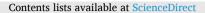
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# Dough rheological properties, physical, consumer acceptability and microbial qualities of wheat and wheat-sorghum biscuits fortified with longhorn grasshopper (*Ruspolia differens*) powder

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#### ABSTRACT

Protein deficiency is a nutritional challenge affecting young children in developing countries. Food fortification with edible insects is a potentially sustainable approach to increasing protein quality in young children's diets. This study investigated the effect of fortifying wheat and wheat-sorghum flours with *Ruspolia differens* powder (RDP) on the pasting properties of flour, rheological characteristics of dough, physical properties, consumer acceptability, and microbial qualities of biscuits. Ten biscuit formulations were made by replacing part wheat and wheat-sorghum flours with 5, 15, 25 and 40 % RDP. Textural and rheological properties of the dough and physical properties, microbial qualities, consumer acceptability and microbial qualities of the biscuit were determined. The gelatinization temperature for the wheat and wheat-sorghum flours increased, while the maximum viscosities, breakdown velocity, and setback viscosity reduced. Substitution of RDP in the biscuits reduced their weight and lightness. There was a significant reduction in springiness, cohesiveness, gumminess, adhesiveness, and dough stability of the doughs, with an increase in dough development time, and a reduction in water absorption with RDP fortification. The biscuits were highly acceptable following repeated exposure. The biscuits are microbiologically safe. Because of the high acceptance by children, these protein-rich biscuits have the potential for use as supplementary food.

## 1. Introduction

Protein deficiency is a major nutritional challenge that negatively affects children in developing countries. For instance, in the year 2020, 149 million children under the age of 5 years were stunted while 45.5 million suffered wasting [1]. Of these, 41 % and 27 % of stunted and wasted children, respectively live in Africa [2]. In young children, protein energy malnutrition (PEM) results in high morbidity, mortality, poor physical growth and cognitive development [3,4]. In Sub-Saharan Africa, millions of children mainly from resource-limited communities subsist on staple foods such as sorghum, millet, and maize as their main source of protein and energy [5]. However, these staples have poor protein quality, limited in essential amino acids, lysine, and tryptophan with poor digestibility [6]. Previous studies have reported lower serum

concentrations of amino acids in stunted children compared to those who are not [7,8].

Animal-sourced foods such as meat, eggs, and milk are the best sources of high-quality protein with the best amino acid profile and protein bioavailability [9] and have been strongly linked to a modest increase in linear growth of children [10]. However, they are unaffordable for many poorly resourced groups [11]. Recently, the COVID-19 pandemic has further exacerbated the situation due to worsening household income among vulnerable populations making protein-rich foods less available and affordable [12]. Additionally, current animal production systems negatively affect sustainable food production due to environmental degradation such as deforestation, desertification, contribution to greenhouse emissions, and climate change [13]. Consequently, the need to identify alternative and

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sustainable protein sources has led researchers to suggest the utilization of edible insects to supply high-quality proteins in the diet [14].

Insects are currently a natural food resource among many ethnic groups in Africa and Asia [15] and have been recognized as one of the sustainable solutions to protein deficiency in developing countries [16]. However, the practise of entomophagy differs across cultures and regions, with its consumption influenced by food taboos and religious/dietary restrictions. For instance, Judaism prohibits the consumption of most insects, allowing only a few specific ones that are deemed 'kosher' [17]. In Central Australia where totemism is practiced, the Arunta tribe has six insect groups including cicadas, beetle larvae, honey ants, and caterpillars. While these insects are forbidden as food, an exception is made during fertility ceremonies, where their consumption is permitted [17]. The East African longhorn grasshopper (Ruspolia differens) is an edible insect widely harvested and consumed in countries around the Lake Victoria crescent [18]. The longhorn grasshopper has a high protein content of 43–46 %, with good ratios of all the eight dispensable amino acids, crude fat of about 48 %, and high unsaturated fats and minerals [19]. One of the innovative ways to encourage its consumption among children is by incorporating popular snacks such as biscuits.

Biscuits are ready-to-eat foods conventionally made from wheat flour, fat, and sugar. Recent technological advancements have been developed to enhance their properties, aiming to address nutritional needs and prevent diet-related diseases [20]. Biscuits are often liked by children due to their portability, affordability, diverse flavors, and nutrient density, and long shelf life [21]. The extensive consumption of biscuits positions them as an ideal candidate for fortification with edible insects to improve their nutritional qualities [22]. In the current study, biscuits were developed from wheat and wheat-sorghum flours fortified with Ruspolia differens powder [23]. Fortifying the biscuits enhanced the protein content by 20-118 % in wheat biscuits, and 15-116 % in wheat-sorghum biscuits. The fortified biscuits met the protein recommended dietary allowance of the children between 50-180 % and 53-200 % for the wheat-sorghum and wheat biscuits, respectively. The biscuits have potential as a supplementary food to combat protein and energy malnutrition in school-going children [23]. As a result, food products designed specifically for school-aged children must be evaluated by children, and methods that reinforce and predict long-term acceptability, such as repeated measures [24] may alternatively be used.

The use of edible insect powder as an alternative food ingredient confers unique functional properties such as solubility, water and oil holding capacity, foaming, gelation, and emulsifying properties in food resulting in diverse rheological, physical, and sensory qualities of the end products [25]. For instance, bread developed from replacing wheat flour with 5 % insect powder from house cricket, mealworm, and black soldier fly resulted in loaves with high protein and fiber contents with decreased water absorption, and increased dough formation and stability [26]. In another study, biscuits enriched with cricket powder found that burnt flavor, and brown colour significantly decreased their overall liking [27]. There is need therefore evaluate the effects of still unexplored insect powders on the technological properties of baked goods such as biscuits produced with these alternative ingredients. There is limited research on the effects of fortifying wheat and wheat-sorghum flours with RDP on the texture and rheological qualities of the biscuit dough and biscuits, and the long-term acceptability of such biscuits using school-aged children. Therefore, this study investigated the effect of fortifying wheat and wheat-sorghum flours with RDP on the pasting properties of flour, rheological characteristics of dough, physical properties, microbial qualities, and consumer acceptability of biscuits.

## 2. Materials and methods

### 2.1. Biscuit formulation and preparation

Wheat and wheat-sorghum biscuits were formulated and developed

using the procedure described by Ronoh et al. [23]. The formulation rationale of the biscuits targeting school-going children was adopted from previous research [6] to supply an estimated 50 % of the protein needs of 3- to 10-year-old school-going children. From the proximate analysis results, preliminary computations were made to establish formulations of both the longhorn grasshopper powder and the cereal flours that would provide 6.5 g of protein in each biscuit.

The rationale for using wheat-sorghum mix at 1:1 was informed by a previous study which established that 100 % sorghum biscuits tended to have a rough and dry texture, which may not be appealing to consumers [6]. To circumvent these undesirable qualities, sorghum was composited with wheat. Wheat and wheat-sorghum flours for biscuits were developed by replacing part of the flours with 5, 10, 15, 25 and 40 % RDP. Biscuits with 0 % RDP served as controls. The basic formulation for the 100 % wheat and 50:50 sorghum-wheat comprised 100 g flour, 25 g sugar, 30 g sunflower oil and, 6 g vanilla essence (Table 1). Water was dependent on the treatment and ranged from 17.8 % (40 % RPD: 60 % wheat biscuits) to 27.1 % (40 % RPD: wheat-sorghum biscuits) of the total weight of ingredients. The amount of baking powder added ranged from 0.1 to 0.2 g in wheat-alone biscuits to 0.7 g in sorghum-based biscuits. The amounts added were based on the results of initial experiments which showed that the amount of water needed for the dough to form was dependent on the amount of RDP substitution in wheat-sorghum or wheat flours.

The biscuit doughs were hand-rolled to 5 mm thickness on a steel tray using a wooden rolling pin. They were then cut into circular shapes with a 6.5 cm diameter biscuit cutter. The cut dough pieces were placed on a baking sheet and baked in a pre-heated air circulation oven at  $185^{\circ}$ C for 30 min before cooling for 20 min at room temperature. Biscuits were stored in Ziploc plastic bags in a freezer at  $-18^{\circ}$ C (Fig. 1).

#### 2.2. Pasting properties of flour

The pasting properties of the flour were determined using a Brabender Viscograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) at 85 rpm and 700 cmg torgue. Slurries made up 40 g flour (adjusted to 14 % moisture content) and 420 ml water was added into the Viscograph-E canister. The canister was then placed in the heating chamber and were spindles attached. The slurry was heated from 30 °C to 93 °C at a rate of 1.5 °C/min; then held at 93 °C for 15 min; cooled to 30 °C at a rate of 1.5 °C/min, and finally held 30 °C for 15 min. The pasting temperature ( $^{0}$ C), peak viscosity (BU), breakdown viscosity (peak viscosity-trough viscosity, BU), setback viscosity (Final Viscosity-Trough viscosity, BU) and final viscosity (BU) were determined using the Viscograph-E correlation software.

#### 2.3. Dough characteristics

#### 2.3.1. Texture of the dough

Texture Profile Analyses of the biscuit dough (5 mm thick, 30 mm diameter) were measured using a 75 mm diameter aluminum cylinder probe (P/75) attached to a TA/XT-plus Texture Analyser with 50 kg load cell (Stable Micro Systems, Surrey, UK). The instrument settings were: 40 mm height calibration, 1 mms<sup>-1</sup> pre-test speed, 5 mms<sup>-1</sup> test speed, 5 mms<sup>-1</sup> post-test speed, 10 mm target mode distance, 0.05 N trigger auto force, 200 pps data acquisition rate, and 5 s pause between the compression cycles. The hardness, adhesiveness, cohesiveness, springiness, resilience, and gumminess of the dough were computed from the Texture Profile Analysis graph using the instrument software.

### 2.3.2. Rheological characteristics of the dough

2.3.2.1. Farinograph evaluation. The rheological characteristics of the dough were evaluated according to AACC 300 Method [28]. Dough development time (DT), stability time (ST), water absorption capacity

#### Table 1

Formulation of the composite biscuit doughs.

Ingredients	100 % whea	t				Wheat: Sorgh	num (50:50)			
	Fortification levels with Ruspolia differens powder									
	0	5	15	25	40	0	5	15	25	40
R. differens powder (g)	0 (0)	5 (2.5)	15 (7.53)	25 (12.68)	40 (20.39)	0 (0)	5 (2.40)	15 (7.09)	25 (11.48)	40 (18.04)
Sorghum flour (g)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	50 (24.55)	47.5 (22.76)	42.5 (20.08)	37.5 (17.23)	30 (13.53)
Wheat flour (g)	100 (49.73)	95 (47.45)	85 (42.67)	75 (38.03)	60 (30.58)	50 (24.55)	47.5 (22.76)	42.5 (20.08)	37.5 (17.23)	30 (13.53)
Sugar (g)	25 (12.43)	25 (12.49)	25 (12.55)	25 (12.68)	25 (12.74)	25 (12.27)	25 (11.98)	25 (11.81)	25 (11.48)	25 (11.27)
Sunflower oil (g)	30 (14.92)	30 (14.99)	30 (15.06)	30 (15.21)	30 (15.29)	30 (14.73)	30 (14.37)	30 (14.17)	30 (13.78)	30 (13.53)
Baking powder (g)	0.1 (0.05)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.7 (0.34)	0.7 (0.34)	0.7 (0.33)	0.7 (0.32)	0.7 (0.32)
Vanilla essence (g)	6 (2.98)	6 (3)	6 (3.01)	6 (3.04)	6 (3.06)	6 (2.95)	6 (2.87)	6 (2.83)	6 (2.75)	6 (2.72)
Water (g)	40 (19.89)	39 (19.47)	38 (19.08)	36 (18.26)	35 (17.84)	42 (20.61)	47 (22.52)	50 (23.61)	56 (25.73)	60 (27.06)
Total dough wt (g)	201.1	200.2	199.2	197.2	196.2	203.7	208.7 (100)	211.7 (100)	217.7 (100)	221.7
	(100)	(100)	(100)	(100)	(100)	(100)				(100)

Figures in parentheses are percentages. Adopted from Ronoh et al. (2024).

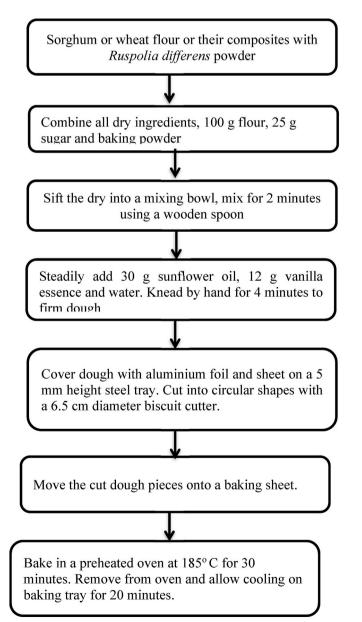


Fig. 1. Flow diagram for preparation of wheat and wheat-sorghum biscuits fortified with *Ruspolia differens* powder.

(%), and Farinograph Quality Number (FQN) at  $500 \pm 20$  FU dough consistency were determined using a Farinograph-AT (Brabender GmbH & Co. KG, Duisburg, Germany) using 300 g flour samples. Water absorption percentages recorded in farinograph trials were used to identify the optimum amount of water to be added to the biscuit dough-making process. DT is defined as the time between the start of the measurement (addition of water) and the point of the torque curve just before weakening begins. ST is defined as the time between the first and second intersection points of the upper trace of the torque curve with the line of consistency, and FQN as the length from the water point to a point 30 FU below the center line of greatest consistency along the time axis [29].

2.3.2.2. Extensograph evaluation. The wheat dough was prepared using the Farinograph according to the ICC-Standard no. 114/1 method using 300g of sample, distilled water, and 6 g analytical salt [30]. The amount of water added to the composite flour was determined from previous Farinograph runs. The dough was prepared using the Farinograph for 5 min. One hundred and fifty (150 g) of the dough was weighed, then homogenized in the balling unit, shaped in the roll fixed with the clamps and, incubated for 20 min [31]. Next, the dough was stretched until rapture using an Extensograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) while placed onto the support system of the device. Dough energy (cm<sup>2</sup>), dough resistance to extension (EU), extensibility, and maximum resistance to extension were recorded. The rheological evaluations were done on the wheat dough only as the farinograph and extensograph are designed and optimized for rheological evaluation of doughs prepared exclusively from wheat flour [29].

# 2.4. Physical characteristics of biscuits

## 2.4.1. Biscuit weight, diameter and thickness

The baking quality of cookie flour Method 10-50D [32] was utilized to measure the width, thickness, and spread factor of the biscuits. A digital Vernier caliper (Model 2002/95/EC, Sealey Professional Tools, UK) was used to obtain the measurements. The width of the biscuits was measured after placing the biscuits edge to edge then rotated 90° and measured again to obtain average width (W) in mm. To measure the thicknesses (T), six biscuits were stuck on top of one another, then re-stacking in a different order and re-measuring to get the average thickness. These measurements were read to the nearest 0.5 mm. Spread factor (SF) was calculated as SF = W/T. The mean weights of six biscuits were taken using Shimadzu electronic balance (Shimadzu Corporation, Japan). The volume was calculated as radius ( $r^2$ ) x (T) x 3.142 and the density of biscuits was calculated as (mass/volume) and expressed as  $g/cm^3$ .

## 2.4.2. Biscuit color

Biscuit colors were measured using the CIE-L\*a\*b\* uniform color space with a Colour Reader (CR-10 Plus, Konica Minolta, Tokyo, Japan) (D65 illuminant and 10° viewing angle) after standardization with a white calibration plate. Measurements were taken with a 30 mm diameter diaphragm inset with optical glass. Color parameters measured were; L\* the lightness, a\* the hue on a green (–) to red (+) axis and, b\* the hue on a blue (–) to yellow (+) axis. Three measurements were made at different points in each biscuit. Additionally, the total color difference ( $\Delta$ E) among the biscuit samples was calculated using the formula;

 $\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ 

Where  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  are differences in the L\*, a\*, and b\* between the reference sample (Wheat biscuits) and the test samples (fortified wheat-sorghum biscuits, and fortified wheat biscuits).

The Browning Index of the biscuits was computed following the formula [33];

Browning Index = 
$$\frac{100*(X-0.31)}{0.17}$$

Where X =  $\frac{(a + 1.75L)}{(5.645L + a - 3.012b)}$ 

## 2.4.3. Biscuit texture analysis

Texture Profile Analysis of the biscuit (20 mm thick, 30 mm diameter) was measured using a 6 mm diameter aluminum cylinder probe (P/ 6) attached to a TA/XT-plus Texture Analyser with 50 kg load cell (Stable Micro Systems, Surrey, UK). The instrument settings were: 40 mm height calibration, 1 mms<sup>-1</sup> pre-test speed, 0.5 mms<sup>-1</sup> test speed, 10 mms<sup>-1</sup> post-test speed, 2 mm target mode distance, 0.05 N trigger auto force, 400 pps data acquisition rate, and 5 s pause between the compression cycles. The hardness of the biscuits was calculated from the Texture Profile Analysis graph using the instrument software.

#### 2.5. Consumer acceptability by children

Six variations of biscuits were evaluated in this study. The first four biscuit samples were (wheat-sorghum 5 % RDP; wheat 5 % RDP; wheatsorghum 40 % RDP, and wheat 40 % RDP). The choice of these biscuits was based on the ability of the biscuits to meet half the protein and energy requirements of children 8-9 years old [23]. The fifth and the sixth samples were 100 % wheat-sorghum and 100 % wheat biscuits used as the controls, as the 100 % wheat biscuit is conventionally consumed by children. The justification for the choice of the six biscuit samples was informed by previous studies [34,35] that demonstrated that children 8-9 years old can comfortably evaluate up to 10 food samples in one session. One hundred children (43 boys and 57 girls) aged 8-9 years old who schooled at the University of Eldoret Primary School in Eldoret, Kenya evaluated the biscuits. A letter was sent the children's parents informing them of the objectives, procedures, activities, risks, and advantages of the study in which children would participate. The study only included children whose parents completed the consent form and willingly consented. Over tea break at 9:30 a.m. on the first day, an orientation session lasting 1 h was conducted in the school's classroom. Through the session, the children learned how to utilize a seven-point scale with stylized faces. The 100 children were divided into four groups of 25 each. For ease of identification, each group was allocated a color: red, yellow, green, or orange. The children were assigned at random to one of 100 stations, each with a tray holding samples for evaluation and name tags displaying the child's number and group number. Each group was allocated a research assistant from the University of Eldoret, who speaks English and Kiswahili.

The children were informed that the various facial expressions showed if they liked what they were eating extremely, very much, a little, not sure, disliked a little, disliked very much, or disliked

extremely. As test samples, two fruits, a sweet banana, and a sour lemon were labeled with three-digit blinding codes. The children were asked to remove the labels from the fruits and place them on the face that represented their feelings about the fruit they had just sampled. With the sticker from the liked banana on a happy face and the sticker from the disliked lemon on a sad face. A small tumbler filled with distilled water was offered for cleaning the palate before and after sampling the biscuits. This session was held in both English and Kiswahili languages. The children were also informed that they could withdraw from the study at any time if they desired. Hedonic categorization was used to measure the children's preference for the biscuits over four days. The biscuits were served on a plate having randomized three-digit codes. A seven-point hedonic facial scale was employed with dislike extremely at 1, neither like nor dislike at 5, and like extremely at 7. The children were given directions to begin with the biscuit on the left side, taste each biscuit, take the coding label, and place it on the scoresheet on the face that corresponded with how they felt after the evaluation. After tasting, they were provided with clean water for cleaning their palates. There were four rounds of the process. Therefore, to determine long-term acceptability, each child assessed each variation of biscuits four times over four days.

#### 2.6. Microbial quality

Determination of the presence of aerobic mesophilic bacteria was done by serial dilution and cultured by pour-plate method [36]. A gram of each of the samples was placed into a test tube containing 10 ml of sterilized normal saline (0.85 % sodium chloride solution). This mixture was thoroughly homogenized using a vortex. Afterward, 1 ml of the mixture was added to the next test tube containing 9 ml of normal saline and the procedure was repeated until the  $10^{-4}$  dilution. This had been earlier established as the most appropriate dilution for plating. In the end, 1 ml of this final dilution was transferred into a sterilized Petri dish. For each sample, there were three replications. A cooled nutrient agar (NA) media was then poured over and left to solidify before being incubated at 28±2 °C for 24 h. After 24 h, observable colony-forming units were counted and documented. A similar procedure was followed when determining the presence of coliforms, Escherichia coli, and Staphylococcus aureus. However, instead of NA, MacConkey agar was used for coliforms, Eosin Methylin Blue (EMB) agar for E. coli, and Mannitol Salt Agar (MSA) for S. aureus. Golden metallic sheen was sought to confirm the presence of E. coli in EMB agar. To determine the presence of spore-forming bacteria, after solidification of NA, there was an initial period where plates containing inoculated media were first incubated at 90 °C for 10 min before being transferred to a mesophilic temperature for 24 h [37]. Saboraud Dextrose Agar (SDA) amended with chloramphenicol was used in the determination of yeast and molds. Incubation was done at 28  $\pm$ 2 °C for 72 h.

#### 2.7. Experimental design and statistical analysis

Each of the experiments followed a single factor completely randomized experimental design. Using one-way analysis of variance, data on physical, instrumental texture, rheological qualities, and consumer acceptability were analyzed using Minitab Release 18 Software (Minitab Inc., Pennsylvania, USA). Fisher's Lease Significant Difference at p < 0.05 was used to compare all pairwise differences between the treatment means.

#### 2.8. Ethical considerations

The Mount Kenya University Ethics Research Committee provided ethical clearance for this study (Application Approval Number: 1131). A research license (number: NACOSTI/P/22/16130) was issued by The National Commission for Science, Technology, and Innovation (NACOSTI). The study only included children whose parents signed a consent form explaining the nature of the biscuit samples and the activities included, and who freely agreed to participate in the research The University of Eldoret Primary School headmaster granted permission for the study to be carried out in the school.

### 3. Results and discussion

#### 3.1. Pasting properties of flour

Results in Table 3 show that the gelatinization temperature (GT) significantly increased with an increase in the RDP. For instance, there was a 3.6-10.78 % and 0.9-9.2 % increase in GT for the wheat and wheat-sorghum flours respectively as the fortification levels with RDP increased. Gelatinization temperature is the minimum temperature required for starch to gelatinize [38]. The increase may be attributed to the fact that as the fortification levels increased, there was a significant increase in protein, crude fiber, and fat, with a decrease in the carbohydrate content in the composite flours (Table 2). High fiber and protein content in cereals delay gelatinization as they prevent hydration and swelling of the starch granule [39]. Also, lipids hinder starch swelling by forming starch-lipid complexes, which cover the starch surface, increasing hydrophobicity and restraining water movement into the granules [40]. As the fortification levels with RDP increased, there was an 11.3 % and 17-85 % reduction in the maximum viscosities of the wheat and the wheat-sorghum flours respectively. The significant reduction is attributed to the decrease of starch in the flours with an increase in the RDP (Table 2). The increased quantity of proteins, lipids, and fiber diluted the starch content of the flour (Table 2), thereby restricting starch swelling and gelatinization during cooking, lowering maximum viscosity [41]. This effect may also have led to a significant reduction in the final viscosities of the flours, with 73 % and 28–82 %reduction in the fortified wheat and wheat-sorghum flours compared to the unfortified flours. The water-binding capacity of flour is dependent on the starch content and the gel formation capacity of these macromolecules [39]. Since the starch content of the flours reduced as the RDP increased, it caused a decrease in the final viscosity. Starch components, amylose, amylopectin, amylose-amylopectin ratio and starch content are the major contributors to the final viscosity [40]. In addition, the association of lipids and the amylose component of the starch, and the amylose-to-amylopectin ratio might have affected the ability of the starches to bind to lipids [42]. Similar findings have been reported on the roti flour enriched with house cricket powder [43].

There was a significant reduction in the breakdown viscosity (BV) in the fortified wheat and wheat-sorghum flours by 50-90 % and 2-98 % respectively compared to the control flours (Table 3). Breakdown viscosity indicates the stability of the cooked flour paste, with low BV implying stronger resistance to the shear-thinning effect of pastes [44]. The reduction may be an indicator that proteins and fiber from RDP provided the starch granules with higher resistance to disintegrate at high temperature, therefore addition of the powder inhibited starch retrogradation of the flours [45]. Due to the high water retention capacity of dietary fiber, it causes a redistribution of water molecules in the flour and therefore inhibits the aging of starch to a certain extent [46]. This may have also led to a reduction in the setback viscosity. Similar results showing a decrease in pasting viscosity, breakdown viscosity, final viscosity, and setback viscosity have been reported by researchers working with brown rice flour fortified with Bombay locust powder [45].

#### 3.1.1. Dough texture parameters

There was a significant increase in the hardness of both wheat dough as the fortification levels with RDP (Table 4). The 40 % fortified wheat dough was significantly harder compared to the other doughs, while there was a 22-31 % reduction in the hardness of the wheat-sorghum composite doughs as the RDP levels increased. This can be attributed to the gluten dilution effect and/or water competition mechanism which may have caused an increase in hardness [44]. The fiber from RDP could have physically disrupted the continuity of the gluten network (Table 2). The reduction in the hardness of the wheat-sorghum dough is attributed gluten dilution effect of RDP. Also, the fat could have mixed with the flour before hydration preventing the formation of gluten network, to produce a less elastic tough dough. This is because sorghum protein bodies, kafirins, have no functional properties in dough development and texture of sorghum-based dough as they are hydrophobic and inert compared to gluten proteins [47]. Increasing hardness of the wheat dough and reduced hardness of wheat-sorghum dough may have correspondingly led to an increase in adhesiveness. The adhesiveness of wheat dough increased with increasing levels of RDP fortification, with 40 % RDP dough being significantly different from the rest of the dough. Conversely, there was a 1-76 % increase in the adhesiveness of the wheat-sorghum dough. An increase in hardness and adhesiveness of dough enriched with Tenebrio molitor and Zophobas atratus powders has been reported [48]. The changes in springiness and resilience of both wheat and wheat-sorghum doughs were not consistent with fortification with RDP at 40 % showing the lowest values for these texture parameters. There was significant a reduction in cohesiveness and gumminess of both wheat and wheat-sorghum doughs can also be attributed to the gluten dilution effect of RDP.

Table 2

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The effect of fortifying sorghum and whe	neat with Ruspolia differens powder (R	DP) on proximate composition of	f biscuits (g/100g) dry weight basis.
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Flour/Biscuits	Moisture	Protein (N $\times$ 6.25)	Fat	Ash	Crude fiber	Carbohydrate	Energy (kJ/g 100g)	Dietary fiber
Flour								
Sorghum flour	$9.48\pm0.4^{\rm b}$	$11.22\pm0.7^{\rm j}$	$3.40^{i}\pm0.3^{i}$	$1.54\pm0.3^{ m bc}$	$2.25\pm0.2^{\rm f}$	$72.11\pm0.7^{\rm a}$	1523 <sup>i</sup>	$6.23\pm0.1^{\rm j}$
Wheat flour	$10.8\pm0.7^{a}$	$13.69\pm0.3^{\rm h}$	$2.17^{j}\pm0.1^{j}$	$0.51\pm0.1^{ m h}$	$0.85\pm0.0^{\rm i}$	$71.99 \pm \mathbf{0.8^a}$	1516 <sup>i</sup>	$11.53\pm0.2^{ef}$
Ruspolia differens powder	$\textbf{7.42} \pm \textbf{0.8}^{c}$	$49.69\pm0.6^a$	$21.7^{\mathrm{f}}{\pm}0.2^{\mathrm{f}}$	$3.98\pm0.4^{a}$	$12.07\pm0.2^{a}$	$5.14\pm0.9^{\rm j}$	1734 <sup>h</sup>	$13.98\pm0.4^{c}$
Wheat: RDP biscuits								
100:0	$6.41\pm0.4^{ m d}$	$12.97\pm0.2^{\rm i}$	$19.30\pm0.9^{h}$	$0.76\pm0.1^{\text{g}}$	$0.84\pm0.0^{\rm i}$	$59.72\pm0.8^{\rm b}$	1943 <sup>g</sup>	$11.37\pm0.3^{\rm f}$
95:5	$4.31\pm0.1^{ef}$	$14.90\pm0.4^{\text{g}}$	$20.97\pm0.6^{\text{g}}$	$0.81 \pm 0.1^{\text{g}}$	$1.38\pm0.1^{\rm h}$	$57.64\pm0.9^{\rm c}$	2004 <sup>f</sup>	$12.32\pm0.1^{\rm d}$
85:15	$4.08\pm0.0^{\text{fg}}$	$18.27\pm0.3^{\rm e}$	$23.87\pm0.5^{\rm d}$	$0.96\pm0.1^{\rm fg}$	$2.58\pm0.1^{e}$	$50.25\pm0.3^{e}$	2045 <sup>ab</sup>	$13.68\pm0.3^{\rm c}$
75:25	$3.73\pm0.0^{gh}$	$25.13 \pm \mathbf{0.2^c}$	$24.66\pm0.2^{b}$	$1.16\pm0.2^{ef}$	$3.51\pm0.3^{\rm d}$	$41.80 \pm 0.5^{\text{g}}$	2049 <sup>ab</sup>	$14.57\pm0.1^{\rm b}$
60:40	$3.23\pm0.1^{\rm i}$	$28.01\pm0.2^{\rm b}$	$26.38\pm0.3^{\text{a}}$	$1.28\pm0.2^{ m de}$	$5.65\pm0.2^{\rm b}$	$35.47 \pm \mathbf{0.7^{i}}$	2055 <sup>a</sup>	$15.70\pm0.0^a$
Wheat- sorghum: RDP bis	scuits							
100:0	$4.56\pm0.0^{e}$	$11.60\pm0.5^{\rm j}$	$23.27 \pm 1.4^{\rm e}$	$1.06^{ m ef}{\pm}0.1$	$1.64\pm0.1^{\text{g}}$	$57.87 \pm 1.3^{\rm c}$	2039 <sup>bc</sup>	$8.45\pm0.3^{i}$
95:5	$\textbf{4.48} \pm \textbf{0.0}^{e}$	$13.91\pm0.3^{\rm h}$	$23.27\pm0.1^{\rm e}$	$1.15^{eg}\pm0.1$	$2.21\pm0.0^{\rm f}$	$54.97\pm0.3^{\rm d}$	2029 <sup>cd</sup>	$9.27\pm0.2^{\rm h}$
85:15	$3.84\pm0.0^{gh}$	$17.44\pm0.5^{\rm f}$	$23.87\pm0.5^{\rm d}$	$1.23^{ m e}{\pm}0.1$	$3.51\pm0.0^{\rm d}$	$50.41\pm0.5^{e}$	2023 <sup>d</sup>	$10.67\pm0.1^{\text{g}}$
75:25	$3.54\pm0.0^{hi}$	$20.33 \pm 0.7^{d}$	$23.93\pm0.1^{cd}$	$1.46^{cd}\pm0.1$	$4.30\pm0.12^{c}$	$46.44\pm0.8^{\rm f}$	2019 <sup>de</sup>	$11.82\pm0.2^{e}$
60:40	$\textbf{3.17} \pm \textbf{0.1}^{i}$	$25.29 \pm \mathbf{0.7^c}$	$24.47\pm0.1^{bc}$	$1.77^{b}\pm0.2$	$5.60\pm0.0^{\rm b}$	$39.70 \pm 0.8^{h}$	2009 <sup>ef</sup>	$12.41 \pm 0.0^{d}$

Values are means  $\pm$  standard deviation of three determinations. Values with the same letter superscript on the same column are not significantly different at (p<0.05) as assessed by Fisher's least significant difference; RDP=*Ruspolia differens* powder. Adopted from Ronoh et al. [23].

Effect of fortification of wheat a		

Flour blends	Gelatinization temperature ( <sup>0</sup> C)	Maximum Viscosity (BU)	Maximum viscosity ( <sup>0</sup> C)	Final viscosity (BU)	Breakdown viscosity (BU)	Setback (BU)
Wheat: RDP flo	our					
100:0	$81.70\pm2.6^{\rm cd}$	$113.5\pm13.4^{\rm c}$	$92.20\pm2.1^{ab}$	$256.0\pm28.3^{\mathrm{b}}$	$36.00\pm1.4^{\rm a}$	$180.0\pm9.9^{\rm b}$
95:5	$80.40\pm1.1^d$	$113.5\pm2.1^{\rm c}$	$92.65 \pm 1.6^{\rm ab}$	$256.5\pm20.5^{b}$	$39.00\pm5.6^{a}$	$182.0\pm19.8^{b}$
85:15	$84.63 \pm 1.5^{\rm bc}$	$69.0\pm17.8^{\rm d}$	$91.50\pm0.4^{\rm b}$	$157.0 \pm 32.1^{c}$	$18.00\pm0.0^{\rm b}$	$114.3\pm22.7^{\rm c}$
75:25	$86.05\pm1.6^{\rm b}$	$35.50\pm4.9^{e}$	$91.90 \pm 1.6^{\rm ab}$	$84.50\pm4.9^{de}$	$1.50\pm0.7^{\rm c}$	$56.50\pm4.9^{de}$
60:40	$90.50\pm0.4^a$	$29.0\pm7.1^{e}$	$93.40\pm0.0^a$	$\textbf{70.00} \pm \textbf{12.7}^{\text{de}}$	$3.50\pm0.7^{\rm bc}$	$50.00\pm7.1^{\rm e}$
Wheat-sorghu	m: RDP flour					
100:0	$84.55 \pm 1.1^{\rm bc}$	$189.5\pm2.8^{\rm a}$	$93.45\pm0.5^{ab}$	$386.00\pm12.7^a$	$46.0\pm21.2^a$	$252.5\pm36.1^{a}$
95:5	$85.30\pm2.1^{\rm b}$	$158.0\pm17.0^{\rm b}$	$93.65\pm0.0^a$	$276.50 \pm \mathbf{4.9^{b}}$	$45.00\pm1.4^{a}$	$214.00\pm5.7^{\rm b}$
85:15	$91.20\pm1.9^a$	$75.50 \pm \mathbf{10.6^d}$	$93.30\pm0.4^{ab}$	$165.00\pm2.8^{\rm c}$	$14.00\pm0.0^{\rm bc}$	$92.50\pm4.9^{cd}$
75:25	$90.85\pm0.0^a$	$65.50\pm9.2^d$	$93.35\pm0.6^{ab}$	$104.50\pm2.1^{d}$	$13.00\pm2.8^{\rm bc}$	$50.50\pm 6.3^{e}$
60:40	$92.30\pm1.3^{\rm a}$	$29.50\pm3.5^e$	$93.00\pm0.1^{ab}$	$70.00 \pm 12.73^{de}$	$1.00\pm1.0^{c}$	$\textbf{37.50} \pm \textbf{2.1}^{e}$

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p<0.05) as assessed by Fisher's least significant difference.

BU: Brabender Unit; RDP: Ruspolia differens powder.

Table 4
The effect of fortifying sorghum and wheat biscuits with defatted <i>Ruspolia differens</i> powder (RDP) on texture parameters of the dough and biscuit.

	Dough						Biscuit
	Hardness (g)	Adhesiveness (g.sec)	Springiness <sup>a</sup>	Cohesiveness <sup>a</sup>	Resilience <sup>a</sup>	Gumminess <sup>a</sup>	Hardness (g)
Wheat: RDP	)						
100:0	$\textbf{7.41} \pm \textbf{0.7}^{b}$	$81.92\pm6.1^{\rm g}$	$0.87\pm0.0^{\rm b}$	$0.72\pm0.0^{\rm a}$	$0.06\pm0.0^{\rm d}$	$5.33\pm0.6^{\rm a}$	$1.94\pm0.7^{\rm e}$
95:5	$6.57\pm0.2^{bc}$	$76.27 \pm \mathbf{4.4^g}$	$0.89\pm0.0^{\rm b}$	$0.72\pm0.0^{\rm a}$	$0.08\pm0.0^{\rm a}$	$4.75\pm0.2^{\rm a}$	$1.81\pm0.3^{\rm e}$
85:15	$7.22\pm0.3^{bc}$	$117.76\pm2.0^{\rm f}$	$0.98\pm0.0^{\rm a}$	$0.69\pm0.0^{\rm b}$	$0.06\pm0.0^{d}$	$5.02\pm0.2^{\rm a}$	$2.07\pm1.1^{\rm e}$
75:25	$7.05\pm0.4^{bc}$	$91.03\pm5.8^{\rm g}$	$0.96\pm0.0^{\rm a}$	$0.71\pm0.0^{\rm a}$	$0.06\pm0.0^{d}$	$5.07\pm0.3^{\rm a}$	$2.57\pm0.7^{\rm e}$
60:40	$8.87\pm0.7^{\rm a}$	$171.72\pm0.9^{\rm e}$	$0.75\pm0.0^{\rm c}$	$0.56\pm0.0^{\rm c}$	$0.05\pm0.0^{\rm e}$	$4.98\pm0.4^{\rm a}$	$4.32\pm1.6^{cd}$
Wheat-sorgh	num: RDP						
100:0	$8.79\pm0.0^{a}$	$255.67\pm3.4^{\rm d}$	$0.63\pm0.0^{\rm d}$	$0.43\pm0.0^{\rm d}$	$0.04\pm0.0^{\rm f}$	$3.82\pm0.0^{\rm b}$	$6.71 \pm 1.1^{\rm a}$
95:5	$6.79\pm0.4^{bc}$	$258.0 \pm \mathbf{35.2^d}$	$0.66\pm0.0^{\rm d}$	$0.43\pm0.0^{\rm d}$	$0.04\pm0.0^{\rm f}$	$2.95\pm0.2^{\rm b}$	$6.13\pm1.5^{\rm ab}$
85:15	$6.06 \pm 1.7^{\rm c}$	$296.78 \pm \mathbf{8.9^c}$	$0.65\pm0.0^{\rm d}$	$0.43\pm0.0^{\rm d}$	$0.05\pm0.0^{\rm e}$	$2.63\pm0.7^{\rm cd}$	$5.38\pm0.9^{bc}$
75:25	$6.35\pm0.4^{bc}$	$360.21\pm15.5^{\mathrm{b}}$	$0.51\pm0.0^{\rm e}$	$0.34\pm0.0^{\rm e}$	$0.06\pm0.0^{d}$	$2.15\pm0.1^{\rm d}$	$4.31 \pm 1.6^{ed}$
60:40	$6.86 \pm 1.07^{bc}$	$450.36\pm10.9^a$	$0.46\pm0.0^{\rm f}$	$0.29\pm0.0^{\rm f}$	$0.06\pm0.0^{\rm d}$	$1.99\pm0.4^{\rm d}$	$3.89 \pm 1.5^{\rm d}$

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p<0.05) as assessed by Fisher's least significant difference.

# 3.2. Rheological properties of the doughs

#### 3.2.1. Farinograph evaluation

Farinograph measurements such as dough development time (DDT), dough stability (DS), and farinograph quality number (FQN) show the tightness of the gluten networks and the strength of the dough, with higher values signifying stronger doughs [49]. The DDT of the biscuit dough increased by 240-327 % as the levels of RDP were increased compared to the control dough (Table 5). DDT is the amount of time required for all the dough components to fully hydrate and form a viscoelastic mass [50]. The increased DDT with RDP fortification is as a result of the hydration properties of dietary fiber (Table 2) or the presence of other hydrophobic compounds such as insect oil. Dough rheology is mostly influenced by non-protein ingredients like starch and non-starch polysaccharides [51], in this case, chitin from the insect powder. These food components increase the elasticity of the dough by combining with other carbohydrates and proteins to produce weak secondary bonds and elastic interactions [51]. This might have led to a delay in the formation of the dough and the following gluten breakdown, which raised the DDT. The increased DDT may have led to reduced dough stability and an increase in the mixing tolerance index.

There was a 1–9 % reduction in water absorption as the levels of RDP substitution increased. This can be ascribed to the fact that increase in the RDP increased the fat and the protein content of the dough (Table 2). For instance, a linear negative relationship between protein and fat contents with moisture absorption in all-purpose bread wheat fortified with mealworm powder has been reported [52]. During mixing, the fat acted as a lubricant and competed with water for the starch granule surface, therefore preventing the formation of a gluten network and

restricting the swelling of the starch granules [53]. When flour contains a high-fat content, the dough's lubrication properties are effective and therefore lea water is needed to create a uniform dough [54]. The reduction in the starch content and increase in the protein content as RDP increased (Table 2) can reduce the water absorption, because of amino acids in insect powders or by the reduction of the amount of gluten which has a high water absorption capacity [55]. This finding corroborates that of other researchers who found that 5 % fortification level of wheat flour with *Acheta domesticus* or *Hermatia illucens* powders decreases the water absorption capacity with increasing the amount of yellow mealworm powder in cereal-based snacks has been reported [55].

The Farinograph quality number (FQN) of the fortified doughs was significantly higher compared to the control dough. As a result of fortification, there was a 78–173 % increase in FQN (Table 5). This unit defines the flour quality in a compressed form where weak flour has a low FQN, while stronger flour samples show higher FQN [56]. There is a strong direct linkage between FQN and other farinograph parameters such as DS [57]. Overall, a 5 % fortification level with RDP caused relatively high changes in farinograph behavior of wheat flour, probably, owed to the strongest disruption of the gluten skeleton.

#### 3.2.2. Extensograph evaluation

Extensograph evaluation was used to elucidate the effect of RDP on the viscoelastic properties of dough. The extensibility of the biscuit dough decreased with an increase in the RDP levels. Resistance to extension was low in the 40 % level of RDP substitution and was significantly different from the other dough samples (Table 5). This can

#### Table 5

The effect of fortifying wheat flour *Ruspolia differens* powder (RDP) on rheological parameters of the dough.

logical parameters	Ũ				
Rheological parameters	100:00	95:5	85:15	75:25	60:40
Farinograph evalua	tion				
Dough	107.00	99.00 $\pm$	457.5 $\pm$	378.3 $\pm$	364.0 $\pm$
development	$\pm$ 7.1 <sup>c</sup>	2.8 <sup>c</sup>	30.4 <sup>a</sup>	54.0 <sup>b</sup>	$0.0^{\mathrm{b}}$
time (sec)					
Water absorption	57.30 $\pm$	56.65 $\pm$	55.10 $\pm$	52.63 $\pm$	52.35 $\pm$
(%)	$0.14^{a}$	$0.1^{\mathrm{a}}$	$0.0^{\mathrm{b}}$	0.9 <sup>c</sup>	0.1 <sup>c</sup>
Dough stability	454.0 $\pm$	717.50	$622.5 \pm$	452.5 $\pm$	226.50
(sec)	$38.2^{bc}$	$\pm 10.6^{a}$	16.3 <sup>ab</sup>	$67.2^{bc}$	$\pm$ 4.9 <sup>c</sup>
Mixing tolerance	$39.0~\pm$	11.00 $\pm$	$47.00~\pm$	81.0 $\pm$	106.50
index (FU) <sup>a</sup>	4.24 <sup>c</sup>	$0.0^{d}$	$0.0^{\rm c}$	$11.31^{b}$	$\pm$ 4.9 <sup>a</sup>
Farinograph	$\textbf{45.00} \pm$	123.50	114.00	87.00 $\pm$	80.00 $\pm$
Quality	9.90 <sup>c</sup>	$\pm$ 2.1 <sup>a</sup>	$\pm 1.4^{a}$	9.9 <sup>b</sup>	$0.0^{\rm b}$
Numbers					
Time to break	$270.5~\pm$	740.50	684.00	522.0 $\pm$	479.0 $\pm$
down (sec)	60.1 <sup>c</sup>	$\pm$ 13.4 <sup>a</sup>	$\pm$ 8.5 <sup>a</sup>	$60.8^{\mathrm{b}}$	$0.0^{\mathrm{b}}$
Extensograph evalu	ation				
Energy (cm <sup>2</sup> )	149.75	130.00	$89.00 \ \pm$	$68.00~\pm$	36.25 $\pm$
	$\pm$ 4.3 <sup>a</sup>	$\pm$ 7.3 <sup>b</sup>	4.6 <sup>c</sup>	8.7 <sup>d</sup>	2.5 <sup>e</sup>
Resistance to	573.3 $\pm$	568.5 $\pm$	560.5 $\pm$	635.8 $\pm$	342.3 $\pm$
extension (EU) <sup>b</sup>	55.8 <sup>a</sup>	23.7 <sup>a</sup>	38.7 <sup>a</sup>	136.4 <sup>a</sup>	21.1 <sup>b</sup>
Extensibility	148.75	138.00	109.50	82.75 $\pm$	67.00 $\pm$
	$\pm$ 8.5 <sup>a</sup>	$\pm 2.8^{\rm b}$	$\pm 2.5^{c}$	$8.0^{d}$	3.8 <sup>e</sup>
Maximum (BU) <sup>c</sup>	796.5 $\pm$	723.3 $\pm$	600.0 $\pm$	643.3 $\pm$	401.8 $\pm$
	54.0 <sup>a</sup>	31.2 <sup>ab</sup>	46.0 <sup>c</sup>	148.0 <sup>bc</sup>	22.5 <sup>d</sup>

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p  $\leq$  0.05) as assessed by Fisher's least significant difference.

<sup>a</sup> FU: Farinograph Unit.

<sup>b</sup> EU: Extensibility Unit.

<sup>c</sup> BU: Brabender Unit.

be attributed to a reduced quantity of gluten in the dough which is responsible for the elasticity of the dough [58]. Similarly, this could have been attributed to the amount of fat and fiber in the dough which inhibited gluten network formation (Table 2). Consequently, this caused a significant reduction in the energy, extensibility and also the maximum Brabender units (Table 5). For instance, there was a 0.8–40 % reduction in the resistance to extenuation of the fortified dough compared to the control dough. Similar results have been reported. For instance, wheat dough fortified with black soldier pupae powder which was less stretchy as the levels of powder increased [25]. In another study, the use of mealworm powder causes the formation of a less developed three-dimensional gluten network, leading to a less viscoelastic dough [59].

### 3.3. Physical characteristics of biscuits

### 3.3.1. Biscuit weight, thickness and spread factor

Table 6 shows that increased substitution of RDP with wheat and wheat-sorghum reduced weights of biscuits by 8-19 % and 22-29 % respectively, compared to the 100 % cereal biscuits. The weight loss in both biscuit types may be explained by a reduction in starch content from sorghum and wheat as RDP has a low carbohydrate content of 4 % [60]. The weight loss in the wheat-sorghum biscuits was higher compared to the wheat biscuits. This is due to the hydrophobic characteristic of sorghum kafirins compared to hydrophilic wheat proteins [61]. Therefore the wheat-sorghum biscuits could have expelled more water during baking [58]. Another cause of higher weight loss in sorghum-wheat biscuits may have been the higher fiber content of sorghum which could have caused greater hydration that made the dough dry and crumbly requiring more water to make it workable as RDP levels increased. Therefore, this may have resulted in a reduction of total solids in the dough lowering the weight of biscuits because they had less dry matter. Consistent findings were reported by studies that established a

## Table 6

The effect of fortifying sorghum and wheat biscuits with defatted *Ruspolia differens* powder on physical characteristics of the biscuits.

Biscuit type	Biscuit weight (g)	Width (mm)	Thickness (mm)	Spread factor	Density (g/ cm <sup>3</sup> )						
Wheat: RD	Wheat: RDP biscuits										
100:0	36.54 $\pm$	57.87 $\pm$	$17.23\pm0.0^{a}$	3.36 $\pm$	$0.81~\pm$						
	2.0 <sup>a</sup>	$0.9^{b}$		0.0 <sup>g</sup>	$0.0^{\mathrm{bc}}$						
95:5	33.75 $\pm$	$53.15^{d} \pm$	$17.08\pm0.1^{a}$	3.11 $\pm$	$0.89~\pm$						
	$0.9^{\mathrm{b}}$	0.4 <sup>d</sup>		$0.0^{\rm h}$	0.0 <sup>ab</sup>						
85:15	32.31 $\pm$	53.16 $\pm$	15.26 $\pm$	3.48 $\pm$	$0.95 \pm$						
	1.6	2.3 <sup>d</sup>	$0.0^{\mathrm{b}}$	$0.2^{g}$	$0.1^{a}$						
75:25	$30.13 \pm$	55.39 $\pm$	$13.63\pm0.0^{\rm c}$	$4.06 \pm$	$0.92 \pm$						
	$0.0^{ m bc}$	$0.5^{\rm c}$		$0.0^{\rm f}$	$0.0^{\mathrm{a}}$						
60:40	$29.49 \pm$	59.42 $\pm$	$13.54\pm0.2^{\rm c}$	4.39 $\pm$	$0.79\pm0.0^{\rm c}$						
	$0.7^{d}$	$0.5^{\mathrm{b}}$		$0.0^{\rm e}$							
Wheat-sorg	ghum: RDP bisc	uits									
100:0	$28.33 \pm$	$63.99~\pm$	$13.47\pm0.2^{c}$	4.75 ±	$0.66 \pm$						
	0.9 <sup>de</sup>	2.7 <sup>a</sup>		$0.1^{d}$	0.1 <sup>d</sup>						
95:5	$26.88 \pm$	$63.82~\pm$	$12.44\pm0.0^{\rm e}$	$5.13 \pm$	$0.68 \pm$						
	$1.3^{ef}$	$0.0^{\mathrm{a}}$		$0.0^{\rm bc}$	$0.0^{d}$						
85:15	$26.26 \pm$	$63.85~\pm$	$12.26\pm0.0^{\rm e}$	$5.20 \pm$	$0.67 \pm$						
	$0.2^{\rm ef}$	$1.6^{a}$		0.1 <sup>b</sup>	$0.0^{d}$						
75:25	26.27 $\pm$	$63.84~\pm$	12.66 $\pm$	5.04 $\pm$	$0.65 \pm$						
	0.0 <sup>ef</sup>	0.4 <sup>a</sup>	0.0 <sup>d</sup>	0.0 <sup>c</sup>	0.0 <sup>d</sup>						
60:40	$25.84 \pm$	$63.50~\pm$	$11.42\pm0.1^{\rm f}$	5.56 $\pm$	$0.71 \pm$						
	$1.1^{ m f}$	$0.1^{a}$		$0.0^{\mathrm{a}}$	0.0 <sup>cd</sup>						

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p  $\leq$  0.05) as assessed by Fisher's least significant difference.

reduction in the weight of biscuits fortified with termite and silkworm pupae powders [36,62].

The width and the spread factor of the wheat-sorghum biscuits were significantly (p < 0.05) higher than that of the wheat biscuits. This could be due to the higher fiber content of wheat-sorghum biscuits compared to wheat biscuits as the sorghum flour used was wholemeal, and also fiber from RDP. The fiber may have acted as a stabilizer in the biscuit dough mixture, allowing the biscuits to maintain their diameter during cooking [54]. Furthermore, there was a 3.6-30.7 % increase in the spread factor of the fortified wheat biscuits compared to the control biscuits. This may be explained by the presence of fiber from RDP that maintained the width and thickness of the biscuits and a decrease in gluten quantity in the biscuits as fortification with RDP increased. Biscuits made using sorghum and defatted termites (Macrotermes subhyalinus), showed an increased spread factor and this was caused by an increase in the high fiber content in sorghum flour and defatted termites [63]. This may also have caused a significant reduction in the thickness of the biscuits.

#### 3.3.2. Colour parameters

The biscuits became significantly darker (p < 0.05) as RDP replaced wheat-sorghum and wheat in composite flours at levels of 5–40 %compared to the 100 % cereal biscuits (Table 7). The L\* (lightness) values decreased in wheat-based biscuits by 7-30 % and 28-36 % in wheat-sorghum-based biscuits. In the wheat-based biscuits, redness values increased by 24-31 % and yellowness values decreased by 5-47 %. The decrease in the L\* values is an indication of high protein content in the fortified biscuits, which was involved in the Maillard reaction to produce the brown color [64]. Aldehydes from Ruspolia differens oil such as hexanal and other lipid oxidation products such as peroxy radicals reacted with amine groups of proteins and free amino acids, yielding vellow intermediary products that polymerize into melanoidins, the dark brown macromolecules responsible for the biscuit color [65]. These findings agree with those previous studies which produced bread with dark crusts enriched with cinereous cockroach and grasshopper powders [66,67].

#### 3.3.2. Biscuit texture

As shown in Table 4, the hardness of the wheat biscuits increased by 6.7–123 % as the percentage of RDP substitution increased compared to the 100 % wheat biscuit. It is possible that as the gluten content of the biscuit dough decreased, protein and carbohydrate interaction weakened the gluten network, leading to poor gas retention ability, and yielding a tight dough [68]. The hardness of the wheat-sorghum-based biscuits decreased by 8.6–42 % as the RDP substitution levels increased compared to the control biscuit. This may be associated with the low levels of gluten in the composite flours as the RDP increased [69]. The high fiber content of RDP (Table 2) and sorghum may have hindered the homogeneity of the dough and the biscuit structure, by introducing course particles, resulting in reduced hardness [70]. Comparable results have been reported in the literature with a reduction in the hardness of biscuits developed from termite powder [62].

#### 3.4. Consumer acceptability

Results on the effect of fortification of the wheat and wheat-sorghum biscuits with RDP on consumer acceptability by children (Table 8). The liking of the wheat biscuit with 5 % RDP was the same as liking of the 0 % RDP wheat-sorghum biscuit. All other biscuits including the 100 % wheat biscuit were liked the same. The best-liked fortified biscuit was the wheat biscuit with 5 % RDP, which was significantly different from all the fortified biscuits. This is ascribed to the fact that the wheat-sorghum biscuits were probably crunchier and crispier as the gluten levels in them were low. Furthermore, they had a brown color, with the 5 % RDP biscuit having low intensity of fishy flavor because of fortification with less quantity of RDP. Wheat buns enriched with edible termites found that wheat buns were more acceptable at 5 % concentration than 20 % [71]. Liking of the 0 % RDP wheat-sorghum biscuit was the

#### Table 7

The effect of fortifying sorghum and wheat biscuits with defatted *Ruspolia differens* powder (RDP) on colour parameters of biscuits.

Biscuits	L*	a*	b*	C* (Chroma)	Color difference (ΔE) <sup>a</sup>	Browning index (BI)
Wheat: R	DP biscuits					
100:0	52.60	5.10	23.58	$24.13~\pm$	_	64.99 $\pm$
	$\pm$ 3.7 <sup>a</sup>	$\pm 0.4^{g}$	$\pm~2.0^{a}$	$2.1^{a}$		2.4 <sup>cd</sup>
95:5	48.80	6.30	22.40	$\textbf{23.70} \pm$	4.50 $\pm$	69.25 $\pm$
	$\pm$ 3.4 <sup>b</sup>	$\pm 0.4^{\mathrm{f}}$	$\pm \ 2.1^a$	$2.2^{\mathrm{a}}$	$2.8^{d}$	$2.1^{a}$
85:15	42.54	6.40	18.94	19.99 $\pm$	$11.17~\pm$	$68.63~\pm$
	$\pm 1.6^{c}$	$\pm 0.3^{ m f}$	$\pm 0.9^{b}$	$1.0^{\mathrm{b}}$	2.8 <sup>c</sup>	2.9 <sup>ab</sup>
75:25	38.74	6.50	16.80	$18.02~\pm$	$15.53 \pm$	$67.96 \pm$
	$\pm \ 0.9^{d}$	$\pm 0.0^{\mathrm{f}}$	$\pm 0.9^{c}$	0.9 <sup>c</sup>	4.7 <sup>bc</sup>	2.4 <sup>abc</sup>
60:40	36.84	6.68	15.74	$17.10 \pm$	$18.71 \pm$	$67.91 \pm$
	±,	$\pm 0.2^{ m f}$	$\pm 0.9^{c}$	0.9 <sup>cd</sup>	4.3 <sup>ab</sup>	3.3 <sup>abc</sup>
	0.8 <sup>de</sup>					
	rghum: RD					
100:0	38.08	10.02	12.58	$16.08 \pm$	$18.94 \pm$	58.70 $\pm$
	$\pm 1.7^{d}$	$\pm 0.2^{a}$	$\pm 0.6^{d}$	0.6 <sup>de</sup>	4.2 <sup>ab</sup>	1.2 <sup>e</sup>
95:5	37.44	9.38	12.60	$15.71 \pm$	19.28 ±	58.76 ±
	±	$\pm 0.3^{b}$	$\pm 0.7^{ m d}$	0.7 <sup>de</sup>	4.0 <sup>ab</sup>	3.7 <sup>e</sup>
	$0.3^{de}$					
85:15	36.56	8.94	13.84	$16.47 \pm$	$19.21 \pm$	64.69 ±
	±	$\pm 0.3^{c}$	$\pm 0.9^{d}$	0.3 <sup>de</sup>	3.9 <sup>ab</sup>	1.6 <sup>cd</sup>
	0.2 <sup>de</sup>					
75:25	35.68	7.90	13.28	15.45 ±	$20.05 \pm$	$62.02 \pm$
	$\pm 0.5^{ m ef}$	$\pm 0.2^{d}$	$\pm 0.5^{d}$	0.6 <sup>e</sup>	4.2 <sup>ab</sup>	2.0 <sup>de</sup>
60:40	33.58	7.08	13.34	$15.10 \pm$	$21.73 \pm$	65.29 ±
	$\pm 0.8^{\rm f}$	$\pm 0.1^{e}$	$\pm 0.2^{d}$	0.3 <sup>e</sup>	3.9 <sup>a</sup>	$3.3^{bcd}$

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p  $\leq$  0.05) as assessed by Fisher's least significant difference.

L\*: Indicates lightness where 0 = darkness and 100 = lightness.

a\*: Indicates redness where -  $a^*$ : = greenness and -  $a^*$  = redness.

b\*: Indicates yellowness where - b\* blueness and - b\* = yellowness.

<sup>a</sup> Change in colour of composite biscuits versus 100 % wheat biscuit.

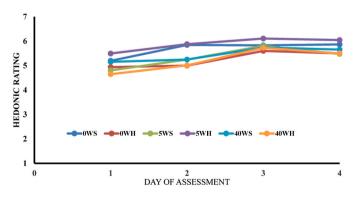
same as 40 % RDP wheat-sorghum. This is because of the dark color of sorghum flour used in the biscuit formulation. Liking of all biscuits was above average between 57 % for the 40 % RDP wheat, the least liked and 65 % for the 5 % RDP wheat biscuit, the best liked.

The children's liking of the biscuits increased with time (Fig. 2). For example, the least liked biscuit 40WH received ratings ranging from 65 % on day 1–78 % on day 4 but the most preferred biscuit 5WH, received ratings from 78 % on day 1–85 % on day 4. On the third day, there was a minor improvement in the liking for every biscuit (except 40WH) in comparison to the previous days. These results suggest that the children's liking of the biscuits gradually increased with repeated exposure. One explanation could be that sorghum and wheat, two staple grains with enduring popularity, were used to make the biscuits. Repeated consumption of moderately liked basic foods does not result in a considerable decline, so their preference curves are flat [72]. These findings are consistent with earlier studies on repeated exposure tests done on school children [6,73]. Consequently, it can be inferred from these results that the children liked the biscuits sustainably.

Furthermore, the children might have been curious in the biscuits after tasting them over time. This is because after tasting an unfamiliar food product, children are less reserved [74]. This is due to the reason that positive emotions could have been more dominant than negative ones after tasting the biscuits. This could also be an indicator that the children may not have been neophobic, leading to an increased willingness to taste the biscuit samples [75]. Among children, novel foods may invoke curiosity and facilitate explorative behavior, that increases liking of the foods [76]. The increased liking with evaluation days is ascribed to the fact that the grasshoppers were incorporated into biscuits, a familiar food among children. The integration of insects into the foods reduces the visibility of insects, consequently increasing the chances of consumption [77]. More than 55 % of the children expressed the desire to consume all the types of biscuits again. In the same manner, children in a previous study expressed positive attitudes about soy-fortified sorghum biscuits where 80 % indicated that they would eat the biscuits again [6]. This could indicate that the children were not bored with the fortified biscuits.

## 3.5. Microbial quality

Results show that mesophilic bacteria were present in both biscuits (Table 9). Spore-forming bacteria such as *Bacillus* spp have been found to be persistently present in *Ruspolia differens* even after being subjected to heat treatments such as deep frying, toasting, and smoking [78]. *Staphylococcus aureus, Escerichia coli* and *Salmonella* were not detected in the biscuits. This may be attributed death of the micro-organisms due to



**Fig. 2.** The effect of compositing wheat and wheat-sorghum with *Ruspolia differens* on children's liking of biscuits over time OWH-100 % wheat, 5WH-5% RDP wheat, 15WH-15 % RDP wheat, 25WH-25 % RDP wheat, 40WH-40 % RDP wheat. OWS-100 % wheat-sorghum, 5WS-5% RDP wheat-sorghum, 15WS-15 % RDP wheat-sorghum, 25WS-25 % RDP wheat-sorghum, 40WS-40 % RDP wheat-sorghum biscuits. RDP=*Ruspolia differens* powder.

#### Table 8

The effect of fortifying sorghum and wheat with RDP on overall liking of biscuits by 8- to 9-year-old children.

Biscuit type	Hedonic score
0 % RDP wheat	$5.25\pm1.9^{\rm c}$
0 % RDP wheat- sorghum	$5.68 \pm 1.7^{\rm ab}$
5 % RDP wheat	$5.87 \pm 1.6^{\rm a}$
5 % RDP wheat- sorghum	$5.33 \pm 1.9^{\rm c}$
40 % RDP wheat	$5.20\pm2.1^{\rm c}$
40 % RDP wheat-sorghum	$5.44 \pm 1.8^{bc}$

Values are mean  $\pm$  SD. Values followed by different letter superscripts in a column are significantly different at p<0.05 as assessed by Fisher's least significant test. RDP=*Ruspolia differens* powder. Overall liking ratings 1 = dislike extremely, 2 = dislike very much, 3 = Dislike a little, 4 = Not sure, 5 = Like a little, 6 = Like very much, 7 = Like extremely. Consumers n = 100.

baking temperature and good hygiene practices in handling the raw ingredients and the biscuits after baking. Similar results to this have been reported in biscuits fortified with insect powder [36]. All the biscuits met the criteria for microbial quality of high energy biscuits for use in supplementary feeding programs [79].

## 3.6. Limitations of the study

Repeated tasting was used to determine the long-term acceptability of biscuits over four days. Studies involving repeated exposure to foods among young children normally consist of repeated consumption of food products over several days or weeks. A limitation of the current study was that four days may have been too short to determine long-term acceptability by repeated consumption. Results showed that even though all the biscuits were highly liked, there was no change in liking over time demonstrating that four days may have been insufficient to bring about change in liking. The sensory evaluation sessions were held in children's classrooms, with four groups of 25 students seated in four distinct rows. A criticism of this arrangement is that peer influence and modeling could be a disadvantage of conducting the study in a school classroom rather than individual sensory booths. Friendship among children may have influenced the study results, despite efforts to randomly assign the children to sitting positions. However, it is improbable that this had an effect on the final results because, as previously stated, the children agreed on the scores and the results were consistent (Fig. 2).

#### 4. Conclusion

The gelatinization temperature for the wheat and wheat-sorghum flours increased, while the maximum viscosities, breakdown velocity, and setback viscosity were reduced. Substitution of RDP in the biscuits reduced their weight and lightness. There was a significant reduction in springiness, cohesiveness, gumminess, and adhesiveness of the doughs, with an increase in dough development time, and a reduction in dough stability, and water absorption with RDP fortification. The biscuits were highly acceptable following repeated exposure. The biscuits are microbiologically safe. Biscuits developed from wheat-sorghum and wheat flours fortified with RDP have reasonably high acceptability and 8-to 9year school children could sustain their acceptability over time on repeated exposure as supplementary-rich sources of protein for alleviating the problem of protein energy malnutrition in Sub-Saharan Africa.

#### CRediT authorship contribution statement

Amos Kipkemoi Ronoh: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Charlotte Atsango Serrem: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Susan Balaba Tumwebaze: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Gertrude Mercy Were: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Gertrude Mercy Were: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

#### Ethics approval statement

The Mount Kenya University Ethics Research Committee provided

## Table 9

The effect of fortifying sorghum and wheat biscuits with defatted Ruspolia differens powder (RDP) on microbial quality of biscuits.

Flour/Biscuits	Mesophilic bacteria count (10 <sup>-3</sup> cfu/g)	Staphylococcus aureus (cfu/g)	Spore Count (cfu/g)	Yeast and molds (cfu/g 10 <sup>4</sup> )	<i>E coli</i> (cfu/ g)	Salmonella (cfu/g)	<i>Bacillus cereus</i> (cfu/g)
Flour							
Sorghum flour	$98.67 \pm 12.5^{\rm d}$	$ND^{b}$	$9.67 \pm 1.2$	$1.66\pm0.58$	ND	ND	ND
Wheat flour	$146.67 \pm 15.2^{ m ab}$	ND	ND	ND	ND	ND	ND
Ruspolia differens powder	$101.0\pm1.7^{d}$	ND	ND	ND	ND	ND	ND
Wheat: RDP biscuits							
100:0	$155.5\pm22.2^{\rm a}$	ND	ND	ND	ND	ND	ND
95:5	$126.33 \pm 16.4^{c}$	ND	ND	ND	ND	ND	ND
85:15	$52.33 \pm 2.1^{\rm ef}$	ND	ND	ND	ND	ND	ND
75:25	$64.00\pm7.8^{\rm e}$	ND	ND	ND	ND	ND	ND
60:40	$136.0\pm5.6^{bc}$	ND	ND	ND	ND	ND	ND
Wheat: sorghum: RD	P biscuits						
50:50	$25.33\pm2.3^{\rm h}$	ND	ND	ND	ND	ND	ND
95:5	$34.33\pm4.5^{gh}$	ND	ND	ND	ND	ND	ND
85:15	$44.0\pm4.6^{fg}$	ND	ND	ND	ND	ND	ND
75:25	$41.67 \pm 10.4^{fgh}$	ND	ND	ND	ND	ND	ND
60:40	$33.67 \pm 1.1^{\rm gh}$	ND	ND	ND	ND	ND	ND
Standard criterion <sup>a</sup>	Max 10,000 cfu/g	<10 cfu/g	Not specified	Max 100 cfu/g	Absent in 10g	Absent in 25 g	Max 10 cfu/g

Values are means  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at (p  $\leq$  0.05) as assessed by Fisher's least significant difference.

<sup>a</sup> Technical specifications for High Energy Biscuits [79].

<sup>b</sup> ND=Not Detected.

ethical clearance for this study (Application Approval Number: 1131). A research license (number: NACOSTI/P/22/16130) was issued by The National Commission for Science, Technology, and Innovation (NACOSTI). The study only included children whose parents signed a consent form explaining the nature of the biscuit samples and the activities included, and who freely agreed to participate in the research The University of Eldoret Primary School headmaster granted permission for the study to be carried out in the school.

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#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Data availability

Data will be made available on request.

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