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Microbiological Assessment and Shelf-Life Determination of Wheat Muffins Enriched with Domesticated African Emperor Moth (*Gonimbrasia zambesina* Walker) Caterpillar Flour

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Abstract

African emperor moth (*Gonimbrasia zambesina*) caterpillars are considered healthy food as they are rich in protein and unsaturated fats. In Kenya, *G. zambesina* caterpillars are predominantly found along the coastal region, where they emerge in during the short and the long rains. The caterpillars forage in the wild on mango (*Mangifera indica*) leaves and *Euclea natalensis* (Ericales: Ebenaceae) leaves. The caterpillars are consumed whole or may be transformed into fine flour. The caterpillars' flour can be utilized in the bakery industry for the enrichment of bakery products since wheat (the major component of bakery products) is low in protein. However, consumers are concerned about the microbiological quality of bakery products enriched with insect flour. There are also concerns about the effect of these insects' flour on the shelf life of bakery products since they have unsaturated fats. Therefore, this study evaluated the microbial quality and shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15%, and 20%. For all the samples analyzed, total viable count (TVC) was <30 cfu/g, total coliform count (TCC) was <30 cfu/g, *Salmonella* spp, and *Staphylococcus aureus* were not detected. The colonies for yeast and moulds were <30 cfu/g throughout the evaluation time of 21 days. The PVs of wheat muffins increased with an increase in the substitution level. The PVs of enriched wheat muffins increased with an increase in storage time and temperature. The shelf-life of the wheat muffins decreased with an increase in the substitution level of wheat flour with *G. zambesina* caterpillar flour. The predicted shelf life of 0%, 5%, 10%, 15%, and 20% wheat muffins was 120.0 days, 111.0 days, 103.0 days,

102.0 days and 90.0 days, respectively. Therefore, wheat muffins enriched with *G. zambesina* caterpillar flour have good microbiological quality and shelf life.

Keywords

Gonimbrasia zambesina, Domestication, Bakery Products, Microbial Quality, Oxidation, Enrichment

1. Introduction

The protein malnutrition situation in sub-Saharan Africa and changing food systems are forcing the food sector players to explore various alternative sources of proteins [1] [2]. Food industries have shifted their attention to the use of edible insects as a high-quality protein product and a nutrient source for other foods through fortification [3]. Fortification with insect flour aims to improve the food product's nutritional value, correct any deficiencies in minerals or vitamins, and enhance its health-protecting substances [4] [5]. Food fortification with insects has been mostly orchestrated by the new technologies that are currently in use by the food industries, the need for transformation that would ensure proper food management, and the cost of production of insects as raw materials due to their high feed conversion efficiency and the rate of reproduction is high in comparison to other animal sources [5] [6]. Some of the insects that have been used for fortification/enrichment include *Rhynchophorus phoenicis* larvae, *Macrotermes subhylanus*, *Acheta domesticus*, *Tenebrio molitor* larvae and *Nauphoeta cinerea* [5] [7] [8] [9] [10]. Lepidoptera such as *G. zambesina* caterpillars can also be used for fortification since they are abundant, easy to rear and are high in protein content with more than 50% of their body weight being protein [11]. In coastal Kenya, there is an initiative by the Kipepeo butterfly centre, Gede, Kilifi to train farmers on the domestication of *G. zambesina* caterpillars to improve production for export and local consumption.

For successful fortification, the chosen food should be that whose consumption is wide and adequate and have a gap deficit in its nutritional elements that need to be bridged [5]. Wheat flour, a major component of bakery products is low in protein with the soft wheat used to make muffins having only 8% - 10% protein content [12]. Therefore, its protein quality can be improved by enriching with an alternative protein source such as insects flour/powder [12]. To literature, bakery products such as bread, cookies, biscuits, and wheat buns enriched with some insect powder have been developed to improve their nutritional value [5] [7] [8] [10] [13] [14]. However, the progress already made towards meeting protein security is under threat as there are fears that gains may have been reversed by the adverse effects of the COVID-19 pandemic on raw material production and transportation, production cost and consumer accessibility of end products [1]. Therefore, for the food industry to cope with the current consumer

demand for insect-based food products, there should be a much more emphasis on combined approaches for conventional food fortification and food to food fortification by optimal utilization of all the edible insects that are underutilized such as *G. zambesina* caterpillar larva.

Fortification of bakery products not only increases their nutritional value but also has got an effect on their microbiological properties and oxidative stability. The consumers and producers are both concerned about the microbiological effects of the addition of insect flour, insect parts, or insect protein into bakery products [15] [16]. This is because previous studies on the microbiological safety of most edible insects, especially those harvested from the wild, have shown that they harbour microbes of health concerns [17]-[22]. Some insects, such as some edible caterpillars have also been reported to harbour both the pathogenic and spoilage microorganism in their gut [23] [24]. Furthermore, wheat muffins are classified as bakery products with a high water activity (a_w) > 0.85, which often are characterized by spoilage bacteria and yeast and moulds. In some instances, they have also been intimated to the outbreak of foodborne illnesses caused by *Salmonella* and *Bacillus cereus* [25]. The presence of bacteria in bakery products may be due to post-process contamination and poor packaging and storage conditions [25]. This is because the high baking temperatures readily destroy vegetative pathogenic microorganisms due to their low thermal resistance (D values), such that they destroy most of the microorganisms in the raw materials [7] [25] [26].

Bakery products may deteriorate in quality either due to the germination of yeasts and mould spores which can survive the baking temperatures since they have high thermal resistance (D values), food chemical reactions, and enzymatic activities [14] [25]. Yeast and moulds are associated with high sugar foods [27]. When the yeast and moulds grow to levels that are above the acceptable limits for bakery products, the quality of these products is compromised [26]. The shelf life of bakery products is also limited by the growth of moulds [27]. Shelf life is defined as the period in which a food product is satisfactorily safe for consumption and appeals to the senses of consumers [14]. The fortification of bakery products with insect powder which is rich in unsaturated lipids makes them susceptible to lipid oxidation which may lead to the formation of rancidity compounds [28]. This, like the yeast and moulds, not only results in the loss of the nutritional value of the bakery products but also affects their shelf life [29] [14].

Due to the increasing use of insects in enriching bakery products, there is a need to take precautions by determining the microbiological properties of these products such that there is a surety about their safety. To ascertain that no form of microbiological contamination took place during production, bakery industries have got a responsibility to carry out microbiological tests on the insect-based bakery products [14]. Furthermore, there is also a need to determine their oxidative stability due to the addition of unsaturated lipids present in insect ingredients [30]. The oxidative stability can be determined by monitoring the

chemical changes such as oxidative rancidity, caused by the oxidation of fats [30] [31]. The oxidation of oils results in the formation of primary products, peroxides, which are measured as peroxide values [31]. The peroxide value can be used to estimate the shelf life either through conventional storage or accelerated shelf life [14]. In performing the conventional storage test, food is stored under conditions that are expected during normal storage for a long time while the accelerated shelf life test is done by storing food products under higher temperatures or oxygen concentrations for some time as tests are frequently performed on selected indicators [30]. Therefore, this study aimed to investigate the microbial safety of wheat muffins enriched with *G. zambesina* caterpillar flour. It also aimed to estimate the shelf life of the wheat muffins based on peroxide accumulation.

2. Materials and Methods

2.1. Study Design and Study Area

The experiments were arranged in a completely randomized design with 5 treatments of wheat muffins enriched with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15%, and 20% substitution levels. *Gonimbrasia zambesina* caterpillars were domesticated and processed between August and December 2020 at Kipepeo butterfly centre, Gede, Kilifi, Kenya. Batter preparation and baking were done in the food processing laboratory of Jaramogi Oginga Odinga University of Science and Technology (JOOUST). Microbiological analysis was done in the microbiology laboratory of JOOUST whereas peroxide value determination was done at the chemistry laboratory of Masinde Muliro University of Science and Technology (MMUST). All analyses were done in triplicate. This study was authorized by Ethical Review Committee (ERC) of JOOUST and a research permit by the National Commission for Science, Technology and Innovation (NACOSTI) was obtained.

2.2. Raw Material Acquisition and Processing

Domestication of *G. zambesina* caterpillars

Gonimbrasia zambesina caterpillars were reared in two rectangular cages made of steel, wire mesh and net. The cages were 1.2 m long, 1.0 m wide and 0.5 high. Each cage was on four stands, every 0.6 m high. The stands were dipped in ice cream boxes filled with water to prevent predators from accessing the cages. Eggs of *G. zambesina* moth deposited on mango tree (*Mangifera indica*) leaves were taken by plucking off gently the leaf. These were transported to the cages where fresh mango leaves were added to protect the eggs from direct sunlight and heavy rains and also act as starter feed for the newly hatched larvae. The doors of the cages were sealed to prevent the hatched larvae from escaping. The eggs of the *G. zambesina* moth were monitored for hatching at 8:00 am, 12:30 pm, and 4:30 pm daily. The eggs took about 2 - 5 days to hatch. However, hatching was not uniform since, during their collection, the exact time and day

of deposition on the leaves were not known. The hatched caterpillars were continuously fed on fresh *M. indica* leaves. The stalks of consumed mango leaves were removed every morning and evening during routine checking and replaced with fresh ones. Moulting of *G. zambesina* larvae from the 1st instar to the 6th instar was at an interval of 4 - 6 days for 3 - 4 weeks depending on the feed consumption rate of each larva and the date on which it was hatched.

Harvesting of *G. zambesina* caterpillars

Mature *G. zambesina* caterpillars (those that have reached the 6th instar stage of larval development) were hand-picked from the cages (Figure 1). *Gonimbrasia zambesina* caterpillars are regarded as mature if, after continuous feeding for 3 - 4 weeks, they stop feeding and burrow in their manure inside the cage. The harvested caterpillar was weighed and 10% of the harvested populations were released to the environment into ensure continuity in biodiversity.

Processing of *G. zambesina* caterpillar

About 5 kg of the harvested *G. zambesina* caterpillars were killed by freezing at −25˚C for 30 min and degutted using a scalpel. The caterpillars were washed in running tap water and drip-dried for 20 min on drying nets. They were then transferred into a locally made wooden electric dryer and dried at 55˚C for 3 days to a constant weight. To consider the sample dry, three samplings were done at an interval of one hour which resulted in less than 1% change in weight. The dry caterpillars were milled using a coffee bean grinder (DeLonghi KG-79, Wuhan, China). The *G. zambesina* caterpillar flour was sieved through a 250 μm sieve to obtain fine flour, packaged and transported to the food processing laboratory of JOOUST in sterilized polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India).

2.3. Wheat Muffins Formulation and Baking

Formulation of wheat muffins enriched with *G. zambesina* caterpillar flour

The recipe and the ingredients for the wheat muffins preparation were according to Purnoma *et al.* [12] with slight modifications. Wheat flour was substituted

Figure 1. Image of harvested *G. zambesina* caterpillars at the 6th instar larval stage.

with *G. zambesina* caterpillar flour at 5 levels; 100:0 (the control), 95:5, 90:10, 85:15 and 80:20 w/w. Approximately 150 g of the wheat flour composited with *G. zambesina* caterpillar flour was weighed into a mixing bowl and mixed using a clean wooden spoon. Melted margarine (98.57 g), water (47.14 g), and whisked eggs (85.71 g) were added into the bowl according to the recipe at an interval of 30 sec, respectively while mixing using a hand mixer (Model: RM/382, Guangdong, China) at high speed (as indicated on the equipment) for 3 min. Baking powder (2.14 g) and refined sugar (91.43 g) were added to the mixing bowls and mixing was done at low speed (as indicated on the hand mixer) for 2 min to form the batter. The muffin batter was poured into the wells of 3 standard 6-well muffin pans. Baking was done in triplicate at 180˚C for 20 min in a preheated forced air circulation oven (Electrolux AR 85, Italy). The baked wheat muffins were then removed from the oven (Electrolux AR 85, Italy) and cooled for 20 min at room temperature. Packaging was done in sterilized polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India), awaiting microbial analysis and peroxide value determination.

2.4. Microbiological Analyses

The microbiological assessment of wheat muffins enriched with *G. zambesina* caterpillar flour stored at 4˚C was done 48 hr after baking. For total viable count (TVC), total coliform count (TCC), *S. aureus* determination, and enumeration of yeast and moulds, 5 g of the grounded sample were diluted into 45 mL of sterilized peptone water to make a stock dilution. The dilution was homogenized by vortexing at 3000 rpm for 5 min and serially diluted three fold. Aliquots (1 mL) from each serial dilution were inoculated in triplicate plates.

TVC

The total viable count was determined according to AOAC [32] Method 966.23 in plates with Plate Count Agar (PCA) (CM0152). The plates were incubated at 35˚C for 48 hr. The results were expressed as cfu/g of the sample.

TCC

The total coliform count was determined according to AOAC [32] Method 966.24 in plates with MacConkey Agar (MA) (Himedia Ref M043). The plates were inverted and incubated at 35˚C for 48 hr. The results were expressed as cfu/g of the sample.

Staphylococcus aureus

Staphylococcus aureus counts were determined according to Nyangena *et al.* [17] in petri dishes with Baird-Parker medium (Himedia Ref M043) enriched with egg-yolk tellurite emulsion (Himedia, India). The plates were incubated at 35˚C for 48 hr. Colonies of typical *S. aureus* appearance (grey-black to jet black, circular, smooth, convex, and moist and 2 - 3 mm in diameter) were monitored for growth.

Detection of *Salmonella*

Detection of *Salmonella* spp was according to Nyangena *et al.* [17]. Briefly, 25 g of ground sample was diluted into 225 mL of nutrient broth (Himedia Ref

M002) in a closable container, gently shaken, and incubated at 35˚C for 24 hr. Then, 25 mL of the enriched homogenate was transferred to 225 mL of tetrathionate broth (Himedia, India) and incubated at 37˚C for 24 hr. A 3mm loopful of incubated culture was streaked in triplicate plates on *Salmonella-Shigella* Agar (Himedia, India). Plates were incubated at 37˚C for 24 hr and observed for the typical salmonella colonies (colourless colonies with black centers).

Enumeration of yeast and moulds

Total yeast and moulds counts were enumerated according to Nyangena *et al.* [17] on days 1, 7, 14, and 21 of baking in plates with Potato Dextrose Agar (PDA) (Himedia Ref M096) acidified with 10% tartaric Acid (Sigma-Aldrich, Schnelldorf, Germany) to pH 3.5 using a sterile micropipette. The aliquot was spread over the media using a sterile spreader until it was absorbed into the media. The plates were incubated at 25˚C for 72 hr. Plates with between 30 - 300 colonies were selected and the colonies were counted. The results were expressed as cfu/g of the sample.

2.5. Peroxide Value (PV) and Shelf Life Estimation

Peroxide value was determined according to AOAC [32] Method 965.33 for triplicate samples stored at 25˚C, 37˚C, and 45˚C over 21 days. Briefly, 0.3 g of oil extracted from the enriched wheat muffins through the Soxhlet extraction method according to AOAC [32] Method 920.39 was weighed into a 250 mL stoppered Erlenmeyer flask, and 30 mL of acetic acid chloroform solvent mixture was added and swirled to dissolve. Saturated potassium iodide solution (0.5 mL) was added using a mohr pipet, left to stand in the dark for 1 min with occasional shaking and then 30 mL of distilled water was added. The solution was titrated against 0.01M sodium thiosulfate with vigorous shaking until the yellow colour almost disappeared. Then, 0.5 mL 1% starch solution was added and titration continued with vigorously shaking until the blue colour disappeared. The peroxide value was calculated as follows:

$$PV_1\left[\text{meq } O_2/\text{kg}\right] = \frac{T \times M \times 100}{W} \tag{1}$$

where: PV_1 is peroxide value expressed in meq O_2/kg, M is the molarity of sodium thiosulfate solution consumed in mol/L, T is the titer of the sodium thiosulfate solution, W is weighed portion of substance in grams.

Data processing

A regression plot of a semi-logarithmic scale was established for the rate constant for each sample at the three temperatures (Lnk) against the absolute inverse of temperature $\left(\frac{1}{T}\right)$ to obtain an Arrhenius equation based on:

$$\ln k = \ln k_o - \frac{E_a}{R}\left(\frac{1}{T}\right) \tag{2}$$

$$\frac{1}{T} = \frac{1}{T^*} - \frac{1}{T_{ref}} \tag{3}$$

where: k is the reaction rate constant; R is the molar gas constant (8.314 J/K/mol), T is the absolute temperature (K); E_a is the apparent activation energy (J/mol), and k_o is the pre-exponential factor; T_{ref} is corresponding to the average of the temperature range used during the experiment where in this case was taken to be 37˚C (310.15 K) and T^* is the temperature to which prediction of shelf life is done, in this case, it was 22˚C (295.15 K).

First-order regression models for wheat muffin enriched with *G. zambesina* caterpillar flour at each substitution level at 25˚C, 37˚C, and 45˚C temperatures as presented in Table 1.

The Arrhenius parameters were obtained to estimate the shelf life according to a formula described by the method of [33]:

$$\text{Shelf-life} = \frac{[A_{\text{lim}}]-[A_o]}{K_{ref}\exp\left[-\frac{E_a}{R}\left(\frac{1}{T^*}-\frac{1}{T_{ref}}\right)\right]} \tag{4}$$

where: A_o is the initial peroxide value at time zero; K_{ref} is the rate constant at the reference temperature; E_a is the apparent activation energy (J/mol); T^* is the temperature to which prediction of shelf life is done, in this case, it was 22˚C (295.15 K); A_{lim} is the standard acceptable limit for peroxide value in processed foods which is 10.0 meq O_2/kg oil according to East African Standard (EAS 795:2013); T_{ref} is corresponding to the average of the temperature range used during the experiment where in this case was taken to be 37˚C (310.15 K).

Table 1. First order regression models for the wheat muffins enriched with *G. zambesina* caterpillar flour at 25˚C, 37˚C, and 45˚C storage temperatures.

Substitution level	Temperature	Regression equation	R^2
0%	25˚C	$Y = 0.0347x + 0.0749$	0.9295
	37˚C	$Y = 0.0388x + 0.0731$	0.9465
	45˚C	$Y = 0.0426x + 0.1219$	0.8984
5%	25˚C	$Y = 0.0311x + 0.1089$	0.8476
	37˚C	$Y = 0.032x + 0.1365$	0.7929
	45˚C	$Y = 0.0431x + 0.1824$	0.8025
10%	25˚C	$Y = 0.0332x + 0.0913$	0.9018
	37˚C	$Y = 0.0378x + 0.0774$	0.9453
	45˚C	$Y = 0.0471x + 0.087$	0.9552
15%	25˚C	$Y = 0.0269x + 0.064$	0.9277
	37˚C	$Y = 0.0327x + 0.0872$	0.9021
	45˚C	$Y = 0.0389x + 0.1363$	0.8497
20%	25˚C	$Y = 0.0266x + 0.0659$	0.9094
	37˚C	$Y = 0.0337x + 0.0477$	0.9685
	45˚C	$Y = 0.0441x + 0.094$	0.9416

Key: y is the peroxide value and x is the temperature.

2.6. Statistical Analysis

Data were analyzed using SAS® software version 8.3. Normality of microbiological and shelf life data was tested using PROC UNIVARIATE and homogeneity was tested using Levene's method. Analysis of Variance (ANOVA) was carried out using PROC GLM to test the hypotheses of the study at a 95% confidence level. Tukey's Honestly Significant Difference (HSD) method was used in the separation of the means at $p \leq 0.05$.

3. Results and Discussion

The TVC and TCC of wheat muffins enriched with *G. zambesina* caterpillar flour were <30 cfu/g for all samples (Table 2). *Staphylococcus aureus* and *Salmonella* were not detected in all the samples analysed. Yeast and moulds counts were low (<30 cfu/g) in wheat muffins enriched with *G. zambesina* caterpillar flour for days 1, 7, 14, and 21 of evaluation. Edible caterpillars have been found to harbour pathogenic and spoilage microorganisms [24]. Therefore, they are of public health concern more so in the utilization of their flour in the bakery industry [17] [23]. However, the utilization of *G. zambesina* caterpillars' flour for the enrichment of wheat muffins during processing was observed to produce microbiologically safe wheat muffins for human consumption. This might be attributed to the domestication of the caterpillars which may have modulated the source to new microorganisms and the intrinsic ones in *G. zambesina* caterpillars [15]. The results of this study corroborate with those by Ayensu *et al.* [7] in their research on the nutritional composition and acceptability of biscuits fortified with *R. phoenicis* larvae and orange-fleshed sweet potato in pregnant women.

The total viable counts were below the maximum permissible limits for edible foods (<10^5 CFU/g) [26]. This might be ascribed to good handling practices of the muffins post baking and proper packaging in sterile polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India) [25]. The absence of coliforms, *S. aureus*, and *Salmonella* in wheat muffins enriched with *G. zambesina* caterpillar flour might be attributed to the dehulling of wheat during wheat flour (major component of the muffins) processing that concentrates 90% of microorganisms

Table 2. Microbiological quality of *G. zambesina* caterpillar flour enriched wheat muffins.

Level of Substitution	TVC (cfu/g)	TCC (cfu/g)	*Staphylococcus aureus*	*Salmonella* spp.	YM cfu/g
0%	<30	<30	Not detected	Not detected	<30
5%	<30	<30	Not detected	Not detected	<30
10%	<30	<30	Not detected	Not detected	<30
15%	<30	<30	Not detected	Not detected	<30
20%	<30	<30	Not detected	Not detected	<30

Key: TVC = Total viable count; TCC = Total coliform count; YM = Yeasts and moulds.

on the bran which is then separated from the germ and pollard [34] [35] Coliforms are indicator organisms [36]. Their absence in wheat muffins enriched with *G. zambesina* caterpillar flour is an indication that good handling and manufacturing practices were adhered to during the processing [36]. The absence of coliforms might further be attributed to proper packaging which was done in sterilized polyethylene zip lock bags (Vinayak Udyog, New Delhi, India). Most coliform contaminations in bakery products occur due to poor post-process handling [26]. The absence of *S. aureus* and *Salmonella* in all the analysed samples might be attributed to good handling and manufacturing practices. *Salmonella* spp is commonly found in the egg content, which was used as an ingredient in the enriched wheat muffins [26]. The absence might be ascribed to the fact that *Salmonella* is a member of enterobacteriaceae family, hence very sensitive to heat. The high baking temperature for the enriched wheat muffins might have killed the *Salmonella* cells [26] [37]. It can further be attributed to degutting and rinsing of the *G. zambesina* caterpillars which might have reduced the gut microorganism [38]. For food to meet the microbiological guidelines on safety, there should be no detection of *Salmonella* in 25 g of the sample tested [17].

At 21 days of storage, the yeast and moulds counts were lower than 30 cfu/g in all the wheat muffins samples. This was way below the maximum acceptable limit for yeast and moulds in foods (1000 cfu/g) [39]. Yeast and moulds are associated with high sugar foods such as wheat muffins which are often responsible for their spoilage thereby limiting their shelf life [27] [39]. Therefore, based on the findings of this study, wheat muffins enriched with *G. zambesina* caterpillar flour are safe microbiologically safe for human consumption over a storage period of 21 days.

As shown in **Figure 2**, the initial PVs for wheat muffins enriched with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15% and 20% substitution levels were 4.50 meq O_2/kg, 5.00 meq O_2/kg, 4.67 meq O_2/kg, 5.50 meq O_2/kg, 5.34 meq O_2/kg, respectively. The PV increased with an increase in storage temperature and time for all the wheat muffin oil samples. The control wheat muffins recorded lower PV compared to those enriched with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15%, and 20% substitution levels.

The initial PVs of all the oils of wheat muffins enriched with *G. zambesina* caterpillar flour was higher than that of fresh amaranth seed oil (0.487 meq O_2/kg oil) and that of fresh coconut oil (0.24 to 0.49 meq O_2/kg oil) [14]. This discrepancy in the initial PVs of these oils might be attributed to the primary oxidation of lipids that might have started immediately after the wheat muffins' processing [14]. During cooling, the enriched wheat muffins were exposed to atmospheric oxygen. Exposure of lipids to oxygen (depending on concentrations) enhances oxidation [14] [40]. Furthermore, the addition of sodium bicarbonate (metal ion) to the wheat muffins as a chemical leavener might have also played a role in their initial PVs. Such metal ions act as a catalyst in an oxidative reaction [40]. The PVs can also be further ascribed to the high baking temperatures (180˚C).

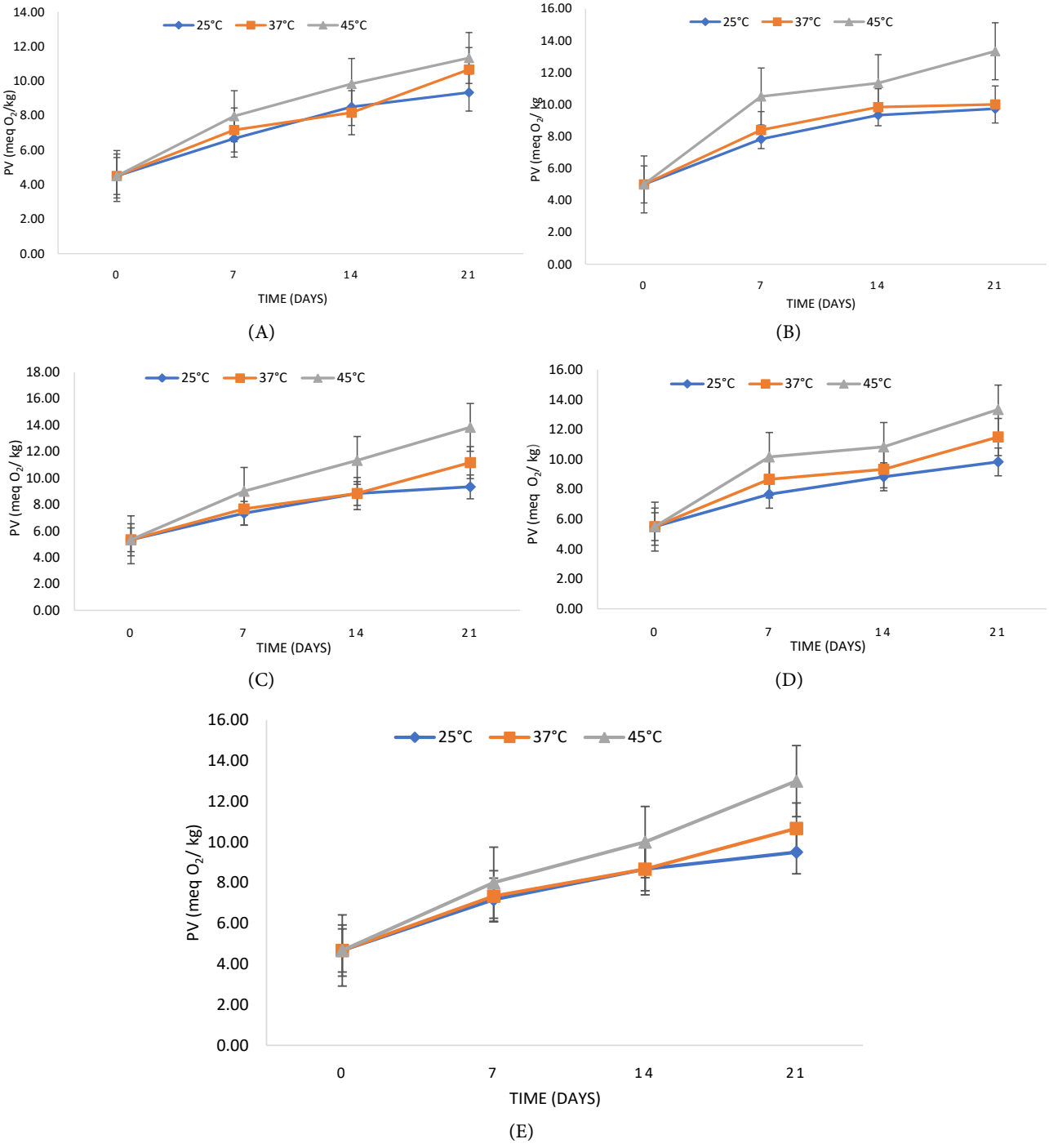

Figure 2. Representative curves showing the peroxide values of wheat muffins enriched with *G. zambesina* caterpillar at different substitution levels. (A) (100% wheat flour), (B) (95% wheat flour; 5% *G. zambesina* caterpillar flour), (C) (90% wheat flour; 10% *G. zambesina* caterpillar flour), (D) (85% wheat flour; 15% *G. zambesina* caterpillar flour), (E) (80% wheat flour; 20% *G. zambesina* caterpillar flour).

There exist a linear relationship between temperature and the rate of oxidation [40].

Based on the initial PVs, wheat muffins enriched with *G. zambesina* caterpillar flour can be classified to be at a low oxidative state or the moderate oxidative

state. This however changed with storage time as the PVs increased. Conventionally, food products are classified into three oxidative states based on their PVs [41]. A food product is classified as a low oxidative state when it has a PV of between 1 and 5 meq O_2/kg oil, a moderate oxidative state when the PV is between 5 and 10 meq O_2/kg oil, and a high oxidative state if the PV is above 10 meq O_2/kg oil [14] [41]. The oxidative level of oils is reflected in the number of hydro peroxides in it which ultimately informs the tendency of an oil to become rancid [41]. A food product with a PV of between 20 to 40 meq O_2/kg oil exhibits noticeable acidity and rancid taste [14].

The increase in the PVs of *G. zambesina* caterpillar flour enriched wheat muffins with an increase in storage temperatures might be ascribed to the linear relationship between temperature and the rate of fat oxidation [40]. Exposure of lipids to higher temperatures enhances the rate of lipid oxidation, while at lower temperatures, the rate of lipid oxidation is slow [29]. The higher PVs for the enriched wheat muffins reported on test day 21 might be attributed to the fact that as lipid oxidation takes place over a period of time, there is a buildup of the hydro peroxides in the food product which leads to deterioration in quality [40]. The higher PVs for the wheat muffins enriched with *G. zambesina* caterpillar flour at higher substitution levels were expected since edible caterpillars flour is rich in unsaturated fatty acids [42]. Unsaturated fatty acids are broken down by oxygen through an autolytic free radical mechanism [25] [41]. Higher substitution levels of wheat flour with *G. zambesina* caterpillar flour translated to higher quantities of unsaturated fatty acids added to the enriched wheat muffins. At 25˚C storage temperature, the highest PV was 9.83 meq O_2/kg oil. According to the East African Standard, the PV limit for refined oil is 10 meq O_2/kg oil (EAS 795:2013) [14]. However, the PV limit for refined oil according to Codex Alimentarius is 15 meq O_2/kg oil [41].

There was a significant difference ($p < 0.05$) in the predicted shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour (**Table 3**). The control wheat muffins had a shelf life of 120.0 days. Wheat muffins with 20% *G. zambesina* caterpillar flour had shorter shelf life (90 days). Generally, the shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour is reduced with an increase in substitution levels. To predict the shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour, the quality reference estimation for a maximum PV was taken to be 10 meq O_2/kg. The longer shelf life reported for the reference wheat muffins might be attributed to the low quantities of unsaturated fatty acid in *G. zambesina* caterpillar flour used in substituting wheat flour [42]. The short shelf life of 20% wheat muffins is ascribed to high amounts of unsaturated fatty acids oxidized at a 20% substitution level. Based on the findings of this study that there was no significant difference ($p < 0.05$) in shelf life among 0%, 5%, and 10% *G. zambesina* enriched wheat muffins, the bakery industry can embrace the utilization of the *G. zambesina* caterpillar flour to enrich bakery products.

Table 3. Predicted shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour.

Substitution level	Arrhenius equation	R^2	Shelf-life (days)
0%	$Y = -962.37x - 3.2398$	0.9934	120.0[a]
5%	$Y = -1424.0x - 3.3292$	0.7023	111.0[a]
10%	$Y = -1597.3x - 3.2195$	0.9234	103.0[a]
15%	$Y = -1726.7x - 3.3996$	0.9903	102.0[ab]
20%	$Y = -2344.2x - 3.3414$	0.9715	90.0[b]

Note: Values with the same letter along the column are not significantly different at $p < 0.05$.

4. Conclusion and Recommendation

Wheat muffins enriched with *G. zambesina* caterpillar flour are microbiologically safe for human consumption. Adhering to good handling and manufacturing effectively controls the growth of yeast and moulds which are the major cause of spoilage in bakery products with a high and intermediate water activity (a_w). The PV of wheat muffins enriched with *G. zambesina* caterpillar flour at room temperature (25˚C) is below the East African Standard for PV limit (10 meq O_2/kg oil). Wheat muffins enriched with *G. zambesina* caterpillar flour generally have a shelf life between 120.0 days to 90.0 days due to their good microbiological quality and PVs. This study recommends that further tests, e.g. moisture content, free fatty acidity, and para-Anisidine value (*p-AV*) should be conducted to the determination of the shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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