



RESEARCH ARTICLE

# Shelf life of the preheated and ready-to-eat long-horned grasshopper *Ruspolia differens* Serville

G. Ssepuuya<sup>1,2\*</sup> , J. Claes<sup>1</sup>, M. Van Der Borgh<sup>1</sup>, L. Van Campenhout<sup>1</sup> and D. Nakimbugwe<sup>3</sup>

<sup>1</sup>KU Leuven, Department of Microbial and Molecular Systems (M2S), Research group for Insect Production and Processing, Campus Geel, Kleinhoefstraat 4, 2440 Geel, Belgium; <sup>2</sup>Kyambogo University, Department of Food Science and Technology, Faculty of Science, P.O. Box 1, Kyambogo, Kampala, Uganda; <sup>3</sup>Department of Food Technology and Nutrition, CAES, Makerere University, P.O. Box 7062, Kampala, Uganda; \*gksepuya@gmail.com

Received 14 December 2022 | Accepted 6 May 2023 | Published online 29 September 2023 |  
Published in print 1 December 2023

## Abstract

*Ruspolia differens* is an edible insect delicacy in sub-Saharan Africa, majorly harvested from the wild, with a very short shelf life (24 hours depending on the handling conditions). Combinations of preheating, roasting and drying, and storage at refrigerated and room temperature were used to prolong its shelf life. Product quality was evaluated using sensory, microbial and chemical analyses. To allow transportation from the harvesting areas to the market, preservation up to 9 hours at room temperature was possible using only preheating (boiling at 100 °C for 6 minutes). Combinations of preheating and roasting (for 25 and 35 minutes at 165 °C) resulted in moisture contents of 21.3 and 10.4% with shelf life at refrigeration temperature of 25 and 54 days, respectively. Aroma was the main factor determining the shelf life. Further drying of preheated and roasted grasshoppers resulted in a product with 4.5% moisture. In combination with vacuum packaging, storage at room temperature was possible for at least 20 weeks. This research therefore contributes to long term storage of the surplus harvested *R. differens*, and thus to increased food security.

## Keywords

edible bush cricket – Nseenene – pre-heating – microbial spoilage – rancidity – storage

## 1 Introduction

The majority of the edible insects regularly consumed in Africa are harvested from the wild in a particular season of the year (Kelemu *et al.*, 2015), often in large though undocumented volumes that cannot be consumed immediately after harvest. Due to their high perishability (Halloran *et al.*, 2018), surplus edible insects, that would otherwise be of nutritional and economic benefit to the insect harvesting communities are lost

to spoilage. For *Ruspolia differens*, even with heat treatment (sautéing or roasting), its shelf life is limited to 24 hours at room temperature post-harvest unless dehydrated, beyond which all the unconsumed volumes are lost (Ssepuuya *et al.*, 2016a). Microbiological deterioration is implicated as the cause of fast spoilage due to its high moisture content (~51%) and high water activity (0.97) (Ssepuuya *et al.*, 2021). This shelf life is too short a time to allow for transportation, processing and profitable sale of raw and processed *R. dif-*

*ferens* to the intended consumers. Within this time, a large proportion of batches of raw grasshoppers under transportation often lose sensory acceptability and ultimately fetch lower prices, that is, if they are still fit for consumption. The surplus is often prepared into dehydrated ready-to-eat products (by either deep-frying plucked *R. differens* or boiling whole *R. differens* in salty water followed by sun-drying) which often have reduced consumer acceptability. This therefore creates a need to extend the shelf life of raw and processed forms of *R. differens* in ways that guarantee high consumer acceptability.

Extending the shelf life of foods (edible insects inclusive) is based on controlling enzymes or chemically active molecules in food, controlling microbial deteriorative processes and avoiding faulty post-harvest handling practices (Adegoke and Olapade, 2012). This therefore implies that the deteriorative mechanisms of a food must be established, to enable the application of appropriate strategies for extending its shelf life (Man, 2008). Our current research has proven oxidative rancidity as the potential cause of spoilage in dehydrated grasshoppers with low water activity (<0.60) and microbial deterioration as the potential cause of spoilage in grasshoppers with high water activity ( $\geq 0.97$ ) (Ssepuyua *et al.*, 2021). Headspace gas chromatography analysis reveals the presence of high concentrations of aldehydes, ketones and organic acids that lead to the off flavors and off odors responsible for the loss of acceptability of dehydrated grasshoppers (Ssepuyua *et al.*, 2021). For *R. differens* with high water activity, microbial deterioration has been identified as the potential cause of spoilage. Microorganisms break down the amino acids such as methionine leading to formation of dimethyl sulphide and dimethyl trisulphide responsible for the unpleasant smell of spoiled grasshoppers with high water activity (Ssepuyua *et al.*, 2021). It is therefore prudent that attempts to increase the shelf life of grasshoppers are based on reducing the rate of progress of these deteriorative mechanisms.

Different strategies have been employed to increase the shelf life of perishable and non-perishable foods. Shelf life extension of perishable food products essentially targets the control of microorganisms by manipulating one or more food conditions including: mild heat treatments, reducing the water content/water activity, and storage at low (refrigeration) temperatures and/or modifying the gas composition in the package atmosphere (Man *et al.*, 2000; Yadav, 2010). Often, each of these control measures is insufficient on its own and thus a combination of them (hurdles) are often applied,

such as a combination of mild heat treatment and low temperature storage (Man *et al.*, 2000; Yadav, 2010). To minimise lipid oxidation, attempts should be made to prevent oxygen from accessing the product during processing and storage, as well as light and heat that catalyze the lipid oxidation reaction (Rahman, 2007; Singh and Cadwallader, 2004). Both spoilage mechanisms, i.e. microbial and chemical (lipid oxidation) influence the sensory quality of *R. differens*. Therefore, identification of the key sensory attributes affected by the deteriorative mechanisms and the descriptive analysis of the changes that occur in these attributes is important in assessing the shelf life of *R. differens* (Man *et al.*, 2000; Nicoli, 2012). This is because consumers always use the sensory attributes to decide as to whether the product is acceptable, especially when making purchase decisions (Nicoli, 2012).

Previous research increased the shelf life of ready-to-eat *R. differens* from 24 hours up to 5.5 months but (a) did not focus on improving the stability of raw grasshoppers as a raw material and (b) resulted in loss of wholesomeness (damage) of vacuum-packed ready-to-eat dehydrated product (Ssepuyua *et al.*, 2016a; Ssepuyua *et al.*, 2016b). Therefore, the aim of the present study was twofold. First, to develop detailed sensory descriptors using fresh and deliberately spoiled *R. differens*. These descriptors were used for shelf stability studies. Second, together with microbial and chemical analyses, to evaluate both short term (~hours) and long term (~weeks and months) spoilage under different conditions. The moisture content of *R. differens* was varied using roasting and/or drying, while the packaged grasshoppers were stored at room and refrigeration temperature, including vacuum packaging for up to five months.

## 2 Materials and methods

### *Experimental approach*

The experiment was designed to study short-term and long-term stability of grasshoppers. For the short term storage experiment (Figure 1A), we examined the role of a preheating step (the independent variable) towards maintaining the sensory and microbial stability (dependent variables) of raw *R. differens* at room temperature for over 6 hours. This is because (a) it takes about 6 hours to transport harvested *R. differens* from the furthest harvesting point of the country to the central market in Kampala, and (b) normally, within 6 hours, raw grasshoppers reach the market with a high degree of

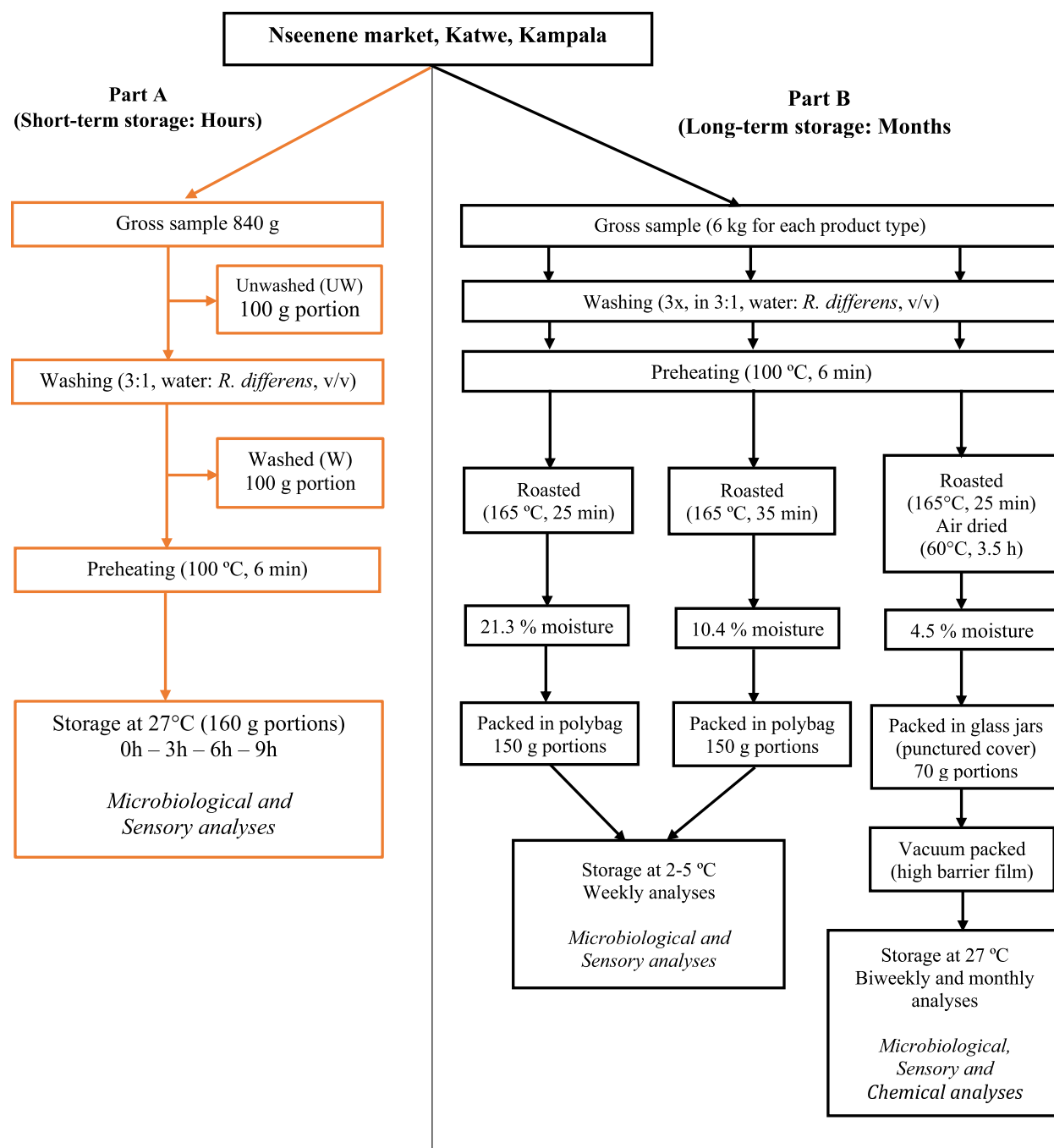


FIGURE 1 Sampling and sample preparation for studying the shelf life of pre-heated (A), roasted, and roasted and further dried *Ruspolia differens* (B).

deterioration. For this reason, there was no control. For the long term storage experiments (Figure 1B), we determined: (a) the effect of moisture content (21.3% and 10.4%), the independent variable, on the refrigerated storage (2-5 °C) sensory and microbial shelf life (dependent variables) of ready-to-eat *R. differens* products. In these experiments, the control (un-refrigerated roasted sample) was not included because it was observed to spoil within 24 hours at 27 °C during qualitative descriptive analysis (Table 1). (b) The effect of vacuum pack-

aging, the independent variable, on the sensory, chemical and microbial shelf life (the dependent variables) of roasted and dehydrated (4.5% moisture content) *R. differens*. Similarly, this was an improvement of an earlier study (Ssepuuya *et al*, 2016a) in which vacuum packed grasshoppers were more shelf stable and hence, no control was included in the study. The product with 21.3% moisture has a high water activity (0.951) while that with 4.5% moisture has a low water activity (<0.600).

### Sampling

To study the shelf stability of preheated *R. differens* (Figure 1A), 840 g of *R. differens* were collected for each of the three biological replicate samples. Biological replicates are parallel measurements of biologically distinct samples that capture random biological variation. To obtain 840 g, 280 g of *R. differens* were collected from each of the three randomly selected traders using the quartering technique (i.e. 70 g of sample picked from four quarters of the area occupied by *R. differens* displayed for sale) were collected from each of the three randomly selected traders. After collection, each replicate was placed in a pre-disinfected (by 70% alcohol) container and the three replicates were delivered to Makerere University. To study the shelf life of each of the three processed types of ready-to-eat *R. differens* products (roasted *R. differens* with 21.3% MC, roasted *R. differens* with 10.4% MC, and dried and vacuum packed *R. differens* with 4.5% MC) based on applied processing treatments (Figure 1B), 6 kg of the gross sample was obtained for processing each type of sample as biological replicates. To obtain each 6 kg batch, 2 kg of *R. differens* using the quartering technique (i.e. ~500 g of sample picked from four quarters of the area occupied by *R. differens* displayed for sale) were obtained from three randomly selected traders.

### Sample preparation

Fresh plucked (with wings, antennae, legs and ovipositor removed) *R. differens* samples were obtained from traders in Katwe, Kampala (Uganda) during the November-December swarming season of 2018 (Figure 1). For short term storage experiments, each replicate sample was: (1) washed by rinsing it three times with thrice its volume of potable water each time; and (2) preheated by placing washed grasshoppers in portable boiling water for six minutes (Figure 1A). Preliminary laboratory tests showed that preheating grasshoppers in boiling water beyond six minutes did not result in any further reduction in microbial counts. The preheating was also used to kill the grasshoppers. For long term storage experiments, roasting was achieved by placing a single layer of *R. differens* in a rotary hot air oven (Falcon 1A, Macadams International, Cape Town, South Africa) for 25 or 35 minutes at 165 °C (Figure 1B). Dried *R. differens* were prepared by placing roasted *R. differens* in a single layer in a convection air dryer (Innotech, Altdorf, Germany) for 3.5 hours at 60 °C. High barrier polythene bags (5-foil film, VAX080200300, 80 MU) were used for packaging refrigerated samples (Figure 1B). Dried samples were packed in glass jars with perforated covers to

provide for rigidity and thus intactness of the grasshoppers. The jars were then packed in a high barrier film (5-foil film, VAX080200300, 80 MU) and vacuum-sealed (99% vacuum) using the DZQ 400/500/600 (Dongguan, Guangdong, China) vacuum packaging machine (Figure S1).

### Sensory stability of *Ruspolia differens*

For sensory description, each type of sample (Figure 1A and 1B) was presented to a trained panel in two forms, i.e. the freshly processed form, and the spoilt form as a reference. Samples with 21.3 and 10.4% moisture content were respectively spoiled by storing them for 24 h at 27 °C in an incubator (CYANLab CL011-110, Cypress Diagnostics, Langdorp, Belgium). Since dried *R. differens* could not spoil within 24 h, an already spoilt processed and dry sample was used instead. It was obtained from a batch that was previously sautéed and dried at 80 °C for 10 h in an air convection dryer (Innotech, Altdorf, Germany) to a moisture content of 5%. It had been on the shelf for 24 months and all the sensory panel members consented that it was spoilt.

Following procedures elaborated by Lawless and Heymann (2010), descriptive analysis of the processed fresh and spoilt samples was carried out using a 10 member trained panel of assessors. The panel already existed at the Department of Food Technology and Nutrition, Makerere University and co-incidentally, members were regular consumers of *R. differens*. Before the descriptive sessions, members were convened and briefed about the purpose of the study and their role, and also introduced to the concept of line scaling that was used to evaluate the products' shelf life. To describe the quality attributes, a freshly processed sample was presented to the members who were tasked to suggest and agree on terms that better describe its colour, aroma, taste, and texture. The following day, the spoilt counterpart of the sample assessed on the previous day was presented for the description of the same attributes. Sessions were always conducted between 2:00 and 5:00 pm under daylight (except for the short-term storage experiment in Figure 1, part A). The descriptions of the fresh and spoilt samples are found in Table 1. These terms were then used as anchor terms during shelf life testing using the same panel on a 15 cm line scale. For each parameter, the 15 cm line was indented by a 1 cm distance from the low (0 cm) and high end (15 cm) of the line to reduce the end-of-scale effects. At the indented points, the low end of the line was marked with the anchor terms of the freshly processed sample while the

high end was marked with the anchor terms of the spoilt sample.

For sensory shelf life monitoring, each of the ten panellists was provided with 5 g of a coded sample and a ballot. The panellist was requested to indicate his/her perception by putting a slash at any point of his/her choice on the 15 cm line between the two anchors of each attribute as provided on the ballot. Below each anchored line for each attribute, space was provided for the panellist to write his/her own description of each attribute that corresponded to the position of the slash point he/she made on the line. Each panellist was provided with drinking water to rinse his/her mouth in case it was important for him/her to reach a judgment.

Preheated samples (Figure 1A) were evaluated for changes in aroma and colour on a 3 h basis. Ready to eat refrigerated samples (Figure 1B) were evaluated for changes in colour, aroma, taste and texture on a weekly basis. Refrigerated samples were only delivered for sensory evaluation if their total aerobic bacterial counts were below 7 log cfu/g. This is the limit above which chilled foods to be consumed immediately after purchase would be considered unfit for human consumption, according to the existing guidelines on the microbial quality of similar ready-to-eat foods (Centre for Food Safety, 2014; Health Protection Agency, 2009). Vacuum-packed samples were evaluated for changes in colour, aroma, taste and texture on a bi-weekly basis and after 12 weeks, on a monthly basis.

#### *Microbial stability of Ruspolia differens*

From each *R. differens* sample, 30 g were pulverised using a hand-held blender (RHSB038, 600W, Russell Hobbs, Middleton, WI, USA) for 3 minutes into a paste. Samples were analysed for total aerobic count (TAC), aerobic spores (AS), and lactic acid bacteria (LAB) according to procedures elaborated by Ssepuuya *et al.*, (2018). Total anaerobic count (TANC) were counted on PCA (Laboratorios CONDA, Torrejón de Ardoz, Spain) and after incubation for 3 days at 30 °C. After a heat treatment step of 10 minutes at 80 °C to kill the vegetative cells, (a) anaerobic bacterial spores (ANS) were counted on PCA (Laboratorios CONDA, Torrejón de Ardoz, Spain) after incubation at 37 °C for 48 hours, and (b) spores of sulphite reducing bacteria (SRC) were counted on reinforced clostridial agar (RCA) (Himedia, Marg, Mumbai, India) after incubation at 35 °C for 24 to 48 hours. To provide for anaerobic conditions, poured plates were placed in anaerobic jars fitted with a gas pack (Thermo Fisher Scientific, Oxoid Ltd, Hampshire, UK) and an indicator strip (Thermo Fisher Scientific,

Oxoid Ltd, Hampshire, UK). Samples were analysed in triplicate. These procedures were used to determine the: (1) effect of washing on the microbial quality of *R. differens* prior to preheating, (2) microbial stability of preheated *R. differens* stored for 0, 3, 6, and 9 hours while being maintained in an incubator (CYANLab CL011-110, Cypress Diagnostics, Langdorp, Belgium) at 27 °C (Figure 1A); and (3) the microbial stability of ready-to-eat refrigerated and dried vacuum packed samples as indicated by TAC. All measurements were made in triplicate.

#### *Fat stability of Ruspolia differens*

The vacuum packed *R. differens* with low moisture content (4.5%) were evaluated for fat rancidity. *R. differens* oil was extracted using the Folch method (Wrolstad *et al.*, 2005). In brief, approximately 70 g of whole *R. differens* was pulverised using a hand-held blender (RHSB038, 600W, Russell Hobbs, Middleton, WI, USA) into a paste. The sample was extracted thrice using a 2:1 chloroform (Suvchem, Mumbai, India) methanol (Loba Chemie Pvt. Ltd., Mumbai, India) mixture (extraction solvent). After separation using a separatory funnel, the fat-containing organic phase was evaporated using a Yamato rotary evaporator (Yamato Scientific Co. Ltd, Tokyo, Japan) under vacuum, at 50 °C for 2 h. The quality parameters, i.e. acid, peroxide and *p*-anisidine values of the extracted oil/fat were determined using procedures described by Nielsen (2010) and the Ethiopian Standards Agency (2012). Changes in fat (acid, peroxide and *p*-anisidine values) quality were assessed on a bi-weekly basis, and after 3 months, on a monthly basis.

#### *Statistical analysis*

Statistical Package for Social Scientists (SPSS) for Windows (Version 20, IBM Corporation, Armonk, NY, USA) software was used to perform statistical analyses. Using descriptive statistics, means and standard deviations were obtained. According to the Levene test, the data was normally distributed. ANOVA (one way) was used to determine the effect of processing and storage conditions (as the independent variable) applied on the microbial, sensory and fat quality (as the dependent variables) of *R. differens* over time. Mean separation was obtained by the Tukey's option of the ANOVA test for microbial results while the Dunnett option was used for sensory results using week 0 as the control category. For all tests, a significance level of 0.05 was considered.

TABLE 1 Description of the aroma, colour, taste and texture of pre-heated and ready-to-eat *Ruspolia differens*.<sup>1</sup>

Processing treatment	Attribute	Description	
		Freshly processed	Spoiled
Pre-heated	Colour	Ghee yellow	Grey
	Aroma	Pleasant	Rotten cabbage
Roasted at 165 °C for 25 min (21.3% MC)	Colour	Fresh ginger yellow	Raw gold colour
	Aroma	Weak roasted meat aroma	Rotting mushroom aroma
	Taste	Meaty	Off-taste
	Texture	Slightly crunchy	Soft
Roasted at 165 °C for 35 min (10.4% MC)	Colour	Fresh ginger yellow	Raw gold colour
	Aroma	Weak roasted meat aroma	Rotting mushroom aroma
	Taste	Meaty	Off-taste (non-meaty)
	Texture	Moderately crunchy	Soft
Roasted at 165 °C for 25 min and air dried for 3.5 h at 60 °C (4.5% MC)	Colour	Dry ginger yellow	Raw gold colour
	Aroma	Weak roasted meat aroma	Spoiled cooking oil aroma
	Taste	Moderately meaty	Off-taste (non-meaty)
	Texture	Crunchy	Soft

<sup>1</sup> The ginger referred to in this table is the flesh and not the skin.

### 3 Results and discussion

#### *Description of the key attributes of preheated and ready-to-eat Ruspolia differens*

According to the sensory panel discussion (section 2.2), the descriptions for aroma, colour, taste and texture of preheated (Figure 1A) and further processed *R. differens* (Figure 1B) are presented in Table 1.

The colour of freshly preheated *R. differens* was predominantly yellow. This is because the predominant green polymorph upon heating changed from green to yellow (Ssepuuya *et al.*, 2020). Upon roasting, the intensity of this yellowness increased from light 'fresh ghee' yellow to a more vivid 'fresh ginger' yellow. On the other hand, drying 'fresh ginger' yellow *R. differens* resulted into a less vivid 'dry ginger' yellow colour. The aroma of preheated *R. differens* was described as pleasant. The aroma of roasted (and dried) *R. differens* was described as a 'weak roasted meat' aroma and the taste as 'meaty'. Drying reduced the meatiness in the taste of *R. differens* and the aroma of dried *R. differens* was then described as moderately meaty. As the moisture content of the *R. differens* decreased with increased roasting time and further drying, the crunchiness increased from slightly crunchy for *R. differens* with 21.3% moisture content, to moderately crunchy for *R. differens* with 10.4% mois-

ture content and being crunchy upon further drying of roasted *R. differens* to 4.5% moisture.

Upon spoilage, the 'ghee yellow' colour of preheated *R. differens* turned greyish, while for the roasted ones, the ginger flesh yellow colour turned into a dull darker yellow colour, expressed as a 'raw gold yellow' colour. *R. differens* assumed a soft texture, lost the meaty taste to a somewhat non-meaty taste that descriptors referred to as an 'off taste' for lack of a better descriptor. Spoilt preheated and roasted *R. differens* was described as having a 'rotting mushroom' or 'rotten cabbage' aroma, both of which are associated with putrid aromas. The 'rotting mushroom' smell is ammonia-like (Balch, 2003) while the cabbage smell is related to the presence of sulphides (Buettner, 2017) and both compounds are associated with the breakdown of amino acids (Bakker and Law, 1994; Ulrich and Halvorson, 1951). Microbial degradation of proteins leading to the production of awful sulphur volatile compounds is the most probable cause of spoilage of *R. differens* with high moisture content/water activity (Ssepuuya, 2019). The aroma of spoiled dried *R. differens* was described as 'spoilt cooking oil' which is indicative of oxidative rancidity (Roehl, 1996). For both preheated and ready-to-eat *R. differens*, the aroma was the most distinctive indicator of spoilage.

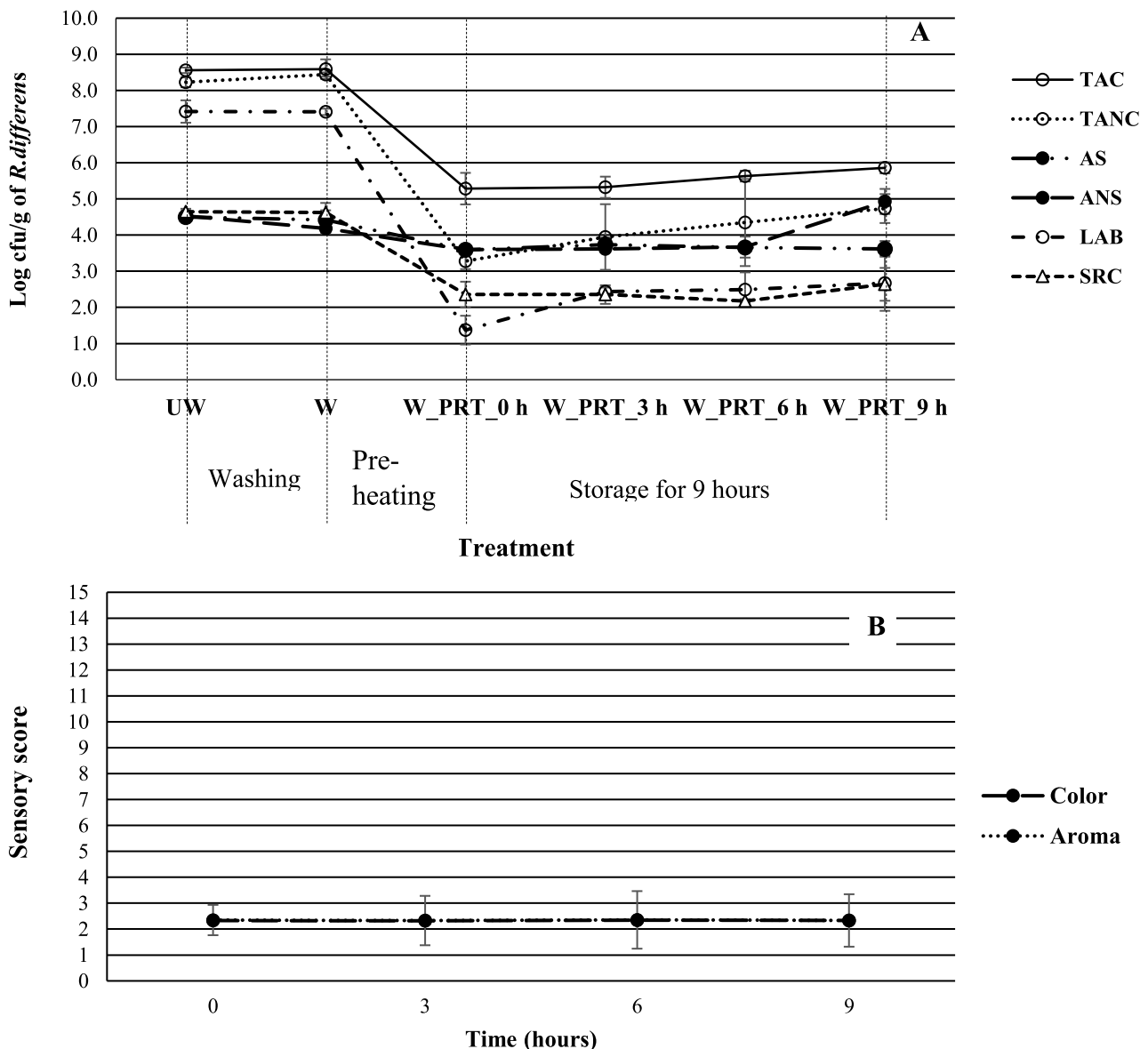


FIGURE 2 Microbial counts (A;  $n = 3$ ,  $P < 0.05$ ) and sensory scores (B;  $n = 10$ ,  $P < 0.05$ ) on a 15 cm line scale of washed and preheated (W\_PRT) *Ruspolia differens* stored for 9 h at 27 °C for 9 h. ANS = anaerobic bacterial spores; AS = aerobic bacterial spores; LAB = lactic acid bacteria; SRC = sulphite reducing clostridia; TAC = total aerobic count; TANC = total anaerobic count; UW = unwashed; W = washed.

### Short term shelf life of *Ruspolia differens* after washing and preheating

The microbial counts of raw unwashed and washed *R. differens* were not significantly different [ $F(1,40) = 0.014$ ,  $P = 0.906$ ], implying that washing did not influence the microbial quality of *R. differens*. This is similar to observations in mealworm larvae (Wynants *et al.*, 2017) where rinsing had no effect on the microbial load. This implies that the microbial quality of *R. differens* and possibly that of other edible insects may not be predominantly determined by the microorganisms on their surfaces. Pre-heating had a significant effect on microbial load [ $F(1,61) = 85.56$ ,  $P < 0.0001$ ]. The effect of

preheating on the counts of the different groups of microorganisms of quality concern in *R. differens* is presented in Figure 2A. In raw *R. differens*, TAC (8.6 log cfu/g), ANS (8.2 log cfu/g), and LAB (7.4 log cfu/g) exhibited the highest counts. Aerobic spores, TANC, and SRC spores showed similar concentrations of about 4.5 log cfu/g. With the exception of TANC, ANS, and spores of SRC that weren't investigated in this matrix before this research, the counts of the other groups of microorganisms are similar to those observed in raw and freshly harvested *R. differens* (Ssepuyua *et al.*, 2018).

LAB showed more sensitivity to preheating, exhibiting the highest reduction (6.0 log cfu/g) followed by

TANC (5.2 log cfu/g), TAC (3.3 log cfu/g), spores of SRC (2.3 log cfu/g), ANS (0.8 log cfu/g) and lastly, AS (0.6 log cfu/g). Similar reductions in microbial loads have been observed in other edible insects such as mealworms, small and large crickets, termites and caterpillars (Klunder *et al.*, 2012; Megido *et al.*, 2017) after a heat treatment step. This implies that preheating by boiling for six minutes and similar heat treatments can effectively improve the microbial quality of edible insects including *R. differens*. However, these reductions are more meaningful if they apply to the specific microorganisms that lead to spoilage of *R. differens*. Notably however, is that AS inhabiting *R. differens* matrix seem to be less sensitive to the applied heat treatment compared to the ANS. Also, the presence of both aerobic and anaerobic sporulating and non-sporulating microorganisms indicates the possibility of *R. differens* microbial quality deterioration by the action of the two groups of microorganisms depending on the prevailing atmospheric environment. For example, DNA sequencing showed the potential presence of spore-forming anaerobic *Clostridium sp* and aerobic *Bacillus sp*, and non-sporeforming bacteria such as lactic acid bacteria (*Lactococci* and *Lactobacilli*) all of which contain enzymes that can degrade protein to sulphur volatile compounds that indicate spoilage in *R. differens* with high water activity (Ssepuuya *et al.*, 2021, 2018). This further makes it important to establish the preferred air requirements for the specific spoilage organisms in *R. differens*.

Overall, storage period had a significant effect on the microbial load [F(3,60) = 2.81,  $P = 0.045$ ]. During the 9 h storage period of preheated *R. differens*, there was no significant increase ( $P > 0.05$ ) in TAC, AS, ANS and spores of SRC but significantly influenced LAB and TANC counts. TANC at 0, 3 and 6 hours was also not significantly different ( $P > 0.05$ ) except after 9 hours of storage when it was significantly higher ( $P > 0.001$ ). After storage for 3 h at room temperature, the lactic acid bacteria significantly ( $P = 0.001$ ) increased by about 1 log cfu/g, which count was not significantly different ( $P = 0.348$ ) from that after 6 hours of storage. After 6 hours, LAB and TANC significantly ( $P > 0.001$ ) increased by 0.4 and 0.2 log cfu/g, respectively. However, from a microbiological point of view, these significant changes are limited. The sensory panel did not find any significant ( $P > 0.05$ ) difference in scores of colour and aroma of preheated *R. differens* stored for 0, 3, 6 and 9 h (Figure 2B). Therefore, after 9 h, the TAC of 5.9 log cfu/g was not associated with a noticeable change in sensory quality of *R. differens*. Also, after 9 hours of storage, the counts of all other groups of microorganisms

assayed were below the TAC count. Preliminarily, 5.9 log cfu/g can be regarded as the TAC below which no negative change in sensory quality is expected when freshly harvested grasshoppers are preheated by boiling for six minutes and stored for between 6 and 9 hours at room temperature. These results also suggest that preheated *R. differens* can be stable for between 6 and 9 h at room temperature without a significant change in sensory and microbial quality, the latter being represented by TAC in this case, but this is also an aspect for further careful investigation.

#### *Effect of refrigeration on the sensory and microbial stability of roasted Ruspolia differens*

The sensory and microbial quality of roasted *R. differens* roasted at 165 °C for 25 minutes with 21.3% moisture ( $a_w = 0.95$ ), was monitored over time (Figure 3) in an attempt to determine its shelf life. By week 5, it was no longer sensorially acceptable to the consumers.

By week 5, the *R. differens* had assumed a dull darker 'raw gold' yellow colour and a soft texture, indicating that the slight crunchiness (Table 1) was lost. There was an off odor tending towards the 'rotting mushroom smell', that was far different from that of freshly roasted grasshoppers as observed by the markings on the line scale. This was accompanied by the loss of the 'meatiness' in the aroma and taste. At week 5, the average scores for aroma, colour, taste and texture were 8.9, 8.8, 8.6 and 10.7, respectively (Figure 3B). The TAC associated with the loss in edible quality was 6.8 log cfu/g (Figure 3A). However, consistent significant ( $P < 0.05$ ) deviations from week 0 for aroma, taste, and texture scores were observed from week 4 onwards, and for colour, from week 5 onwards.

For the sample roasted at 165 °C for 35 min with 10.4% moisture content, sensory testing was stopped at week 11 because the panel deemed the sample as unacceptable even when, by this time, the TAC (5.08 log cfu/g) was still below the limit of 7 log cfu/g. Of the 10 panelists, 7 agreed that by week 11, the colour had become 'duller and less appealing', the taste was 'off', the meaty aroma was completely lost and the grasshoppers were only slightly crunchy. At week 11, the aroma, colour, taste and texture scores were 9.3, 10.4, 10.6, and 12.7, respectively (Figure 4B). Based on this information, the sample at week 12 could not be presented for scoring. The TAC associated with the noticeable loss of edible quality at week 12 was 5.9 log cfu/g (Figure 4A). At week 5, texture scores began to significantly ( $P < 0.05$ ) differ consistently from week 0. Aroma, colour and



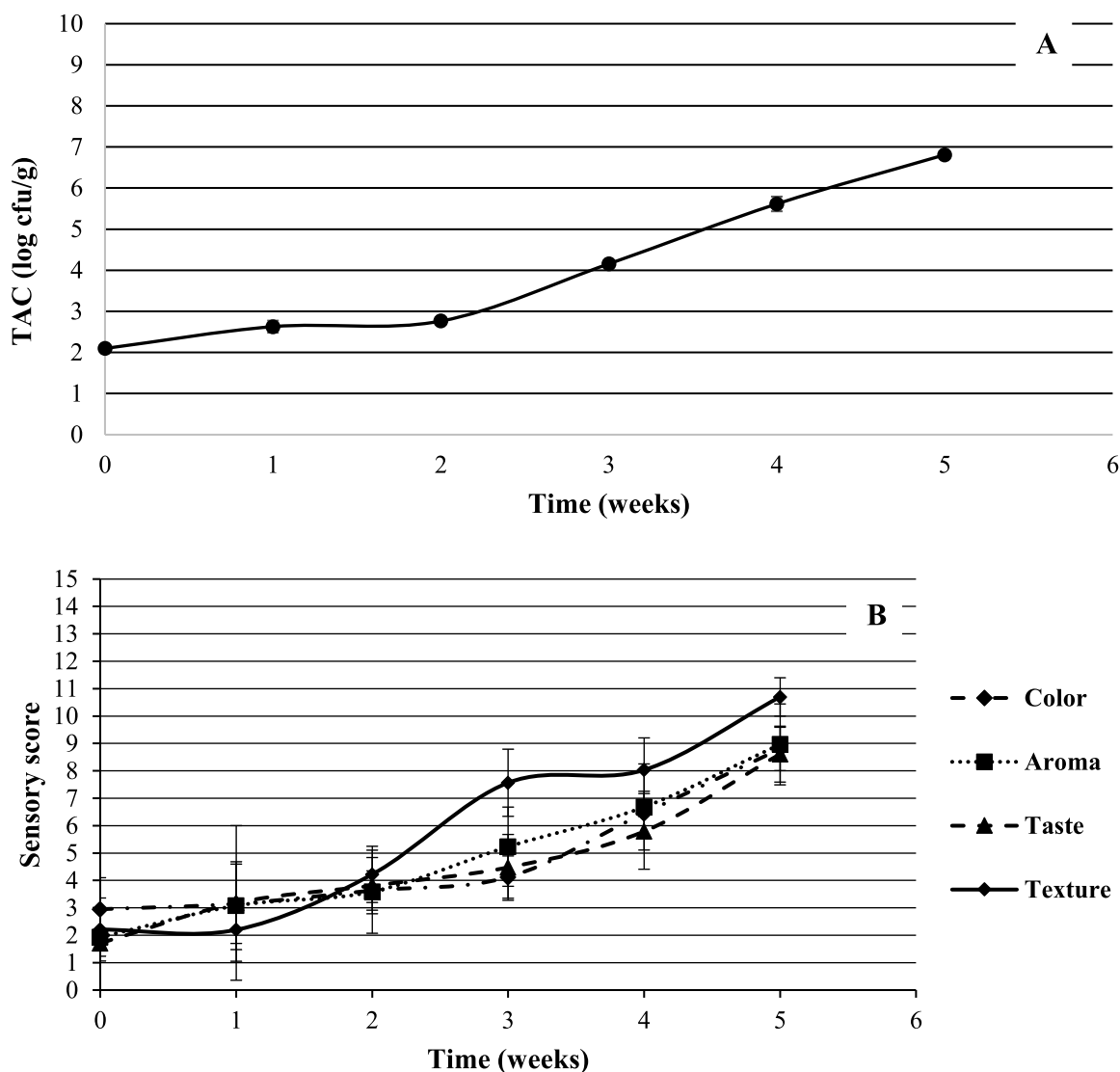


FIGURE 3 Total aerobic count (A;  $n = 3$ ,  $P < 0.05$ ) and sensory scores on a 15 cm line scale (B;  $n = 10$ ,  $P < 0.05$ ) of ready-to-eat *Ruspolia differens* (roasted at 165 °C for 25 min, 21.3% moisture content) stored for 5 weeks at a temperature ranging from 2 to 5 °C. Anchor terms on the 15 cm line scale of each parameter are described in Table 1.

taste scores began to significantly differ from week 0 at week 8.

To determine the shelf life, 70% of the time to spoilage is taken to be the storage life so as to allow for a safety margin (Kilcast and Subramaniam, 2010). In this case, *R. differens* with 21.3% and 10.4% were considered spoiled by the consumer panel at 5 and 11 weeks of storage, respectively. Therefore, *R. differens* with 21.3% and 10.4% moisture content were accorded a storage life of 3.5 weeks (25 days) and 7.7 weeks (54 days), respectively. This period also coincides with the duration of 4 and 8 weeks when the sensory scores of aroma, colour and taste of *R. differens* with 21.3% and 10.4% moisture content began to consistently differ significantly ( $P < 0.05$ ) from those of the freshly processed products at week 0. For both products, texture showed the

highest score, implying that it exhibited the greatest deviation from scores at time 0. It is possible that the loss in crunchiness was a result of breakdown of tissue by for example (microbial) enzymes, absorption of moisture from the surrounding environment, or both. It should, however, be noted that softness in texture is mainly of concern to the processor but not the consumer, as it does not indicate spoilage to the consumer. This is because preparation methods other than roasting result in acceptable *R. differens* with a soft texture. Therefore, crunchiness is rather an indicator of quality deterioration for crunchy *R. differens*. The changes in aroma towards rotting mushrooms, loss of the meatiness in taste (off taste) and loss of the fresh ginger yellow colour to a dull raw gold yellow were the main indicators for spoilage.

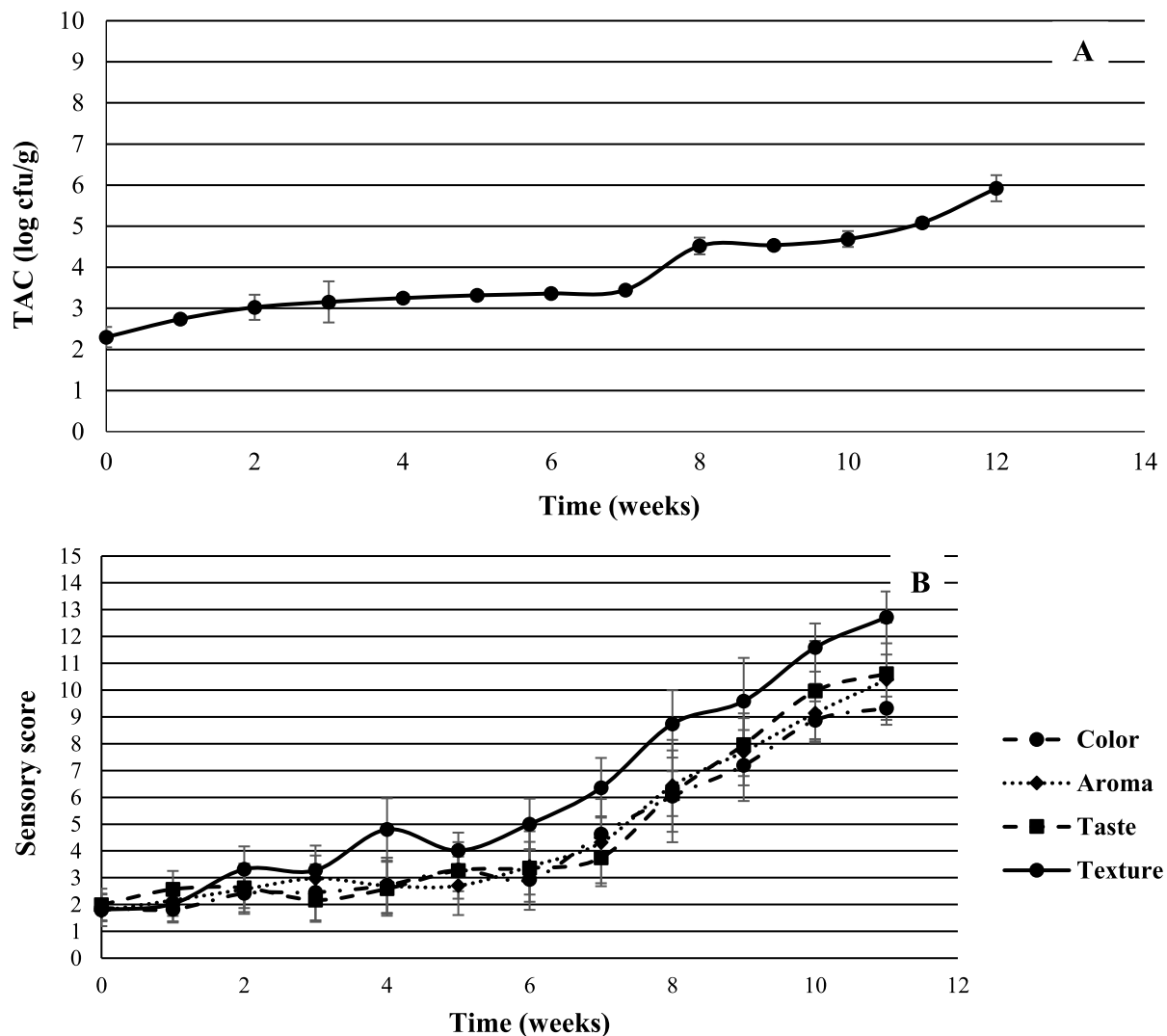


FIGURE 4 Total aerobic count (A;  $n = 3$ ,  $P < 0.05$ ) and sensory scores (B,  $n = 10$ ,  $P < 0.05$ ) of ready-to-eat *Ruspolia differens* (10.4% moisture content) stored for 11 weeks at a temperatures ranging from 2 to 5 °C. Anchor terms on the 15 cm line scale of each parameter are described in Table 1.

The shelf life of 3.5 weeks (25 days) and 7.7 weeks (54 days), respectively, correspond to a TAC of 4.9 and 3.9 log cfu/g, respectively. Even though no microbial criteria and limits have been established for ready-to-eat edible insects in Uganda, guidelines stating microbial limits for different categories of ready-to-eat foods with a high water activity (i.e.  $a_w \geq 0.95$ ) have been developed in Hong Kong and England (Centre for Food Safety, 2014; Health Protection Agency, 2009). According to the latter, ready to eat cooked foods that are chilled but with minimum handling prior to sale or consumption, a category under which these refrigerated ready-to-eat *R. differens* products would fall, are considered microbiologically acceptable for consumption if they have a TAC corresponding to  $<7.0$  log cfu/g. These values, 4.9 and 3.9 log cfu/g associated with refrigerated *R. differens* of edible quality are less than 7.0 log cfu/g, the upper

limit for acceptable ready-to-eat foods according to the aforementioned guidelines. According to the European Commission criteria, (EC) No 2073/2005, for ready-to-eat products placed on the market during the shelf life, the presence of *Listeria monocytogenes* is an important quality criteria. Although the presence of *L. monocytogenes* and other pathogens in *R. differens* is a possibility as indicated by DNA sequencing (Ssepuyya *et al.*, 2018), classical analyses have not confirmed the growth and survival of such pathogens in the *R. differens* matrix.

#### *Effect of vacuum packaging on the chemical, sensory and microbial stability of roasted and dried Ruspolia differens*

The microbial load of roasted and dried *R. differens* remained rather constant around 5 log cfu/g. There was a slow but significant ( $P < 0.05$ ) increase of TAC in the

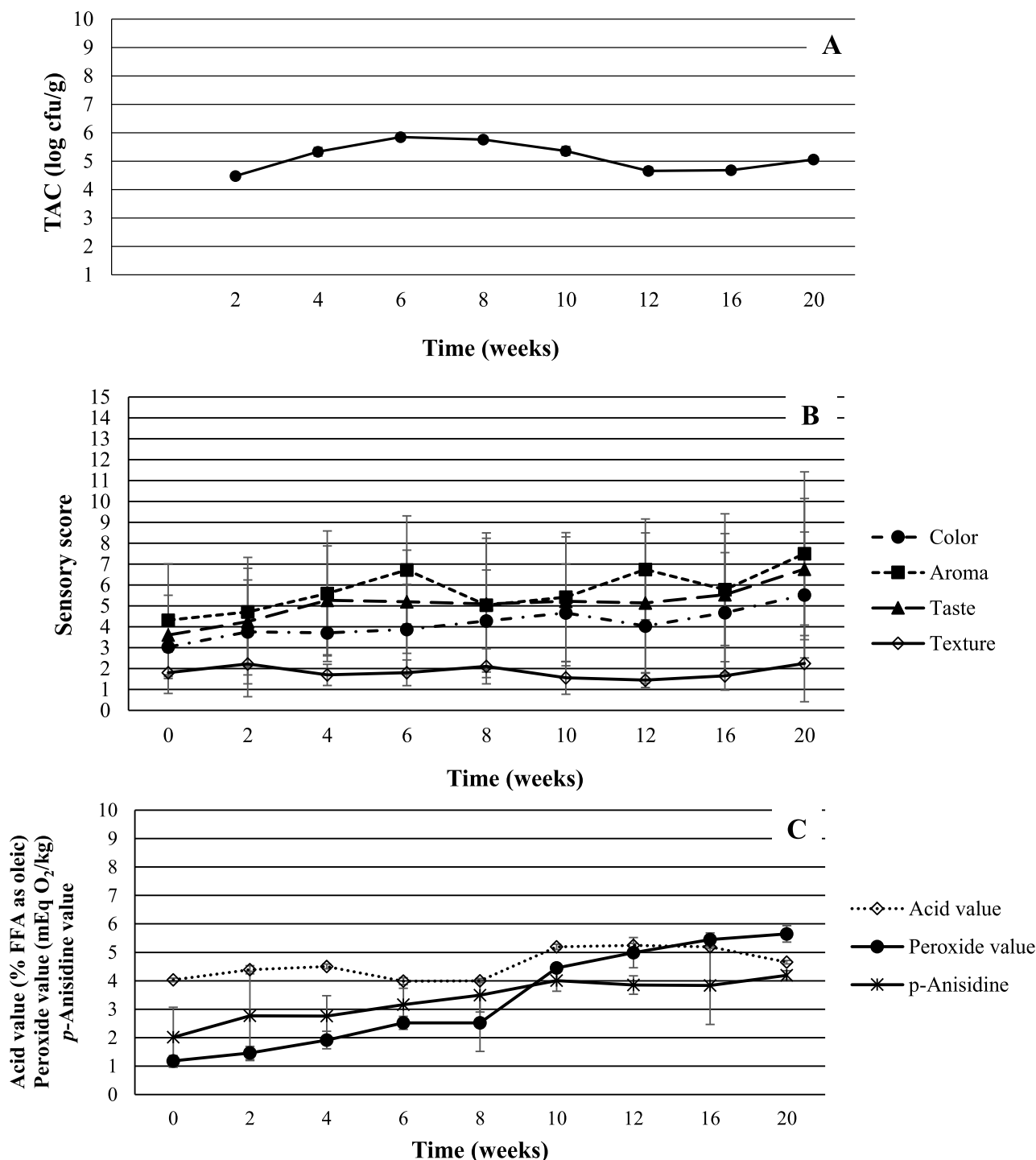


FIGURE 5 Total aerobic count (A;  $n = 3$ ,  $P < 0.05$ ), sensory scores on a 15 cm line scale (B;  $n = 10$ ,  $P < 0.05$ ) and fat quality (C;  $n = 3$ ,  $P < 0.05$ ) of ready-to-eat *Ruspolia differens* (preheated, roasted and dried, 4.5% moisture content) stored for 20 weeks at a room temperature (27 °C). Anchor terms on the 15 cm line scale of each parameter are described in Table 1.

first 6 weeks from 4.48 log cfu/g at week 2 up to 5.84 log cfu/g at week 6 (Figure 5A).

The latter value was not significantly ( $P = 0.98$ ) different from that at week 8 (5.76 log cfu/g). After week 8, the TAC decreased to almost a constant value of 4.66 log cfu/g at week 12, which was not significantly different from 4.68 log cfu/g at week 16 ( $P = 0.10$ ) and 5.05

log cfu/g at week 20 ( $P = 0.10$ ). Although an explanation for these changes is not straightforward, it should be mentioned as well, that from a microbiological point of view, the TAC could be considered as rather constant around 5 log cfu/g over the studied time period. This constant value is similar to the concentration of spores associated with *R. differens* (Ssepuyya *et al.*, 2018). Given

the low moisture content (4.5%), and hence low water activity, it is possible that spores highly contribute to the observed TAC of dried *R. differens*.

The point of sensory rejection was not reached indicating that the samples may still be acceptable beyond week 20 (Figure 5B). The texture of the dried *R. differens*, as indicated by crunchiness, was the least affected. The score for texture in the 20th week was not significantly different ( $P < 0.05$ ) from that at week 0. Though the crunchiness was maintained until week 20, panelists agree that *R. differens*' aroma was very weak. At week 20, aroma exhibited the highest score (7.5) followed by taste (6.5) and colour (5.5), indicating the lowest consumer acceptance. Sensory testing was stopped at week 20 because some of the products lost vacuum and the experiment could not continue. Notably, air drying also had an effect on sensory quality of *R. differens*. Though it increased the crunchiness, as the moisture was driven off, the meatiness of the taste reduced from being 'meaty' in roasted grasshoppers to only 'moderately meaty' after drying; and, from a 'fresh ginger yellow' colour to a more dull 'dry ginger yellow colour' (Table 1). Hence, unlike for the roasted *R. differens*, the combination of roasting and drying *R. differens* reduced the sensory acceptability already from the start of the shelf life experiment.

Low acid, peroxide and *p*-anisidine values were observed at the end of the testing period. The acid, peroxide and *p*-anisidine values associated with the scores at week 20 were 4.65% FFA, 5.65 mEq O<sub>2</sub>/kg and 4.19, respectively (Figure 5C). These values are below those associated with: (a) rancid fatty foods, i.e. peroxide of 20 mEq O<sub>2</sub>/kg and a *p*-anisidine value 10 (Kiokias *et al.*, 2010; Nielsen, 2010); and (b) spoilt *R. differens*, i.e. a peroxide value of more than 17.09 mEq O<sub>2</sub>/kg and a *p*-anisidine value of 11.84 (Ssepuuya *et al.*, 2021). Though the acid value rather assumed a fairly constant value, the peroxide and *p*-anisidine values gradually increased over time indicating continued formation of primary and secondary oxidation products. Although the experiment could not be continued beyond week 20, it can be concluded that vacuum packaging can be used to increase the shelf life of grasshoppers at room temperature by over 5 months. Vacuum packaging not only modifies the atmosphere by removing all the air from the package, without precautionary measures, it also leads to physical disintegration of dry *R. differens* (Ssepuuya *et al.*, 2016). A promising alternative which requires further research is the use of modified atmosphere packaging where the gas composition in the head space is

changed using flushing instead of vacuum compensation.

#### 4 Conclusions

The study results show the potential of different processing and storage conditions to prolong the shelf life of raw and processed wild harvested *R. differens*. Short-term storage at room temperature of preheated *R. differens* is possible for up to 9 hours, with an acceptable microbial and sensorial quality. Given that the furthest point of harvesting grasshoppers to the major grasshopper market in Kampala is about 6 hours, the preheating step enables transportation at ambient temperature without loss of shelf stability. Reducing the moisture content of roasted grasshoppers from 21.3 to 10.4% doubled the shelf life under refrigeration from 25 days to 54 days, implying that the shelf life of ready-to-eat roasted grasshoppers is moisture (water activity) dependent. Further reducing the moisture content (and thus the water activity) by drying, increased the shelf life of *R. differens* by another 15 weeks at room temperature, further indicating the potential role of moisture content/water activity in influencing the shelf stability of *R. differens*. Although the texture change was most prominent, for a consumer, the loss of the meaty aroma is probably more important. The microbial counts of acceptable ready-to-eat grasshoppers, i.e. 4.9 and 3.9 log cfu/g for preheated and roasted *R. differens*, and 5.05 log cfu/g for dried and vacuum packed *R. differens* were below 7.0 log cfu/g, the upper limit for acceptable ready-to-eat foods, and hence of appreciable consumption quality. Rancidity as measured by the peroxide and *p*-anisidine values had no effect on the shelf stability of dried *R. differens*.

#### Acknowledgements

This work was financially supported by a scholarship from the KU Leuven Interfaculty Council for Development Cooperation (IRO), the INSBIZ Project and by an FWO travel grant.

#### Conflict of interest

The authors declare no conflict of interest.

## References

- Adegoke, O.G. and Olapade, A.A., 2012. Preservation of plant and animal foods: an overview. In: Bhat, R., Alias, A.K. and Paliyath, G. (eds.) *Progress in food preservation*. Wiley-Blackwell, West Sussex, UK, 318 pp. 603-604.
- Bakker, J. and Law, B.A., 1994. Cheese flavor. In: Piggott, J.R. and Paterson, A. (eds.) *Understanding natural flavors*. Springer Science & Business Media, Dordrecht, the Netherlands, pp. 294-296.
- Balch, P.A., 2003. *Prescription for dietary wellness*. Penguin group (USA) Inc, New York, 320 pp.
- Buettner, A., 2017. *Springer handbook of odor*. Springer International Publishing AG, Cham, Switzerland, 1151 pp.
- Center for Food Safety, 2014. Microbiological guidelines for food. Available at: [https://www.cfs.gov.hk/english/food\\_leg/files/food\\_leg\\_Microbiological\\_Guidelines\\_for\\_Food\\_e.pdf](https://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf).
- Ethiopian Standards Agency, 2012. Animal and vegetable fats and oils-determination of anisidine value ISO 6885:2012. Available at: [http://www.questin.org/sites/default/files/intl-codes/et.iso\\_6885.2012.pdf](http://www.questin.org/sites/default/files/intl-codes/et.iso_6885.2012.pdf).
- Halloran, A., Flore, R., Vantomme, P. and Roos, N., 2018. Edible insects in sustainable food systems. Springer International Publishing AG, Cham, Switzerland, pp. 97-100.
- Health Protection Agency, 2009. Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. Available at: [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1259151921557](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1259151921557).
- Kelemu, S., Niassy, S., Torto, B., Fiaboe, K., Affognon, H., Tonang, H., Maniania, N.K. and Ekesi, S., 2015. African edible insects for food and feed: inventory, diversity, commonalities and contribution to food security. *Journal of Insects as Food and Feed* 1: 103-119. <https://doi.org/10.3920/JIFF2014.0016>
- Kilcast, D. and Subramaniam, P., 2010. Introduction. In: Kilcast, D. and Subramaniam, P. (eds.) *The stability and shelf-life of food*. CRC Press LLC, Boca Raton, Florida, pp. 11-12.
- Kiokias, S., Varzakas, T.H., Arvanitoyannis, I.S. and Labropoulos, A.E., 2010. Lipid oxidation and control of oxidation. In: Yildiz, F. (ed.) *Advances in food biochemistry*. CRC Press, Boca Raton, Florida, pp. 384-402.
- Klunder, H.C., Wolkers-Rooijackers, J., Korpela, J.M. and Nout, M.J.R., 2012. Microbiological aspects of processing and storage of edible insects. *Food Control* 26: 628-631. <https://doi.org/10.1016/j.foodcont.2012.02.013>
- Lawless, H.T. and Heymann, H., 2010. *Sensory evaluation of food*. 2nd ed. Springer Science & Business Media LLC, New York, NY, 596 pp.
- Man, D., Man, C.M.D. and Jones, A.A., 2000. Shelf life evaluation of foods. Aspen Publishers Inc, Gaithersburg, MD, 272 pp.
- Man, D., 2008. *Shelf life*. John Wiley & Sons, Hoboken, NJ, 128 pp.
- Megido, R.C., Desmedt, S., Blecker, C., Béra, F., Haubruge, É., Alabi, T. and Francis, F., 2017. Microbiological load of edible insects found in Belgium. *Insects* 8(1). <https://doi.org/10.3390/INSECTS8010012>
- Nicoli, M.C., 2012. *Shelf life assessment of food*. CRC Press, Boca Raton, FL, 316 pp.
- Nielsen, S.S., 2010. *Food analysis laboratory manual*. 2nd ed. (Vol. 1). Springer Science + Business Media LLC, New York, USA, 171 pp.
- Rahman, M.S., 2007. *Handbook of food preservation*. 2nd ed. CRC Press, Boca Raton, FL, 1088 pp.
- Roehl, E., 1996. *Whole food facts: the complete reference guide*. Healing Arts Press, Rochester, Vermont, 296 pp.
- Singh, K.T. and Cadwallader, K.R., 2004. Ways of measuring shelf-life and spoilage. In: Steele, R. (ed.) *Understanding and measuring the shelf-life of food*. Woodhead Publishing, Cambridge, UK, 448 pp.
- Ssepuuya, G., Aringo, R.O., Mukisa, I.M. and Nakimbugwe, D., 2016b. Effect of processing, packaging and storage-temperature based hurdles on the shelf stability of sautéed ready-to-eat *Ruspolia nitidula*. *Journal of Insects as Food and Feed* 2: 245-253. <https://doi.org/10.3920/JIFF2016.0006>
- Ssepuuya, G., 2019. Shelf Life, sensorial, and nutritional quality of the long-horned grasshopper *Ruspolia differens* Serville. Available at: [https://kuleuven.limo.libis.be/discovery/fulldisplay?docid=lirias2835228&context=SearchWebhook&vid=32KUL\\_KUL:Lirias&search\\_scope=lirias\\_profile&tab=LIRIAS&adaptor=SearchWebhook&lang=en](https://kuleuven.limo.libis.be/discovery/fulldisplay?docid=lirias2835228&context=SearchWebhook&vid=32KUL_KUL:Lirias&search_scope=lirias_profile&tab=LIRIAS&adaptor=SearchWebhook&lang=en)
- Ssepuuya, G., Mukisa, I.M. and Nakimbugwe, D., 2016a. Nutritional composition, quality, and shelf stability of processed *Ruspolia nitidula* (edible grasshoppers). *Food Science & Nutrition* 5: 103-112.
- Ssepuuya, G., Nakimbugwe, D., De Winne, A., Smets, R., Claes, J. and Van Der Borght, M., 2020. Effect of heat processing on the nutrient composition, colour, and volatile odour compounds of the long-horned grasshopper *Ruspolia differens* serville. *Food Research International* 129: 108831. <https://doi.org/10.1016/j.foodres.2019.108831>
- Ssepuuya, G., Nakimbugwe, D., Van Campenhout, L., De Winne, A., Claes, J. and Van der Borght, M., 2021. Towards establishing the spoilage mechanisms of the long-horned grasshopper *Ruspolia differens* Serville. *European Food Research and Technology* 2021: 1-12. <https://doi.org/10.1007/S00217-021-03824-9>

- Ssepuyya, G., Wynants, E., Verreth, C., Crauwels, S., Lievens, B., Claes, J., Nakimbugwe, D. and Van Campenhout, L., 2018. Microbial characterisation of the edible grasshopper *Ruspolia differens* in raw condition after wild-harvesting in Uganda. *Food Microbiology* 77: 106-117. <https://doi.org/10.1016/J.FM.2018.09.005>
- Ulrich, J.A. and Halvorson, H., 1951. Chemical and microbial studies on sliced canned bacon. In: Mrak, E.M. and Stewart, G.F. (eds.) *Advances in food research*. Academic Press, New York, USA, pp. 304-305.
- Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D. and Sporns, P., 2005. *Handbook of food analytical chemistry*. John Wiley & Sons Inc, Hoboken, NJ, 597 pp.
- Wynants, E., Crauwels, S., Lievens, B., Luca, S., Claes, J., Borremans, A., Bruyninckx, L. and Van Campenhout, L., 2017. Effect of post-harvest starvation and rinsing on the microbial numbers and the bacterial community composition of mealworm larvae (*Tenebrio molitor*). *Innovative Food Science & Emerging Technologies* 42: 8-15. <https://doi.org/10.1016/J.IFSET.2017.06.004>
- Yadav, L., 2010. *The effect of ozone on the growth and development of selected food spoilage fungi*. Universal-Publishers, Irvine, CA, 70 pp.