O group	1239
N-H bend C-N stretch	1555
N-H bend, C-N stretch	1650
C- H stretch	2925
O-H (alcohol) stretch	3420
O-H (alcohol) bending	1379
O-H (acids) stretch	2854, 2925
C-O stretch	1079.91, 1158

Table-2: Amide band assignment of A. discoidalis edible insect flour

Amide band	Wavenumber (cm^{-1})
Amide I	1650
Amide II	1555
Amide III	1379

The amide I band generally causes stretching vibrations of C=O bond and bending vibrations of N-H are linked to Amide II band. Studies have shown that the amide I band generally arises in the frequency range of 1600-1700 cm^{-1} and the amide II band is linked to a frequency ranges between 1480-1600 cm^{-1} [33]. A strong absorbance peak was observed due to C=O stretching around 1650 cm^{-1} and is a region that represents amide I band. The results obtained were comparable with the FTIR results reported by [34] where amide I band gave a peak wavelength around 1633 cm^{-1} in a whey protein isolate. Furthermore, another strong absorbance peak was observed at wavelength 1555 cm^{-1}. This peak is related to amide II and is a result of bending and stretching vibrations of N-H and C-N functional groups respectively. This result is consistent to work reported by Berkay [35] where the amide II band hand absorbance peaks at a wavelengths 1516 cm^{-1} and 1508 cm^{-1} for mealworm and house cricket flour respectively. The amide II band peak observed from a pure MPB70 recombinant protein produce purified by Reis et al., [36] was also around a wavenumber of 1550 cm^{-1}. According to Muyonga et al., [37], broad absorbance range with low spectral density in the range of 1200-1400 cm^{-1} is associated with amide III band. In this study the amide III band peak was observed at a wavelength of 1379 cm^{-1}. Amide III band was constant to results reported by Berkay [35] where the peak absorbance was obtained around 1396 cm^{-1} and 1392 cm^{1} for the house cricket and mealworm respectively. Another peak was obtained around 1079 cm^{-1}, and this could be because of C-O stretching in hydrogels and dry powders emphasising the presence of carboxylic acid units as reported by Ozel et al., [34] and Yang et al., [38]. Vibrations at frequency between 700-900 cm^{-1} and 900-1200 cm^{-1} are associated with C-O stretching of bonds found in carbohydrates [39]. An absorbance peak at a wavelength of 2854 cm^{-1} was observed. Yang et al., [38] states that the absorbance range between 2850 and 2980 cm^{-1} indicate the stretching groups of $-CH_2$ and $-CH_3$. A broad peak was observed in the range between 3000-3600 cm^{-1} and this could be because of the vibrations of O-H group stretching. The peak observed in range around 3420 cm^{-1} was because of O-H group stretching. These results are similar to work done by other researchers [34, 40]. The intensity of protein related compounds as a result of N-H bending, C-N stretching C-O stretching and vibrations of C=O bonds increase with increase in protein content.

Protein solubility

Figure-4 shows the solubility of A. discoidalis protein extract at different temperature and pH conditions.

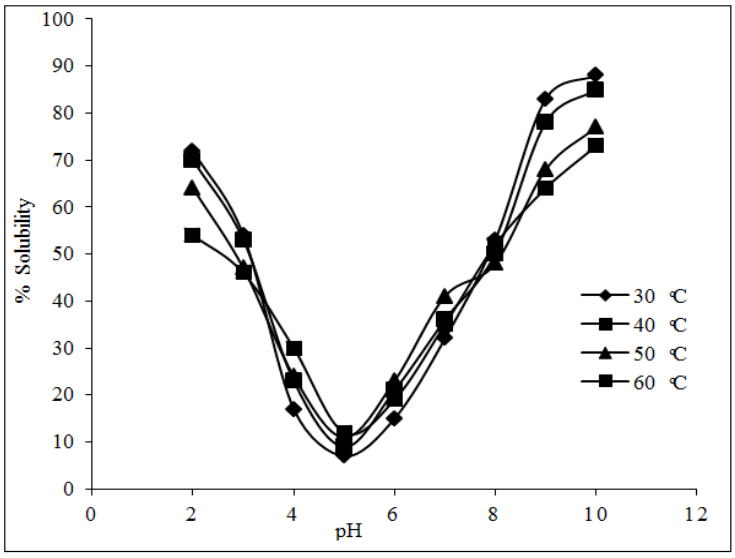

Fig-4: Protein solubility of A. discoidalis extract

The solubility values of A. discoidalis protein extract at different temperatures were minimum at around pH 5. High solubility values at any temperature were observed at either high acid or high alkaline conditions. Also at these high acid or high alkaline conditions it was observed that the solubility decreased with the temperature which could indicate that thermal protein denaturation had occurred. The results are consistent with work done by Zielińska et al., [41] on Tenebrio molitor, Gryllodes sigillatus and Schistocerca gregaria in which protein solubility was minimum at around pH 5. However, the pI in the work done by Mishyna et al., [42] of S. gregaria was determined at pH 4, while Apis mellifera at pH 5. Whey proteins were recorded to have a minimum solubility at pH 4.5 [43]. Minimum solubility of Patanga succinta, Temebrio molitor, Locusta migratoria, Chondracris rosea and Hermetia illucens protein concentrates occurred at pH 4 [42, 44, 45]. Good protein solubility is linked to formation of better emulsions, foams, and gels in designing of food products. Protein solubility is greatly dependent on pH amongst other factors such as amino acid composition and structure, protein size and three dimensional structure. A protein's net charge is neutral close to the isoelectric point (pI). Generally, at this pI there is greater protein-protein interactions which consequently results in protein precipitation. Findings therefore on the effects temperature and pH conditions which change during processing can be of importance in predicting the foaming and emulsion properties as these are affected by protein solubility.

Particle size distribution

The particle size reduction distribution of the sun dried and oven dried flour is represented in Figure-5.

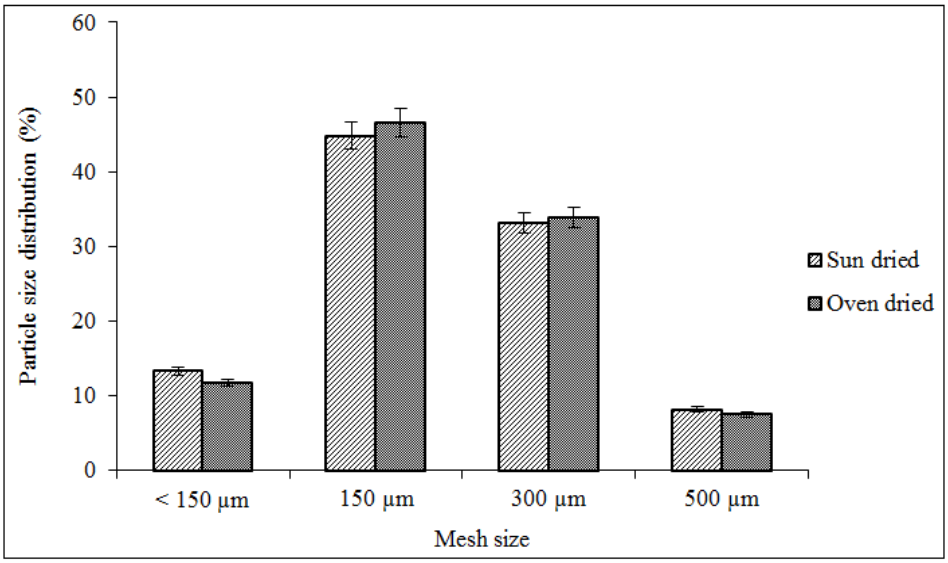

Fig-5: Particle size distribution of milled A. discoidalis flour

The highest yields for both the oven dried and sun dried insect flour were observed at mesh size 150 µm thus, 47% and 45% respectively. This was followed by mesh size 300 µm were the observed yields were 35% and 33% for the oven dried and sun dried insect flour respectively. Particle size affects the functional properties which in turn have a considerable bearing on the application in the production of various food products [23].

Functional properties A. discoidalis flour

Table-3 shows the functional properties of sun dried and oven dried A. discoidalis flours.

Table-3: Functional properties A. discoidalis flour

Parameter	Oven dried 150µm	Oven dried 300µm	Oven dried 500µm	Sun dried 150µm	Sun dried 300µm	Sun dried 500µm	Protein isolate
BD (g/mL)	82.67 ± 2.52^{c}	67.33 ± 0.58^{b}	63.67 ± 1.53^{b}	64.33 ± 2.52^{b}	56.33 ± 1.15^{a}	52.33 ± 1.53^{a}	
WHC (g/g)	215.33 ± 4.93^{b}	220.00 ± 6.25^{b}	236.00 ± 3.61^{c}	183.73 ± 3.52^{a}	192.90 ± 2.85^{a}	219.67 ± 5.03^{b}	236.67 ± 6.51^{c}
OHC (g/g)	144.33 ± 5.13^{b}	160.00 ± 4.00^{c}	179.67 ± 4.51^{d}	110.57 ± 1.25^{a}	118.53 ± 3.23^{a}	149.97 ± 3.59^{b}	291.67 ± 5.51^{e}
FC (%)	33.83 ± 1.04^{f}	22.40 ± 1.22^{e}	14.37 ± 0.64^{c}	18.70 ± 1.04^{d}	6.83 ± 0.57^{b}	4.00 ± 0.00^{a}	42.90 ± 2.41^{g}
FS (%)	24.00 ± 2.00^{d}	22.33 ± 1.53^{d}	17.33 ± 1.53^{c}	13.63 ± 0.55^{b}	9.13 ± 0.32^{a}	6.67 ± 0.58^{a}	7.33 ± 0.35^{a}
EC (%)	84.07 ± 1.10^{g}	76.67 ± 0.58^{f}	71.27 ± 0.93^{e}	53.53 ± 1.36^{c}	47.27 ± 0.55^{b}	43.33 ± 1.15^{a}	66.43 ± 2.87^{d}
ES (%)	41.83 ± 0.76^{e}	36.13 ± 0.81^{d}	28.07 ± 1.10^{b}	33.03 ± 0.65^{c}	28.43 ± 0.67^{b}	24.73 ± 0.64^{a}	3 5.20^{f}

$^{a-g}$: Different superscripts indicate significant differences between samples per parameter ($p < 0.05$)

A few studies have documented the effects of drying method on the functional properties of insect proteins [46-48]. In this study, BD increased with a decrease in mesh size for both the air dried and sun dried flour samples. BD of A. discoidalis flour ranged from 52.33 to 82.67g/mL with oven dried samples having high values compared to sun dried samples. Significant differences ($p < 0.05$) were observed for the air dried and oven dried samples using aperture size 150µm for all properties. BD is used as specification for some food products derived from drying/or grinding. The results for the BD were consistent with those reported by Ekpo [49] for Imbrasia belina where the bulk density increased with increasing in mesh size. When particle decreases, pore space decreases and the bulk density increases. Variations in BD are attributed to variations in particle size, expansion ratio, wall support and presence of oil [50]. Samples that were used in this study were not defatted. A higher BD is an important characteristic in lowering product viscosity in foods for the convalescent and children [48].

WHC of A. discoidalis flour values ranged from 236 to 183.73g/g with oven dried samples having high values compared to sun dried samples. No significant difference was observed between WHC of the protien isolate and oven dried A. discoidalis flour ground to 500µm. is Significant differences ($p < 0.05$) were observed between air dried and oven dried flour. In this study, a comparison between the WHC with relation to mesh size revealed that the finer the flour, the lower the water binding capacity. WHC values of 168±6% [51], 129±19% [41] and 218±7% [45] were observed for egg white, Tenebrio molitor and Schistocerca gregaria flour respectively. The significance of WHC is that, higher values of WHC, the higher maintenance on the moisture content of the product since moisture content has a significant effect on product quality. It is documented that intrinsic factors affecting water binding properties of flour with relatively higher protein content contents include the protein conformation, amino acid composition and surface polarity [52]. Large particle size has been linked to higher chitin content as observed in T. molitor [53] which entraps more water as the flour would be course. Fiber content influences the WHC, higher dietary fiber may result in higher WHC [54]. High water absorption values are desirable for ready-to-use foods.

Values for OHC of A. discoidalis flour ranged from 110.57 to 179.67g/g with oven dried samples having high values compared to sun dried samples. The results demonstrated that decrease in particle size caused a decrease in OHC of the armoured cricket flour. Significant differences ($p < 0.05$) for OHC were observed between air dried and sun dried flour samples of different particle sizes. OHC is important because oil is a flavor retainer and improves palatability of food products [55]. Higher OHCs could again be due to larger particle size and porous structure in course flour which causes physical entrapment of oil [56]. The values obtained were lower than that of Cirna forda reported by Omotoso [57] which exhibited higher OHC. According to Zayas [58], OHC is higher when the protein is insoluble and hydrophobic and there is a correlation between protein surface hydrophobicity and protein solubility. In this study, the OHC was determined when no chemical modification was not conducted.

FC values of A. discoidalis flour ranged from 4 to 33.83% with high values being observed for oven dried samples. FC decreased with an increase in mesh size for both the oven dried and sun dried flour samples. FC of proteins is described as the amount of interfacial area that can be created by protein and FS is the ability of a protein to stabilise against mechanical stress [59]. FS values of A. discoidalis flour ranged from 24 to 6.67% with high values being observed for oven dried samples. No significant difference ($p < 0.05$) was observed between the FS of protein isolate and that of sun dried flour ground to 300 and 500µm. Foams are

used to improve appearance, consistency, and texture of foods [60]. Good foam ability is linked to flexible protein molecules, which reduces surface tension [61]. Low FCs on the other and can be attributed to highly ordered globular proteins, which resist denaturation.

EC and ES values ranged from 43.33 to 84.07% and 24.73 to 41.83% respectively. EC and ES decreased with an increase in mesh size for both the oven dried and sun dried flour samples with significant differences ($p < 0.05$) being observed for the different particle sizes. Formation and stability of emulsions is important in food systems such as salad dressing [62, 63] Ability of proteins to form emulsions is attributed to the flexibility of solutes and exposure of hydrophobic domains. The formation and stability of emulsions is very important in food systems such as salad dressings. The capacity of proteins to enhance formation of emulsions and stabilising the emulsions is important in food applications such as manufacturing of cakes, frozen desserts and coffee whiteners.

This work showed the functional properties of A. discoidalis flour and protein extract. The obtained results could be valuable to industries that would want to take up insects in their formulation of improved foods. Insect flours are rich in protein, good extenders, thickeners and gelling agents. González et al., [64] suggests that Acheta domesticus is a suitable flour for bread formulation because of its desirable functional properties. The addition of 5% cricket powder into wheat pasta displayed no significant difference compared to whole wheat pasta [65]. Replacing 10% of lean pork in emulsion sausages with T. molitor or Bombyx mori insect proteins produced high valued added emulsion sausages [66]. Incorporation of T. molitor flour (10 and 20%) to cereal snacks incorporation increased the protein content and digestibility of the snacks [67].

CONCLUSION

The solubility values of A. discoidalis protein extract were minimum at around pH 5. The work demonstrated that A. discoidalis could be processed into flour. The functional properties of the flour were found to be comparable to those of other insect species. Data obtained in this study showed that drying method and mesh size have an effect on functional properties of A. discoidalis flour. Significant differences ($p < 0.05$) were observed for BD for sun dried and oven dried samples using aperture size 150µm. Furthermore, the foaming properties of the insect flour ranged from 4.00 to 33.83% for the dried samples.

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