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Characterisation of Lacto-Fermented Cricket (*Acheta domesticus*) Flour and Its Influence on the Quality Parameters and Acrylamide Formation in Wheat Biscuits

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Abstract: The aim of this study was to evaluate the influence of different amounts (40, 80 and 100 g) of non-fermented and fermented (with *Lactiplantibacillus plantarum* No. 122 and *Lactiacaseibacillus casei* No. 210) cricket flour (Cr) on the quality characteristics and acrylamide formation in wheat biscuits (WB). The main formula for WB preparation consisted of 280 g of wheat flour, 100 g of margarine, 50 g of saccharose, 3 g of vanilla sugar, 50 g of eggs, 1.5 g of salt and 2.0 g of baking powder. It was established that the highest lactic acid bacteria (LAB) number was achieved in 48 h with No. 122 fermented Cr (11.8 log₁₀ CFU/g) and the lowest pH (4.34) was obtained after 48 h of Cr fermentation with both the tested LAB strains. The total colour differences were in the range of 17.54 to 22.08 and, in every case, fermented samples were clearly distinguished from untreated ones. Fermentation increased tyramine content in Cr (from 13.0 to 29.2 times). The main FAs in Cr were palmitic acid, stearic acid, octadec-9-enoic acid and linoleic acid. The lowest acrylamide content (84.1 µg/kg) was found in WB with 40 g of Cr fermented with No. 210. Significant differences in WB overall acceptability were not found. However, the highest intensity of emotion “happy” was elicited by WB with 80 g of Cr fermented with No. 122. Due to the demonstrated decrease of acrylamide content, fermented Cr can be considered a beneficial ingredient for the manufacture of WB.

Keywords: cricket flour; fermentation; biscuits; acrylamide; emotions; biogenic amines

1. Introduction

Insects can provide a valuable source of proteins, fatty acids, vitamins and minerals, and in many parts of the world they are culturally accepted and valued as traditional parts of the human diet [1]. Because of the problems associated with climate change, new strategies are being sought to ensure an adequate quantity of sustainable proteins in human as well as in animal diet. Additionally, dietary diversification, such as the inclusion of edible insects as an ingredient to the main food formulations, becomes very attractive and are nutritionally desired.

Crickets (*Acheta domesticus*) contain proteins [2,3], including bioactive peptides with desirable beneficial health effects to the consumers [4]. However, possible allergenic reactions, induced by insects’ proteins, is still a concern [5]. Also, insects are still considered a primitive food source in many Western countries, and the major obstacles to acceptability

and consumption of insect-based food seems to be related to anticipated consumer's aversion, neophobia and disgust, even after an initial informative session about entomophagy thought to increase the willingness to taste an insect-based product [6].

Despite that most of the consumers still do not accept insects' consumption [7], insects, as a food ingredient, are an alternative that is likely to increase consumer's acceptance [8–11]. Indeed it is reported that the inclusion of insects in the main recipe of products significantly improves their acceptability, and these changes are associated with the modification of volatile compound profiles of the products [12,13], among other technological parameters.

Biscuits are a very popular ready-to-eat food and can be a good candidate to develop innovative products enriched with insect flour(s). The global biscuit market is estimated to grow according to the projections for 2021–2026 [14], and new sorts and higher variability of these baking products becomes very attractive for consumers to fulfil their demand for broader assortment, as well as for the baking companies that are constantly on the lookout for innovation in a particularly very competitive global market.

However, biscuit-processing technology includes thermal treatment at high temperatures. During the thermal processing, the Maillard reaction, caramelization, lipid oxidation, nutrient leaching and other reaction occurs [15]. From one point of view, these reactions lead to a positive effect on the product sensory profile but, from another one, some compounds are also produced and having harmful effect on consumer's health.

Acrylamide (a toxic and carcinogenic compound) can be formed during high-temperature processes [16,17]. The latest regulation from European Commission (EC) 2017/2158 standardizes the presence of acrylamide in various food products [18]. Bakery products are one of the food categories at acrylamide risk [19].

Despite asparagine in flours being the main acrylamide precursor, resulting in biscuits with higher concentrations of this toxic compound [20], Žilić et al. [21] established the opposite: that asparagine concentration did not significantly correlate with acrylamide content in biscuits. These observations indicated that other compounds and dough properties can influence the extent of Maillard reaction and the acrylamide formation [22].

Some protein traits can influence the acrylamide content in products [23–26]. Therefore, when adding proteinaceous ingredients to the main biscuits' formulation, acrylamide concentration shall be investigated, so as to avoid the formation of high concentrations of this toxic compound in the end-products.

It was reported that some lactic acid bacteria (LAB) strains can potentially reduce non-desirable chemicals in food [27]. However, the mechanisms of acrylamide reduction have been linked to specific LAB strains and food products. In cereal products, LAB decreases pH—leading to the reduction of acrylamide levels. Finally, asparagine hydrolysis, sugar metabolism and pH reduction are the most significant indirect approaches for reducing acrylamide levels [27]. In biscuits, the addition of amino acids in the recipe prevented the formation of acrylamide by up to 97% because cysteine and glycine have the capacity to compete with asparagine for the carbonyl group reaction [28]. Additionally, the microbial approaches to reduce acrylamide formation may also greatly improve the organoleptic properties of foods.

Fermentation with selected LAB is a tool used for enhancing the bioactive compounds of the fermentable substrates [29]. In this regards, insect flour can represent an ingredient that may be able to fulfil the requirements of LAB growth [30].

The overall sensory quality of fermented products may also be dependent on the type of fermenting LAB strain [31]. During fermentation, LAB strains produce secondary metabolites that enhance organoleptic quality of food [32], because the metabolism of LAB leads to a broader spectrum of volatile compounds in fermentable substrates and better sensory properties [33]. It is important to underline that a large number of metabolites can be synthesised by microorganisms, however, not all of them are desirable.

Another important aspect is that despite LAB being considered Generally Regarded As Safe (GRAS) microorganisms, all fermented foods are subjected to the risk of biogenic

amine (BA) contamination [34]. The BAs derive from amino acid decarboxylation and can cause toxic effects on humans [34]. However, their toxic effect depends on some factors, including the type of BA, the individual sensitivity, etc. [35,36].

Based on the above, the main aim of this study was to evaluate the influence of cricket flour (Cr) on the quality characteristics and acrylamide formation in wheat biscuits. Additionally, to reduce acrylamide formation in biscuits, fermentation of cricket flour with *Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210, as a pre-treatment, was tested. Considering that during fermentation both desirable and undesirable changes in fermentable substrates can occur, the pH, LAB counts, colour coordinates, fatty acid (FA) and volatile compound (VC) profiles of the non-treated and fermented cricket flour, as well as BA concentration were analysed. The tested biscuit groups were prepared by addition to the main recipe different contents of the non-treated (non-fermented) and fermented cricket flour. The biscuit samples were subjected to analysis of colour coordinates, acrylamide concentration, volatile compounds and overall acceptability. Taking into consideration that insect flour was added as a non-traditional ingredient, emotions induced by the tested biscuit samples for judges were also evaluated.

2. Materials and Methods

2.1. Materials

Cricket flour was provided by Bugsandus Ltd. (Vilnius, Lithuania). Cricket flour composition is protein 62.6%, fat 26.5%, ash 3.8%, moisture 2.0%, saturated fatty acids 8.7%, total carbohydrates 5.1%, sugars < 0.6%, salt (sodium \times 2.5) 0.69%. LAB strains *Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210, previously isolated from spontaneous sourdough [37], were used for cricket flour fermentation. Wheat flour (type 550D, falling number 350 s, gluten 270 g/kg, ash 6.8 g/kg) was obtained from Kauno Grudai mill (Kaunas, Lithuania), margarine (fat content 80%) was obtained from Vilniaus margarine factory (Vilnius, Lithuania), and sugar, vanilla sugar, eggs, salt and baking powder (composition: sodium pyrophosphate, sodium bicarbonate, wheat flour; by weight 40/40/20, respectively) were obtained from a local supermarket and used for biscuit preparation.

The experiment was performed over two stages. A schematic representation of the experimental design is given in Figure 1. Firstly, cricket flour was fermented and the resulting samples with the most appropriate parameters were selected for biscuit preparation (*viz.*, with the lowest pH and the highest LAB counts). During the second stage, biscuits were manufactured and further analysed.

2.2. Cricket Flour (Cr) Fermentation

Before the experiments, LAB strains were incubated and multiplied in De Man, Rogosa, and Sharpe (MRS) broth culture medium (Biolife, Milano, Italy) at 30 °C under anaerobic conditions for 24 h. A total of 3 mL of fresh LAB biomass grown on MRS broth (average cell concentration of 8.6 log₁₀ CFU/mL) was inoculated in 100 g of cricket flour/water mass (cricket flour/water ratio of 1:1, *w/w*). Afterwards, the cricket flour was fermented in a chamber incubator (Mettler GmbH Co. KG, Schwabach, Germany) for 24 and 48 h, at 30 °C. Non-fermented cricket flour was analysed as control.

2.3. Analyses of Cricket Flour (Cr)

The pH of samples was evaluated with a pH meter (Inolab 3, Hanna Instruments, Venet, Italy) by inserting the pH electrode into the cricket flour/water (1:1, *w/w*) mixture. The colour coordinates of the cricket flour were evaluated on the surface using the CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Marunouchi, Tokyo, Japan). Total colour difference (ΔE) between untreated cricket flour and fermented ones was calculated using the equation according to Foucher et al. [38]. LAB counts were determined according to the method described by Bartkiene et al. [39]. Sample preparation and determination of BAs—including tryptamine (TYR), phenylethylamine (PHE), putrescine (PUT), cadaverine

(CAV), histamine (HIS), tyramine (TYR), spermidine (SPRMD) and spermine (SPRM)—in samples was conducted following the procedure reported by Ben-Gigirey et al. [40] with some modifications described in the Supplementary File S1. Extraction of lipids for fatty acid (FA) analysis was undertaken with chloroform/methanol (2:1 *v/v*) and FA methyl esters (FAME) were prepared according to Pérez-Palacios et al. [41]. All procedures are described in detail in Supplementary File S2. The volatile compounds of non-treated (non-fermented) and fermented cricket flour samples were analysed by gas chromatography-mass spectrometry (GC-MS). All the procedures for VC determination are described in detail in Supplementary File S3.

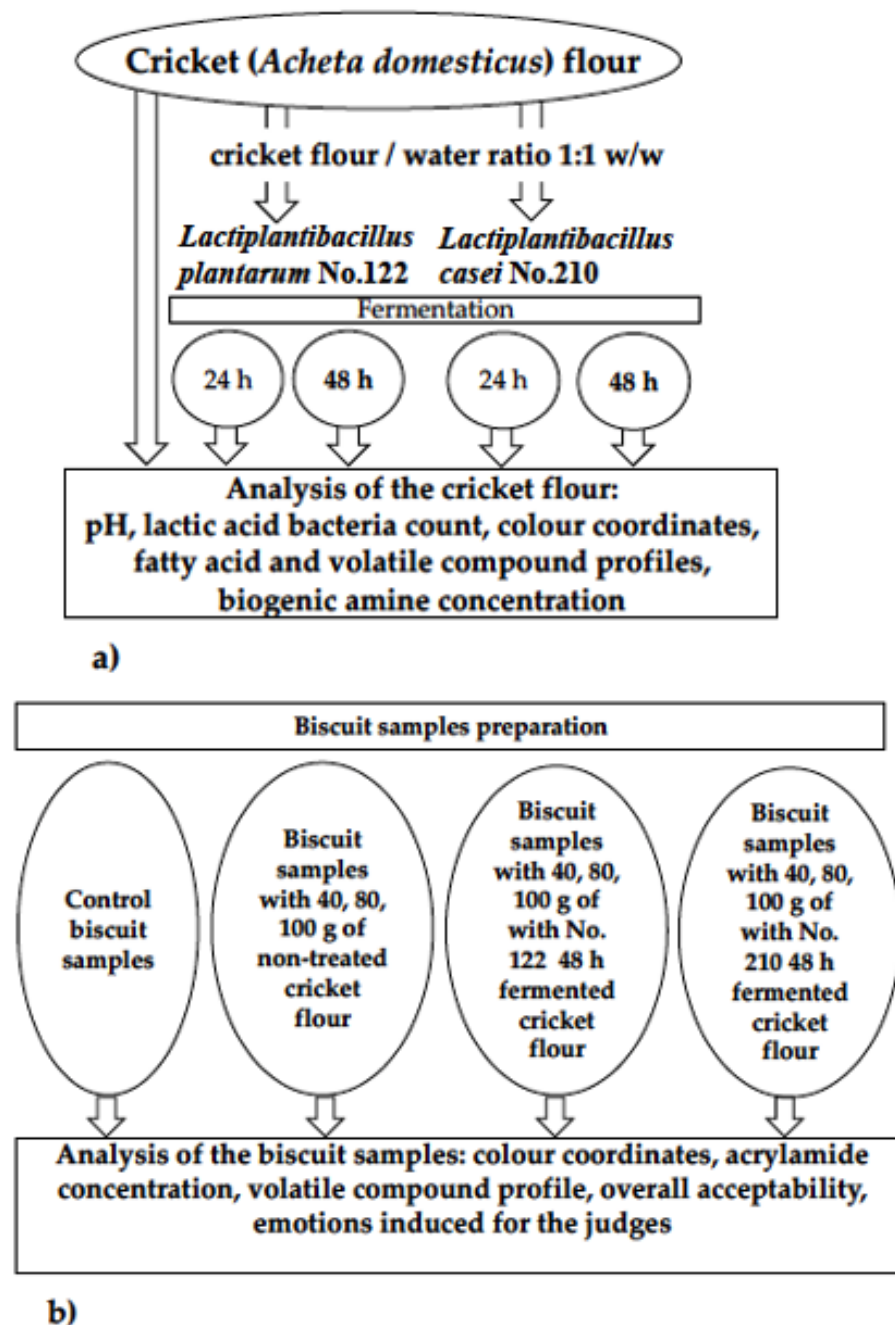


Figure 1. Schematic representation of the experimental design in this study: (a) scheme of the experiment with cricket flour; and (b) scheme of the experiment with biscuit samples.

2.4. Biscuit Formulation and Preparation

The main formula for biscuit preparation consisted in 280 g of wheat flour, 100 g of margarine, 50 g of saccharose, 3 g of vanilla sugar, 50 g of eggs, 1.5 g of salt and 2.0 g of baking powder. Biscuit samples were prepared by the addition of non-treated and fermented cricket flour (with each of LAB strains) to the main biscuit dough at levels of 40, 80 and 100 g. In total, ten biscuit dough samples were prepared: C—control (biscuit) samples, without addition of cricket flour; C-CF40, C-CF80 and C-CF100—biscuit samples with 40, 80 and 100 g of non-fermented cricket flour, respectively; C-CF40-122 and C-CF40-210, C-CF80-122 and C-CF80-210 and C-CF100-122 and C-CF100-210—biscuit samples with 40, 80 and 100 g of the fermented cricket flour with *Lactiplantibacillus plantarum* No. 122 and *Lactiacaseibacillus casei* No. 210 strains, respectively. Sugar, vanilla sugar and margarine were creamed in a mixer (Guangzhou R & M Machinery, Guangdong, China). Eggs were added to this cream and mixed for 0.5 min to obtain a homogeneous mass. Finally, flour was added and mixed for 1 min to obtain a homogeneous dough. Non-treated (non-fermented) and fermented cricket flour was added to this mass. Biscuits were formed manually by rolling (the thickness of the dough was 3.0 mm) and stamping was carried out manually. Biscuits were baked in a deck oven (MIWE, Michael Wenz, Germany) at 220 °C for 7 min.

2.5. Analysis of Biscuit Samples

Biscuit colour parameters were evaluated using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Japan) [42]. The total colour difference (ΔE) between control biscuits and those with cricket flour was calculated as mentioned in Section 2.3. The acrylamide concentration was determined according to the method of Zhang et al. [43] with modification (Supplementary File S4). The volatile compounds of biscuit samples were analysed by GC-MS. All procedures for VC determination are described in detail in Supplementary File S3.

The overall acceptability of biscuits was tested by a panel formed by 10 trained judges using a 10-point Likert scale ranging from 0 (extremely disliked) to 10 (extremely liked). Additionally, biscuit samples were tested by applying *FaceReader 8* software (Noldus Information Technology, Wageningen, The Netherlands), scaling the 8 emotion patterns (neutral, happy, sad, angry, surprised, scared, disgusted and contempt). The judges were asked to taste the biscuit samples one by one in front of a Microsoft LifeCam Studio webcam (Microsoft Corporation, Redmond, Washington, USA). Recordings were analysed with *FaceReader 8* software and the intensity of facial expressions was expressed in a scale from 0 to 1. Judges were asked to rinse their mouths with water between samples.

2.6. Statistical Analysis

For cricket flour and biscuit data interpretation, results are expressed as mean values \pm standard error (SE). Regarding sampling dimensions (n), for cricket flour and biscuit samples physicochemical parameters, $n = 6$, and for biscuits overall acceptability and emotions induced for trained judge panel, $n = 10$. Two parallel fermentations of cricket flour were performed, and from each sample group three replicates were analysed. Also, biscuit preparation was repeated 2 times, and from each group three samples were taken for analysis. In order to evaluate the effects of fermentation and different amounts of cricket flour on biscuit quality parameters, data were analysed by one-way ANOVA and Tukey-HSD tests as post-hoc tests (using statistical package SPSS for Windows (Ver.15.0, SPSS, Chicago, IL, USA). In addition, Pearson correlations were calculated between various parameters. The results were recognised as statistically significant at $p \leq 0.05$.

3. Results and Discussion

3.1. pH, Colour Coordinates (a^* , b^* and L^*) and Lactic Acid Bacteria (LAB) Counts in Non-Treated and Fermented Cricket Flour (Cr)

The pH values, colour coordinates and LAB counts in fermented and non-treated cricket flour are given in Table 1. When comparing the pH of cricket flour after 24 and

48 h of fermentation with the control samples, it was possible to observe that after 24 h of fermentation the pH of cricket flour decreased on by average 13.3 and 15.5%, and after 48 h of fermentation it decreased on average by 28.3 and 25.6%, with *Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210 strains, respectively. Significantly higher (on average 15.8% higher) LAB counts were detected in 48 h fermented samples, in comparison with 24 h fermented samples. It was reported that edible insects (rich in proteins) can be more difficult to degrade by using the same fermentation technologies as for carbohydrate-rich sources [44]. However, acidification potentials and growth capabilities are the most important parameters for a microbial starter to be used in sourdough fermentation [45], and were applied in the current study. Galli et al. [45] stated that LAB strains *Lp. plantarum* CR L1 and *Latilactobacillus curvatus* CR L13 were suitable starter cultures for sourdough fortified with 20% of cricket powder. The drop in pH of insect flour during fermentation is mainly influenced by the organic acid production [46]. Moreover, higher ash content in the insect flour can lead to an higher buffering capacity of insect-based sourdough—thus influencing the pH drop [45]. In addition, the higher number of LABs after 48 h of fermentation, in comparison with 24 h fermented samples, can be explained by the increase of fermentation by itself and (as a cause and/or consequence) the deeper substrate degradation and higher concentration of free nutritive compounds released to the substrate medium to be further used for LAB growth, including free amino acids. This study showed that the used LAB strains are undoubtedly suitable for cricket flour fermentation, though further studies are needed to indicate more detailed changes in the chemical profile of cricket flour during the fermentation.

Table 1. pH, lactic acid bacteria (LAB) counts, colour coordinates and total colour difference (ΔE) of fermented and non-treated cricket flour (Cr).

Samples	pH	LAB Counts, log ₁₀ CFU/g	Colour Coordinates			ΔE
			L*	a*	b*	
CoCr	5.94 ± 0.43 c	na	53.4 ± 1.9 b	2.25 ± 0.13 a	15.7 ± 1.2 b	—
24 h Cr ₁₂₂	5.15 ± 0.03 b	11.6 ± 0.90 b	35.1 ± 1.28 a	2.20 ± 0.12 a	7.13 ± 0.32 a	18.75 ± 0.24 a
48 h Cr ₁₂₂	4.26 ± 0.04 a	11.8 ± 1.0 b	36.3 ± 1.12 a	2.12 ± 0.17 a	8.24 ± 0.41 a	17.54 ± 0.37 a
24 h Cr ₂₁₀	5.02 ± 0.04 b	8.29 ± 0.63 a	32.6 ± 1.93 a	1.95 ± 0.16 a	6.99 ± 0.41 a	22.08 ± 0.79 b
48 h Cr ₂₁₀	4.42 ± 0.62 a	8.44 ± 0.71 a	32.7 ± 2.13 a	1.97 ± 0.13 a	8.11 ± 0.39 a	21.94 ± 0.89 b

Co—control; Cr—cricket flour; 122—cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 strain; 210—cricket flour fermented with *Lacticaseibacillus casei* No. 210 strain; LAB—lactic acid bacteria; 24 h and 48 h—duration of fermentation; CFU—colony-forming units; L* lightness; a* redness or—a* greenness; b* yellowness or—b* blueness; na—not analysed; NBS—National Bureau of Standards units; ΔE —total colour difference, calculated with the control cricket flour sample as reference. The data are expressed as mean values ($n = 6$) ± SE; SE—standard error. a,b Mean values between CoCr, Cr₁₂₂, and Cr₂₁₀ samples with different letters are significantly different ($p \leq 0.05$).

Regarding colour coordinates, statistically significant differences after 24 and 48 h of fermentation were not established. However, comparing non-fermented and fermented samples, fermented samples showed, in all the cases, lower L* values (on average 35.9%). Additionally, fermented samples showed lower b* values, in comparison with non-fermented ones (on average 25.0% lower in fermented samples with *Lp. plantarum* No. 122 strain; on average 46.3% lower on samples fermented with *Lc. casei* No. 210 strain). These changes can be theoretically explained by the degradation of insect flour particles and the release of pigments from the walls, causing the reduction of L* values. Furthermore, pigments can be degraded during fermentation with the consequent reduction of substrate yellowness. Values of total colour difference (ΔE) were in the range of 17.54 to 22.08 and, in every case, clearly distinguished fermented samples from untreated ones. Higher values of ΔE were found for cricket flour fermented with *Lc. casei* No. 210, although these values were similar between different durations of fermentation. However, correlations between pH and colour characteristics of samples were not established. Statistical tests of between-subjects effects showed that LAB strain used for fermentation and the interaction effect

of analysed factors were significant for a* and b* values of cricket samples ($p = 0.010$ and $p \leq 0.0001$, respectively), as well as the effect of duration of fermentation on L*, a* and b* values ($p \leq 0.0001$).

3.2. Biogenic Amine (BA) Concentration in Non-Treated and Fermented Cricket Flour (Cr)

Biogenic amine concentrations in non-treated and fermented cricket flour are displayed in Table 2. The highest tryptamine content was found in non-fermented and 48 h fermented formulations with *Lc. casei* No. 210 cricket flour (on average 43.4 mg/kg). Other samples showed values from 53.5 (48 h fermented samples with *Lp. plantarum* No. 122) to 35.0% (24 h fermented samples with both tested LAB strains) of lower tryptamine concentration. Phenylethylamine content decreased (on average 30.9%) in cricket flour after 24 h of fermentation with both LAB strains, in comparison with control samples. However, opposite trends were found after 48 h of fermentation, and the highest phenylethylamine concentration was attained in 48 h cricket flour fermented with *Lc. casei* No. 210 (50.2 mg/kg). After 48 h of fermentation, samples showed lower putrescine content in comparison with non-fermented and 24 h fermented samples. Moreover, during fermentation, cadaverine content showed a tendency to decrease, and after 48 h of fermentation cadaverine in cricket flour was absent. Histamine was not found in either (non-treated and fermented) sample; however, a significant increase of tyramine content was observed during fermentation (from 13.0 times in 24 h fermented samples with *Lp. plantarum* No. 122, to on average 29.2 times in 24 and 48 h fermented cricket flour with *Lc. casei* No. 210). Unlike tyramine, fermentation reduced spermidine and spermine concentrations in cricket flour, on average by 2.21 and 1.96 times, respectively. A positive moderate correlation between LAB counts and phenylethylamine concentration was observed ($r = 0.679$, $p = 0.005$), as well as negative strong and moderate correlations between LAB counts and putrescine and cadaverine concentrations ($r = -0.845$, $p \leq 0.0001$ and $r = -0.672$, $p = 0.006$, respectively).

Table 2. Biogenic amine (BA) concentration (mg/kg) in non-treated and fermented cricket flour (Cr).

Biogenic Amine Concentration, mg/kg	Samples				
	CoCr	Cr ₁₂₂			Cr ₂₁₀
	Duration of Fermentation, h				
	0	24	48	24	48
Tryptamine (TRP)	45.87 ± 4.75 c	27.31 ± 3.55 b	20.16 ± 1.99 a	29.11 ± 5.51 b	40.83 ± 5.56 c
Phenylethylamine (PHE)	30.16 ± 2.25 b	20.67 ± 2.25 a	40.76 ± 2.85 c	21.03 ± 2.58 a	50.17 ± 6.09 d
Putrescine (PUT)	40.77 ± 7.08 c	46.22 ± 7.66 c	15.15 ± 2.38 a	56.98 ± 6.13 c	23.44 ± 2.98 b
Cadaverine (CAV)	69.34 ± 5.76 c	35.83 ± 3.91 b	nd	21.89 ± 1.37 a	nd
Histamine (HIS)	nd	nd	nd	nd	nd
Tyramine (TYR)	15.4 ± 1.70 a	199.56 ± 23.2 b	291.03 ± 42.5 c	455.01 ± 35.4 d	443.87 ± 23.0 d
Spermidine (SPRMD)	284.72 ± 37.6 b	138.32 ± 11.5 a	150.31 ± 18.7 a	116.43 ± 11.7 a	111.02 ± 19.6 a
Spermine (SPRM)	307.19 ± 27.8 b	161.68 ± 15.6 a	172.21 ± 24.4 a	146.02 ± 17.4 a	147.91 ± 19.6 a

Co—control; Cr—cricket flour; 122—cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 strain; 210—cricket flour fermented with *Lactocaseibacillus casei* No. 210 strain. The data are expressed as mean values ($n = 6$) ± SE; SE—standard error; nd—not determined. a–d Mean values between samples within a column with different letters are significantly different ($p \leq 0.05$).

Histamine and cadaverine are formed during histidine and lysine decarboxylation. Tyramine is formed from tyrosine, and the latter can react with phenylalanine to obtain 2-phenylethylamine [34]. Additionally, putrescine can be formed during the decarboxylation of ornithine [47]. Putrescine formation is also explained by agmatine conversion to urea and putrescine, or by agmatine transformation [48]. Accumulation of spermine and spermidine proceeds through complex pathways, starting from putrescine released from ornithine or agmatine [49,50]. Decarboxylase activity by LAB can induce tyramine production [51,52]. Moreover, the ability to produce histamine, cadaverine and putrescine by LAB has also been reported [48,53,54]. However, the characteristic production of BA

is generally strain-specific [34]. A statistical test of between-subjects effects showed that the LAB strain used for fermentation was a significant factor on phenylethylamine, putrescine, tyramine and spermidine contents in cricket flour ($p = 0.037$; $p = 0.016$; $p \leq 0.0001$; $p = 0.037$, respectively). Duration of fermentation was also a statistically significant factor influencing phenylethylamine, putrescine, cadaverine and tyramine concentrations in samples ($p \leq 0.0001$; $p \leq 0.0001$; $p = 0.009$; $p = 0.036$, respectively). Likewise, the interaction between the LAB strain used for fermentation and process duration was statistically significant for tryptamine, phenylethylamine and tyramine concentrations ($p = 0.026$; $p \leq 0.05$; $p = 0.011$, respectively).

3.3. Fatty Acid (FA) Composition in Cricket Flour (Cr)

Fatty acid (FA) composition (percentage of total fat content) in cricket flour is tabulated in Table 3. The main FAs in cricket flour were palmitic acid (C16:0), stearic acid (C18:0), octadec-9-enoic acid (C18:1) and linoleic acid (C18:2). Palmitoleic acid (C16:1) was only found in non-fermented samples, i.e., those without pre-treatment. Additionally, the lowest α -linolenic acid (C18:3 α) content was obtained in non-treated samples.

Table 3. Fatty acid (FA) composition (percentage of total fat content) in cricket flour (Cr).

Fatty Acid Composition, %	Samples				
	CoCr	Cr ₁₂₂			Cr ₂₁₀
	Duration of Fermentation, h				
	0	24	48	24	48
C16:0	26.5 ± 0.21 b	26.8 ± 0.29 b	24.6 ± 0.42 a	24.3 ± 0.12 a	24.7 ± 0.78 a
C16:1	0.138 ± 0.010	nd	nd	nd	nd
C18:0	8.72 ± 0.20 b	8.40 ± 0.29 b	7.81 ± 0.10 a	7.31 ± 0.32 a	7.65 ± 0.72 a,b
C18:1 <i>cis, trans</i>	27.4 ± 0.11 a	28.9 ± 0.17 b	30.5 ± 0.28 c	31.7 ± 0.53 d	30.0 ± 0.27 c
C18:2	35.7 ± 0.31 c	33.8 ± 0.94 a	35.2 ± 0.30 a	33.9 ± 0.18 a	34.7 ± 0.33 b
C18:3 α	1.52 ± 0.02 a	1.99 ± 0.02 b	1.92 ± 0.05 b	2.71 ± 0.19 c	2.98 ± 0.16 c

C16:0—palmitic acid; C16:1—palmitoleic acid; C18:0—stearic acid; C18:1 *cis, trans*—*cis, trans* octadec-9-enoic acid; C18:2—linoleic acid; C18:3 α — α -linolenic acid; Co—control; Cr—cricket flour; 122—cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 strain; 210—cricket flour fermented with *Lactiplantibacillus casei* No. 210 strain. The data are expressed as mean values ($n = 6$) ± SE; SE—standard error; nd—not determined. a–d Mean values between samples within a line with different letters are significantly different ($p \leq 0.05$).

Linoleic, oleic and palmitic acids are predominant in insect flours [55]. Williams et al. [56] reported that most insects contain linoleic, oleic, palmitic and linolenic acids but FA profile can vary with different insects, stages of development, origin and species. Unlike our study, Castro-López et al. [57] reported that fermentation increases FA content in insect flour, and Ogawa et al. [58] explained that LAB could perform different FA transformations through isomerisation, hydration, dehydration and saturation. Additionally, some LABs may have lipolytic activity [59]. However, in our study, the analysed factors (LAB strain used for fermentation and fermentation duration) as well as their interactions did not significantly impact FA content in cricket flour.

3.4. Volatile Compound (VC) Profile in Cricket Flour (Cr)

The volatile compound profile of non-treated and fermented cricket flour is given in Supplementary Table S1. Main volatile compounds in cricket flour are shown in Table 4. The highest acetic acid content in cricket flour was obtained in non-treated and 24 h fermentation with *Lc. casei* No. 210 strain samples (on average, 18.7% from the total volatile compound content). Other tested samples showed, on average, 2.43 times lower acetic acid content. It was reported that acetic acid is the main volatile organic acid in cricket (*Acheta domesticus*) digesta, because of the specificity of their feeding, which includes both fermentable and non-fermentable saccharides [60]. Acetic acid possesses a pungent, sour, overripe, fruit odour. Acetoin was detected just in 24 h fermented samples (with both LAB

strains), however, 24 h fermented samples with *Lp. plantarum* No. 122 strain showed, on average, 3.58 times higher acetoin content in comparison with 24 h fermented samples with *Lc. casei* No. 210 strain. Duration of fermentation was a statistically significant factor affecting acetoin content in cricket flour ($p = 0.013$). Acetoin is excreted by many microorganisms, including LAB strains, via the Embden–Meyerhof pathway [61]. Acetoin is strongly odorous [62] and possesses sweet, buttery, creamy, dairy, milky and fatty notes.

Table 4. Volatile compounds (VC) (percentage of total volatile compound content) in non-treated and fermented cricket flour (Cr).

Retention Time (RT), min	Volatile Compounds	Samples				
		CoCr	Cr ₁₂₂			Cr ₂₁₀
		Duration of Fermentation, h				
		0	24	48	24	48
2.315	Acetic acid	17.7 ± 2.39 b	6.82 ± 1.39 a	7.76 ± 1.19 a	19.6 ± 2.01 b	8.48 ± 1.26 a
3.754	Acetoin	nd	28.3 ± 5.44 b	nd	7.90 ± 0.71 a	nd
5.926	Hexanal	15.7 ± 2.33 c	3.46 ± 0.60 a	nd	5.98 ± 0.92 b	nd
7.861	3-Methylbutanoic acid	nd	23.6 ± 4.53 d	7.52 ± 0.96 b	14.2 ± 2.95 c	3.99 ± 0.62 a
8.089	1-Hexanol	nd	4.09 ± 0.91 a	nd	9.35 ± 1.01 b	nd
8.765	2-Heptanone	6.18 ± 1.06 b	2.45 ± 0.49 a	35.4 ± 4.82 d	2.10 ± 0.23 a	17.5 ± 2.51 c
12.493	Decane	28.1 ± 5.65 d	7.34 ± 0.78 a	13.3 ± 1.28 b	10.4 ± 1.20 b	16.3 ± 1.50 c
18.967	Dodecane	9.75 ± 1.88 b	2.87 ± 0.52 a	4.10 ± 0.75 a	4.32 ± 0.82 a	5.05 ± 0.97 a

Co—control; Cr –cricket flour; 122 –cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 strain; 210—cricket flour fermented with *Lacticaseibacillus casei* No. 210 strain; RT—retention time. The data are expressed as mean values ($n = 6$) ± SE; SE—standard error; nd—not determined. a–d Mean values between samples within a column with different letters are significantly different ($p \leq 0.05$).

Hexanal was found in non-treated and 24 h fermented samples; however, after 48 h of fermentation this volatile compound was not perceived. Hexanal is greatly responsible for the occurrence of an off flavour, which is known as beany flavour [63]. This volatile compound can be formed during enzymatic process where FAs are degraded by lipoxygenase [64], and fermentation with LAB is a well-known technology to reduce the concentration of this volatile compound with the intention to improve sensory characteristics of fermentable substrates [65].

In opposite to hexanal, 3-methylbutanoic acid was just found in fermented samples; however, this compound’s concentration was reduced with an increasing duration of fermentation (3.14 times on average in fermented group with *Lp. plantarum* No. 122 strain, and 3.56 times on average in fermented group with *Lc. casei* No. 210 strain). It was reported that 3-methylbutanoic acid was found in wheat bread made from sourdough fermented with *Lp. plantarum* [66]. The 3-methylbutanoic acid odour is described as sour and sweaty [67]. Additionally, it was reported that 3-methylbutanoic acid could be formed from the oxidation of 3-methylbutanal [68].

The compound 1-hexanol was only found in 24 h fermented samples. Moreover, the LAB strain used for fermentation, fermentation duration as well as interaction between these factors were proved to be statistically significant factors on the content of 1-hexanol in samples. 1-Hexanol can be originated from the reduction of corresponding aldehydes [69]. This volatile compound odour is described as pungent, ethereal, fusel oil, fruity and alcoholic, and sweet with a green top note.

The highest 2-heptanone content was formed in 48 h fermented cricket flour samples. Comparing 2-heptanone content in samples fermented with different LAB strains, a higher content of 2-heptanone was found in samples fermented for 48 h with *Lp. plantarum* No. 122 strain (on average 2.02 times higher), and fermentation duration was a statistically significant factor on the content of this volatile compound in cricket flour ($p = 0.013$). 2-Heptanone possesses a fruity, cinnamon, intense green, fruity and aldehydic odour [55].

In all cases, fermentation showed a tendency to decrease decane and dodecane contents in samples. Furthermore, Rasi et al. [70] reported a close relationship between the *Lactobacillus* genus and lipid metabolism; in line with this, the formation of volatile compounds such as aldehydes (hexanal, heptanal and octanal), ketones and alcohols can also be a result of lipid oxidation by lipoxygenase enzymes [71]. Yet, volatile compounds can be formed during amino acid transamination, deamination, decarboxylation and side chain modifications, which end with the development of alcohols, aldehydes and organic acids [72].



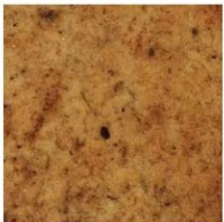
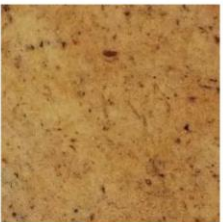
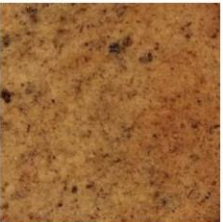
3.5. Colour Coordinates (a^* , b^* and L^*) and Acrylamide Concentration in Biscuit Samples

Colour coordinates (a^* , b^* and L^*), ΔE and acrylamide concentration in biscuit samples are given in Table 5. In all cases, the addition of cricket flour to the main biscuit formulation reduces their lightness (L^*), redness (a^*) and yellowness (b^*), in comparison with the control. Values of ΔE were in the range of 7.39 to 22.44 and, in every case, clearly distinguished biscuits with cricket flour from control biscuits. The highest values of ΔE were found in biscuit samples with 80 and 100 g of non-fermented cricket flour, while the lowest were found in biscuits with 40 g of cricket flour fermented with *Lp. plantarum* No. 122 and *Lc. casei* No. 210. On the other hand, the highest acrylamide concentration was found in control samples (302.4 $\mu\text{g}/\text{kg}$). Acrylamide content in samples showed a positive weak correlation with a^* coordinates ($r = 0.369$, $p = 0.045$), although correlations between acrylamide content in biscuits and L^* and b^* coordinates were not statistically found. Cricket flour content was a significant factor on a^* coordinate and acrylamide concentration in biscuit samples ($p = 0.022$ and $p \leq 0.001$, respectively). Comparison of acrylamide content in sample groups prepared with 40 g of non-treated and fermented cricket flour led us to conclude that the lowest acrylamide content was found in biscuits prepared with 40 g of cricket flour fermented with *Lc. casei* No. 210 strain, chiefly; in comparison with biscuits prepared with non-treated cricket flour, it was on average 76.2% lower, and, in comparison with biscuits prepared with 40 g of cricket flour fermented with *Lp. plantarum* No. 122 strain, it was on average 128.3% lower. Regarding acrylamide content in sample groups prepared with 80 g of non-treated and fermented cricket flour, biscuits prepared with the later showed on average 14.6% lower acrylamide concentration. Likewise, similar tendencies were established in sample groups prepared with 100 g of non-treated and fermented cricket flour; nevertheless, significantly lower acrylamide content was found only in biscuits prepared with 100 g of cricket flour fermented with *Lp. plantarum* No. 122 strain (on average 15.7% lower).

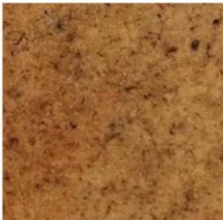




Despite the reaction of asparagine with reducing sugars being the main mechanism for acrylamide formation in cereal products, it was stated that the modelling of biscuit recipes can be a valuable strategy to reduce this contaminant concentration in the end-product [73]. Additionally, it was recognised that the pH value was the most relevant factor on the final acrylamide concentration, and the pH increasing was responsible for an acceleration of the reaction between asparagine and the reducing sugars, followed by the baking time/temperature parameters. González-Gómez et al. [74] conveyed that alternative formulations of innovative insect-based crackers were needed in their design to reduce exposure to acrylamide. Our study showed that the lowest acrylamide content was formed in biscuit samples prepared with 40 g of cricket flour fermented with *Lc. casei* No. 210 strain. EC Regulation (2017/2158) [18] reduced the acrylamide reference level from 500 to 350 $\mu\text{g}/\text{kg}$, and all biscuit groups in this study had an acrylamide concentration below this regulatory value. Furthermore, all biscuit sample groups prepared with cricket flour (non-treated and fermented) showed lower acrylamide concentrations in comparison with the control group.

Table 5. Colour coordinates (a*, b* and L*), total colour difference (ΔE), acrylamide concentration in biscuit samples, and images of biscuit samples.

Biscuit Samples	Colour Coordinates, NBS			ΔE	Acrylamide Content, μg/kg
	L*	a*	b*		
C	67.8 ± 2.22 f	9.06 ± 1.31 d	28.4 ± 0.31 e		302.4 ± 24.3 e
C-CF40	58.3 ± 0.52 c	4.57 ± 0.11 a	19.8 ± 0.37 a	13.58 ± 0.27 b,c	148.2 ± 11.9 b
C-CF40-122	60.2 ± 0.40 d	7.47 ± 0.53 c	22.7 ± 0.19 c,d	9.63 ± 0.38 a	192.0 ± 13.5 d
C-CF40-210	67.0 ± 0.63 f	4.82 ± 0.27 a	22.4 ± 0.22 c	7.39 ± 0.43 a	84.1 ± 7.20 a
C-CF80	46.1 ± 0.39 a	7.23 ± 0.38 c	23.0 ± 0.24 d	22.44 ± 0.61 d	165.4 ± 12.3 c
C-CF80-122	59.9 ± 0.88 d	6.38 ± 0.46 b	21.0 ± 0.37 b	11.15 ± 0.29 b	140.3 ± 11.0 b
C-CF80-210	61.3 ± 0.36 e	4.60 ± 0.24 a	19.9 ± 0.37 a	11.59 ± 0.57 b	142.1 ± 10.9 b
C-CF100	47.6 ± 1.13 a	6.05 ± 0.43 b	22.3 ± 0.19 c	21.31 ± 0.47 d	172.0 ± 14.6 c
C-CF100-122	58.9 ± 0.28 c	5.84 ± 0.39 b	20.1 ± 0.25 a	12.59 ± 0.14 b	145.6 ± 13.8 b
C-CF100-210	55.9 ± 1.13 b	6.44 ± 0.42 b	19.8 ± 0.14 a	14.91 ± 0.21 c	156.5 ± 14.6 b,c

C
C-CF-40
C-CF40-122
C-CF40-210
C-CF80

C-CF80-122
C-CF80-210
C-CF100
C-CF100-122
C-CF100-210

C—control; C-CF40, C-CF80, C-CF100—biscuit samples with 40, 80 and 100 g of non-fermented cricket flour; C-CF40-122, C-CF80-122, C-CF100-122—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122; C-CF40-210, C-CF80-210, C-CF100-210—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lacticaseibacillus casei* No. 210. L* lightness; a* redness or –a* greenness; b* yellowness or –b* blueness; NBS—National Bureau of Standards units; ΔE—total colour difference, calculated with the control biscuit sample as reference. The data are expressed as mean values (n = 6) ± SE; SE—standard error. a–f Mean values between samples within a column with different letters are significantly different (p ≤ 0.05).

3.6. Volatile Compound (VC) Formation in Biscuits and Their Relationship with Acrylamide Content

The volatile compound profile in biscuit samples is given in Table 6. The main volatile compound in biscuit samples was sorbic acid. This finding can be explained by the high margarine content in biscuit recipe, which can be a source of sorbic acid. Vanillin was also identified in all the tested biscuit samples. However, correlations between contents of these volatile compounds and acrylamide concentration in biscuit samples were not found. In all cases, a higher content of benzaldehyde was observed in biscuit groups prepared with cricket flour, and a positive moderate correlation between acrylamide and benzaldehyde content in biscuit samples was established (r = 0.554, p ≤ 0.001). The amount of cricket flour was a significant factor on benzaldehyde content in biscuits (p = 0.002).

Table 6. Volatile compound (VC) profile of the biscuit samples (percentage of total volatile compound content).

Volatile Compound	RT (min)	C	C-CF40	C-CF40-122	C-CF40-210	C-CF80	C-CF80-122	C-CF80-210	C-CF100	C-CF100-122	C-CF100-210
Benzaldehyde	11.8	0.98 ± 0.025 a	1.65 ± 0.19 b	1.84 ± 0.17 c	1.67 ± 0.17 b	1.51 ± 0.14 b	1.93 ± 0.18 c	1.76 ± 0.16 b	1.77 ± 0.18 b	1.66 ± 0.15 b	2.01 ± 0.19 c
Hexanoic acid	12.51	1.1 ± 0.12 a	2.71 ± 0.24 b	2.46 ± 0.23 b	2.81 ± 0.28 b	2.87 ± 0.27 b	3.21 ± 0.29 c	3.46 ± 0.32 c	3.41 ± 0.32 c	3.74 ± 0.29 d	3.21 ± 0.31 c
D-Limonene	14.09	0.099 ± 0.011 a	nd	1.52 ± 0.14 d	0.219 ± 0.019 b	0.235 ± 0.022 b	0.196 ± 0.18 a	0.578 ± 0.042 c	2.23 ± 0.21 e	2.36 ± 0.19 e	0.304 ± 0.28 a
Phenylacetaldehyde	14.53	1.76 ± 0.016 a	2.62 ± 0.25 b	2.01 ± 0.18 a	2.43 ± 0.23 b	4.11 ± 0.39 c	2.61 ± 0.25 b	2.78 ± 0.26 b	1.71 ± 0.16 a	1.9 ± 0.17 a	2.39 ± 0.22 b
(E)-2-Octen-1-al	15	0.283 ± 0.03 d	0.225 ± 0.021 c	0.183 ± 0.014 b	0.189 ± 0.017 b	0.155 ± 0.014 a	0.219 ± 0.02 c	0.275 ± 0.024 d	0.3 ± 0.022 d	0.3 ± 0.026 d	0.223 ± 0.21 c
Sorbic acid	16.36	86.3 ± 9.32 a	81.1 ± 7.85 a	81.3 ± 6.98 a	81.7 ± 7.82 a	81.3 ± 7.52 a	81 ± 6.89 a	79.9 ± 7.81 a	79.6 ± 7.67 a	78.3 ± 7.58 a	78.6 ± 7.45 a
Nonanal	16.44	0.193 ± 0.023 a	0.921 ± 0.052 d	0.76 ± 0.32 c	0.748 ± 0.041 c	0.771 ± 0.038 c	0.806 ± 0.051 c	0.832 ± 0.059 d	0.651 ± 0.041 b	0.733 ± 0.028 c	0.73 ± 0.38 c
Non-(2E)-enal	18.16	0.351 ± 0.04 b	0.321 ± 0.029 a	0.375 ± 0.18 c	0.423 ± 0.036 c	0.4 ± 0.027 c	0.476 ± 0.036 d	0.483 ± 0.03 d	0.396 ± 0.021 c	0.439 ± 0.028 c	0.473 ± 0.031 d
Octanoic acid	18.68	4.27 ± 0.32 a	4.89 ± 0.41 a	4.7 ± 0.43 a	4.85 ± 0.35 a	4.52 ± 0.41 a	4.56 ± 0.36 a	4.6 ± 0.28 a	4.52 ± 0.42 a	4.71 ± 0.31 a	4.82 ± 0.027 a
Dodecane	19.36	1.12 ± 0.25 b	0.942 ± 0.036 b	1.04 ± 0.09 b	1.22 ± 0.12 c	1.2 ± 0.11 c	1.08 ± 0.09 c	0.854 ± 0.041 a	1.01 ± 0.1 b	1.46 ± 0.15 d	1.22 ± 0.11 c
Decanal	19.52	0.226 ± 0.018 b	0.238 ± 0.021 b	0.266 ± 0.022 b	0.204 ± 0.018 a	0.19 ± 0.018 a	0.239 ± 0.018 b	0.243 ± 0.022 b	0.221 ± 0.019 a	0.251 ± 0.022 b	0.344 ± 0.018 c
4,6-dimethyldodecane	21.68	0.34 ± 0.026 f	0.347 ± 0.031 f	0.302 ± 0.027 f	0.082 ± 0.008 b	0.03 ± 0.004 a	0.184 ± 0.017 d	0.299 ± 0.019 f	0.253 ± 0.023 e	0.219 ± 0.019 e	0.152 ± 0.012 c
Undecan-2-one	22.04	0.688 ± 0.031 b	0.994 ± 0.043 e	0.825 ± 0.035 c	0.705 ± 0.025 b	0.45 ± 0.032 a	0.786 ± 0.036 c	0.8 ± 0.037 c	0.8 ± 0.041 c	0.8 ± 0.031 c	0.876 ± 0.036 d
5-hexyldihydro-2(3H)-Furanone	23.97	0.398 ± 0.022 a	0.596 ± 0.039 c	0.457 ± 0.028 b	0.45 ± 0.014 b	0.41 ± 0.028 a	0.413 ± 0.039 a	0.514 ± 0.028 b	0.499 ± 0.031 b	0.501 ± 0.045 b	0.462 ± 0.033 b
1-Tetradecene	24.69	0.267 ± 0.023 b	0.501 ± 0.034 e	0.284 ± 0.019 b	0.487 ± 0.027 e	0.52 ± 0.035 e	0.37 ± 0.022 d	0.265 ± 0.024 a	0.41 ± 0.02 d	0.4 ± 0.029 d	0.324 ± 0.027 c
Vanillin	24.96	0.746 ± 0.041 c	0.824 ± 0.027 d	0.562 ± 0.023 a	0.66 ± 0.024 b	0.6 ± 0.041 a	0.816 ± 0.032 c	1.06 ± 0.11 e	0.853 ± 0.034 d	0.829 ± 0.043 c	2.66 ± 0.025 f
Ethyl dodecanoate	29.69	0.848 ± 0.039 a	1.15 ± 0.12 b	1.15 ± 0.12 b	1.17 ± 0.11 b	1.18 ± 0.1 b	1.1 ± 0.11 b	1.22 ± 0.12 b	1.37 ± 0.14 b	1.39 ± 0.12 b	1.18 ± 0.12 b

C—control; C-CF40, C-CF80, C-CF100—biscuit samples with 40, 80 and 100 g of non-fermented cricket flour; C-CF40-122, C-CF80-122, C-CF100-122—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122; C-CF40-210, C-CF80-210, C-CF100-210—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lactocaseibacillus casei* No. 210. RT—retention time; nd—not detected. The data are expressed as mean values ($n = 6$) ± SE; SE—standard error. a–f Mean values between samples within columns with different letters are significantly different ($p \leq 0.05$).

It was reported the formation of benzaldehyde from phenylalanine [75] is generally thought to be associated with phenylacetaldehyde, the Strecker aldehyde of phenylalanine [76]. Phenylacetaldehyde in biscuit samples showed a moderate positive correlation with acrylamide concentration ($r = 0.573$, $p \leq 0.001$), and the amount of cricket flour was a significant factor on phenylacetaldehyde content in biscuits ($p \leq 0.001$).

In all cases, a higher hexanoic acid content was found in biscuits prepared with cricket flour addition. Strictly anaerobic bacteria such as *Clostridium kluyveri*, *Clostridium* spp. BS-1 and *Megasphaera elsdenii* have been described to be hexanoic acid producers [77]. Additionally, it was reported that some of LAB species produce hexanoic acid and its respective esters [78]. Additionally, hexanal—which was obtained in cricket flour (Table 4)—was possibly oxidised to hexanoic acid. Also, hexanoic acid showed negative moderate correlations with acrylamide content in biscuits ($r = -0.450$, $p = 0.013$), and the amount of cricket flour added to the main biscuit formula was a statistically significant factor on hexanoic acid concentration. This negative correlation can be explained by the antioxidant characteristics of hexanoic acid, as well as inhibitory activities against browning reaction products [79]. D-Limonene was found in most of the biscuit samples (except biscuits prepared with 40 g of non-treated cricket flour), and the highest content of D-limonene was formed in biscuits with 100 g of non-treated as well as fermented cricket flour with *Lp. plantarum* No. 122 strain (on average 2.30% of total volatile compound content). It was reported that d-limonene concentration can be increased during the LAB fermentation of cloudy apple juice made of the different apple cultivars [80]. These changes can be related with the cells breaking down during the fermentation. Conversely, D-limonene contributed little to the sweet orange odour [81].

The analysed factors were not significant for (E)-2-octen-1-al and non-(2E)-enal formation in biscuits. In contrast to nonanal, for which all the analysed factors and their interaction were statistically significant, *viz.*: quantity of cricket flour added $p \leq 0.0001$, LAB strain used for cricket flour fermentation $p = 0.009$, and interaction of factors $p \leq 0.0001$. Besides, between nonanal and acrylamide content in biscuits a weak negative correlation was established ($r = -0.380$, $p = 0.038$). Nonanal has a GRAS status [82], possesses anti-fungal properties [83] and possesses a waxy, aldehydic, citrus, with a fresh slightly green lemon peel like nuance, and a cucumber fattiness odour. However, nonanal is also a marker compound indicating the degree of lipid oxidation [84].

Octanoic acid content in biscuits encompassed on average 4.64% of total volatile compound content, and all analysed factors and their interactions were statistically significant on octanoic acid content in biscuits, *viz.* amount of the cricket flour added $p = 0.049$, LAB strain used for cricket flour fermentation $p = 0.035$, and interaction of factors $p = 0.006$. The octanoic acid odour is described as fatty, waxy, rancid, oily, vegetable and cheesy. The lowest dodecane content was obtained in biscuit samples prepared with 80 g of cricket flour fermented with *Lc. casei* No. 210 strain, and all analysed factors and their interactions were statistically significant with respect to the content of this compound in biscuits ($p \leq 0.0001$). The highest amount of decanal was found in biscuits prepared with 100 g of cricket flour fermented with *Lc. casei* No. 210 strain, and the LAB strain used for cricket flour fermentation was a significant factor on the content of this compound in biscuits ($p = 0.002$). Interaction of analysed factors was statistically significant ($p = 0.010$) for 4,6-dimethyldodecane content in biscuits. Undecan-2-one, 5-hexyldihydro-2(3H)-furanone and 1-tetradecene were found in all groups of biscuit samples, and their content was lower than 1.0% of total volatile compound content. However, in all cases, ethyl dodecanoate content was higher in biscuits with non-treated and fermented cricket flour, in comparison with the control samples. A moderate positive correlation was found between ethyl dodecanoate and acrylamide content in biscuits ($r = 0.717$, $p \leq 0.0001$). Ethyl dodecanoate enhanced black cherry aroma [85].

3.7. Overall Acceptability (OA) and Emotions Induced by Biscuit Samples in the Judge Panel

Overall acceptability (OA) and emotions induced by biscuit samples in the judge panel are tabulated in Table 7. Significant differences between biscuit overall acceptability were not established. Nevertheless, some of the emotions' intensity expressions varied. Despite the main expressed emotion being "neutral" and significant differences between this emotion induced by different biscuit sample groups not being found, the highest intensity of emotion "happy" was expressed by judges when testing biscuit samples prepared with 80 g of cricket flour fermented with *Lp. plantarum* No. 122 strain. The highest intensity of emotion "angry" was obtained when testing biscuits prepared with 40 g of non-treated and fermented cricket flour with *Lp. plantarum* No. 122 strain (on average 0.057). Moreover, the highest intensity of emotion "surprised" was induced when testing biscuits with 40 g of cricket flour fermented with *Lp. plantarum* No. 122 and *Lc. casei* No. 210, as well as with 80 g of cricket flour fermented with *Lc. casei* No. 210 (on average 0.052). The highest intensity of emotion "scared" was induced by biscuit groups prepared with 80 g of cricket flour fermented with *Lp. plantarum* No. 122 and with 100 g of cricket flour fermented *Lc. casei* No. 210 (on average 0.0016). The most intensive emotion "disgusted" was induced by biscuits prepared with 80 g on non-treated cricket flour, and the highest intensity of emotion "contempt" was induced by the group of biscuits prepared with 80 g of cricket flour fermented with *Lc. casei* No. 210. Analysed factors and their interactions were statistically significant as regards the expression intensity of the emotions "happy", "angry", "surprised", "scared" and "disgusted" (Table 8). Additionally, the analysed factors were significant regarding the expression of the emotion "sad". A correlation between overall acceptability and acrylamide concentration in biscuits was not found, neither between acrylamide content or the intensity of emotions.

Table 7. Overall acceptability (OA) and emotions induced by biscuit samples in the judge panel.

Biscuit Samples	OA	Emotions Induced by Biscuit Samples in the Judge Panel (from 0 to 1)							
		Neutral	Happy	Sad	Angry	Surprised	Scared	Disgusted	Contempt
C	7.5 ± 2.3 a	0.811 ± 0.079 a	0.036 ± 0.004 c	0.020 ± 0.002 c	0.019 ± 0.002 b,c	0.038 ± 0.004 e	0.0008 ± 0.0002 c,d	0.006 ± 0.001 b	0.0010 ± 0.0003 a
C-CF40	6.3 ± 3.1 a	0.731 ± 0.061 a	0.059 ± 0.005 d	0.064 ± 0.006 e	0.052 ± 0.005 d	0.033 ± 0.003 d,e	0.0006 ± 0.0001 b,c	0.019 ± 0.003 d	0.0010 ± 0.0002 a
C-CF40-122	9.1 ± 0.9 a	0.845 ± 0.078 a	0.041 ± 0.004 c	0.014 ± 0.002 b	0.061 ± 0.006 d	0.052 ± 0.005 f	0.0006 ± 0.0002 b,c	0.005 ± 0.001 b	0.0010 ± 0.0003 a
C-CF40-210	7.9 ± 2.5 a	0.799 ± 0.075 a	0.036 ± 0.004 c	0.008 ± 0.001 a	0.024 ± 0.002 c	0.054 ± 0.006 f	0.0006 ± 0.0001 b,c	0.007 ± 0.002 b,c	0.0020 ± 0.0004 b
C-CF80	6.5 ± 1.7 a	0.706 ± 0.069 a	0.043 ± 0.004 c	0.028 ± 0.003 d	0.015 ± 0.001 b	0.023 ± 0.002 c	0.0011 ± 0.0003 d	0.029 ± 0.003 e	0.0020 ± 0.0004 b
C-CF80-122	7.0 ± 1.9 a	0.807 ± 0.075 a	0.084 ± 0.007 e	0.032 ± 0.003 d	0.017 ± 0.002 b,c	0.028 ± 0.003 c,d	0.0017 ± 0.0002 e	0.017 ± 0.004 d	0.0020 ± 0.0005 b
C-CF80-210	6.4 ± 2.3 a	0.755 ± 0.074 a	0.020 ± 0.002 a	0.013 ± 0.001 b	0.022 ± 0.003 c	0.051 ± 0.005 f	0.0002 ± 0.0001 a	0.009 ± 0.002 b,c	0.0030 ± 0.0002 c
C-CF100	5.3 ± 2.5 a	0.825 ± 0.079 a	0.023 ± 0.003 a	0.014 ± 0.002 b	0.010 ± 0.001 a	0.012 ± 0.001 a	0.0004 ± 0.0001 a,b	0.008 ± 0.001 b,c	0.0010 ± 0.0002 a
C-CF100-122	6.3 ± 2.5 a	0.885 ± 0.081 a	0.043 ± 0.004 c	0.017 ± 0.001 b,c	0.009 ± 0.001 a	0.026 ± 0.003 c	0.0006 ± 0.0002 b	0.010 ± 0.002 c	0.0010 ± 0.0001 a
C-CF100-210	7.1 ± 2.6 a	0.863 ± 0.084 a	0.028 ± 0.003 a,b	0.009 ± 0.001 a	0.024 ± 0.002 c	0.018 ± 0.002 b	0.0015 ± 0.0003 d,e	0.002 ± 0.001 a	0.0020 ± 0.0005 b

OA—overall acceptability; C—control; C-CF40, C-CF80, C-CF100—biscuit samples with 40, 80 and 100 g of non-fermented cricket flour; C-CF40-122, C-CF80-122, C-CF100-122—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122; C-CF40-210, C-CF80-210, C-CF100-210—biscuit samples with 40, 80 and 100 g of cricket flour fermented with *Lactocaseibacillus casei* No. 210. The data are expressed as mean values ($n = 10$) ± SE; SE—standard error. a–f Mean values between samples within a column with different letters are significantly different ($p \leq 0.05$).

Table 8. Influence of analysed factors and their interaction on overall acceptability (OA) of biscuits and emotions induced by biscuit samples in the judge panel.

Parameters	Factors and Their Interaction		
	Quantity of the Cricket Flour	LAB Strain Used for Cricket Flour Fermentation	Amount of the Cricket Flour Interaction with LAB Strain Used for Cricket Flour Fermentation
Overall acceptability (OA)	0.814	0.583	0.947
Neutral	0.331	0.213	0.742
Happy	0.003	0.0001	0.0001
Sad	0.036	0.004	0.068
Angry	0.0001	0.038	0.0001
Surprised	0.001	0.013	0.027
Scared	0.005	0.043	0.0001
Disgusted	0.0001	0.0001	0.0001
Contempt	0.370	0.228	1.000

OA—overall acceptability; LAB—lactic acid bacteria. Influence of analysed factors (LAB strain used to ferment the cricket flour and amount of the fermented cricket flour) on biscuit parameters is significant when $p \leq 0.05$. Statistically significant values are marked in bold.

Lower acceptability of insect-based food is mainly related with the cultural background and sensory properties (e.g., odour, texture and colour), as well as health and food safety issues [86]. Blending edible insect powder with flour in familiar foodstuffs, where it is not visible to the consumer's eyes, has a high potential to overcome these challenges and enhance consumer's acceptance [87]. Similar to our results, Bas and El [88] replaced the wheat flour in the standard biscuit recipe with 20% of cricket (*Acheta domesticus*) flour, and this had no significant influence on the acceptance compared with the control biscuit. Biró et al. [89] found that a control oat biscuit and an oat biscuit containing 5% of cricket flour had similar overall acceptability, whereas increasing the content of cricket flour in biscuits significantly reduced liking values. In the study of Tedjakusuma et al. [90], cookies made with 5 and 10% of cricket flour had a similar overall acceptance compared to wheat cookies. Other study showed that for Mexican customers, acceptance of chocolate chip cookies supplemented with 30% of cricket flour was on par with the control sample [91]. When compared to more comprehensive models that include information on the emotions that a product elicits, models based solely on hedonic information may not have sufficient predictive power to reliably assess consumers' food choices [92]. Therefore, the inclusion of emotional data can enhance the benefits of product analysis in consumer studies. Several studies also analysed the emotional profile of baked products with crickets but using different techniques for emotion evaluation. Gurdian et al. [92] evaluated emotions with Cochran's-Q test and according to him, chocolate brownies with cricket protein evoked emotions "happy" and "satisfied", which were important indicators of overall liking for both genders. Aleman et al. [93] reported that after hearing a beneficial information statement about cricket powder, panellists' emotions of "happy" and "satisfied" dramatically dropped, while "disgusted" increased for chocolate chip cookies with 7.5 and 10% cricket powder substitute levels. In the latter study, using a check-all-that-apply (CATA) questionnaire, emotional phrases from the EsSense Profile[®] were assessed and taken into consideration for the cookies' evaluation. Pambo et al. [94] used the EmoSemio questionnaire to assess the emotions and he found out that cricket-flour-containing buns elicited more positive emotions, where "curiosity" was the most prevalent emotion.

4. Conclusions

In this study, *Lactiplantibacillus plantarum* No. 122 and *Lacticaseibacillus casei* No. 210 were applied for the fermentation of cricket flour, which was further used in different amounts to manufacture wheat biscuits. Quality characteristics of cricket flour and biscuits as well as biogenic amines and acrylamide formation were examined. According to the drop

in pH and LAB counts, cricket flour was a suitable substrate for fermentation. Fermented cricket flour had lower lightness and yellowness values, as well as a lower biogenic amine content than untreated cricket flour. Fatty acid profile was not affected by fermentation, while most volatile compounds were influenced by duration of fermentation and LAB strain on demand. Analysis of wheat biscuits with cricket flour revealed in all cases that addition of cricket flour reduced the values of lightness, redness and yellowness. All biscuit groups with cricket flour (non-treated and fermented) displayed lower acrylamide contents than the control group, with the lowest value belonging to biscuits with 40 g of cricket flour fermented with *Lc. casei* No. 210. Higher contents of benzaldehyde and hexanoic acid were detected in biscuit groups prepared with cricket flour, and a positive moderate correlation between acrylamide and benzaldehyde in biscuits was established ($r = 0.554$, $p \leq 0.001$). The overall acceptability of biscuits did not differ significantly, although the intensity of certain emotions did. The analysed factors (quantity of cricket flour and LAB strain used for cricket flour fermentation) and their interactions were statistically significant for the intensity of emotions “happy”, “angry”, “surprised”, “scared” and “disgusted”. Considering the results obtained in this research effort, one may say that fermented cricket flour can be a valuable material for supplementing wheat biscuits because of the reduction in acrylamide.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation9020153/s1>. Supplementary File S1—Analysis of biogenic amine (BA) concentration in cricket flour (Cr) samples; Supplementary File S2—Analysis of fatty acid (FA) profile in cricket flour (Cr) samples; Supplementary File S3—Analysis of volatile compound (VC) profile in cricket flour (Cr) and biscuit samples; Supplementary File S4—Analysis of acrylamide concentration in biscuits; Supplementary Table S1—Volatile compounds (VC) in non-treated and fermented cricket flour (Cr) samples.

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Abbreviations

α -Linolenic acid (C18:3 α), Biogenic amine(s) (BA), Biscuit samples with 100 g of cricket flour fermented with *Lacticaseibacillus casei* No. 210 (C-CF100-210), Biscuit samples with 100 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 (C-CF100-122), Biscuit samples with 100 g of non-fermented cricket flour (C-CF100), Biscuit samples with 40 g of cricket flour fermented with *Lacticaseibacillus casei* No. 210 (C-CF40-210), Biscuit samples with 40 g of non-fermented cricket flour (C-CF40), Biscuit samples with 40 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 (C-CF40-122), Biscuit samples with 80 g of cricket flour fermented with *Lacticaseibacillus casei* No. 210 (C-CF80-210), Biscuit samples with 80 g of cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 (C-CF80-122), Biscuit samples with 80 g of non-fermented cricket flour (C-CF80), Blueness ($-b^*$), Cadaverine (CAV), Check-all-that-apply (CATA), *cis, trans*-Octadec-9-enoic acid (C18:1 *cis, trans*), Colony forming units (CFU), Control (biscuit) samples (C), Control (Co), Cricket flour (Cr), Cricket flour fermented with *Lactiplantibacillus casei* No. 210 strain (Cr₂₁₀), Cricket flour fermented with *Lactiplantibacillus plantarum* No. 122 strain (Cr₁₂₂), De Man, Rogosa, and Sharpe (MRS), European Commission (EC), Fatty acid methyl ester(s) (FAME), Fatty acid(s) (FA), Gas chromatography-mass spectrometry (GC-MS), Generally Regarded As Safe (GRAS), Greenness ($-a^*$), Histamine (HIS), Lactic acid bacteria (LAB), *Lacticaseibacillus casei* (*Lc. casei*), *Lactiplantibacillus plantarum* (*Lp. plantarum*), *Latilactobacillus curvatus* (*Lat. curvatus*), Lightness (L^*), Linoleic acid (C18:2), National Bureau of Standards units (NBS), Not analysed (na), Not determined (nd), Overall acceptability (OA), Palmitic acid (C16:0), Palmitoleic acid (C16:1), Phenylethylamine (PHE), Putrescine (PUT), Redness (a^*), Retention time (RT), Sampling dimensions (n), Spermidine (SPRMD), Spermine (SPRM), Standard error (SE), Stearic acid (C18:0), Total colour difference (ΔE), Tryptamine (TRP), Tyramine (TYR), Volatile compound(s) (VC), Yellowness (b^*).

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