Supplementary File 1S – Analysis of biogenic amine (BA) concentration in edible cricket flour (Cr) samples

Sample preparation and determination of the biogenic amines (BAs), including tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine in samples was conducted by following the procedure reported by Ben-Gigirey et al. [19] with some modifications described below.

Briefly, the standard BA solutions were prepared by dissolving known amounts of each BAs (including internal standard) in 20 mL of deionized water. The extraction of BAs in samples (5 g) was done by using 0.4 mol/L perchloric acid. The derivatization of sample extracts and standards was performed using a dansyl chloride solution (10 mg/mL) as a reagent.

The chromatographic analyses were carried out using a Varian ProStar HPLC system (Varian Corp., Palo Alto, California, USA) with two ProStar 210 pumps, a ProStar 410 auto-sampler, a ProStar 325 UV/VIS Detector and Galaxy software (Agilent, Santa Clara, California, USA) for data processing. For the separation of amines, a Discovery ® HS C18 column (150 × 4.6 mm, 5 μm; SupelcoTM Analytical, Bellefonte, Pennsylvania, USA) was used.

The eluents were ammonium acetate (A) and acetonitrile (B) and the elution program consisted of a gradient system with a 0.8 mL/min flow-rate. The detection wavelength was set to 254 nm, the oven temperature was 40 °C and samples were injected in 20 μL aliquots.

The target compounds were identified based on their retention times (RT) in comparison to their corresponding standards.

Supplementary File S2 – Analysis of fatty acid (FA) profile in cricket flour (Cr) samples

The extraction of lipids for fatty acid (FA) analysis was done with chloroform/methanol (2:1 v/v) and FA methyl esters (FAME) were prepared according to Pérez-Palacios et al. [20].

The FA composition of the edible cricket flour samples was identified using a gas chromatograph GC -2010 Plus (Shimadzu Europa GmbH, Duisburg, Germany) equipped with Mass Spectrometer GCMS-QP2010 (Shimadzu Europa GmbH, Duisburg, Germany).

Separation was carried out on a Stabilwax-MS column (30 m length, 0.25 mmID, and 0.25 μm df) (Restek Corporation, Bellefonte, US). Mass spectrometer operated at full scan mode. Analyte was injected in split mode at 1:60 split ratio. The following parameters were used: MS ion source temperature: 240°C, MS interface temperature 240°C, helium (carrier gas) flow: 0.90 mL/min, injector: 240°C, oven temperature 50°C (4 min), 10°C/min to 110°C (1 min), 15°C/min to 160°C (2 min), 2.5°C/min to 195°C (1 min), 2°C/min to 230°C (1 min), 2°C/min to 240°C (12 min).

The individual FAME peaks were identified by comparing their retention times with FAME standards (Merck & Co., Inc., Kenilworth, NJ, USA). Composition was evaluated using corrected area normalization method.

Supplementary File S3 – Analysis of volatile compound (VC) profile in cricket flour (Cr) and biscuit samples

The volatile compounds (VC) of the edible cricket flour and biscuit samples were analysed by gas chromatography-mass spectrometry (GC-MS).

A solid phase microextraction (SPME) device with Stableflex™ fibre coated with a 50 μm PDMS-DVB-Carboxen™ layer (Supelco, USA) was used for analysis. For headspace extraction of biscuit samples, 2 g of sample, 10 mL of 1M phosphate buffer (pH = 3) and 50 μl of internal standard solution (0.939 mg/ml of valeric acid) were transferred to the 20 mL extraction vial, mixed, sealed with a polytetrafluoroethylene septum, and thermostatted at 60°C for 30 min before exposing the fibre in the headspace. For headspace extraction of cricket flour samples, 2 g of sample were transferred to the 20 mL extraction vial, mixed,

sealed with a polytetrafluoroethylene septum, and thermostatted at 60°C for 30 min before exposing the fibre in the headspace.

The fibre was exposed to the headspace of the vial for 10 min and desorbed in an injector liner for 2 min (splitless injection mode). Prepared samples were analysed with a GCMS-QP2010 (Shimadzu, Japan) gas chromatograph and mass spectrometer.

The following conditions were used for analysis: injector temperature 250°C, ion source temperature 220°C and interface temperature 260°C. Helium was used as a carrier gas at 0.65 mL/min flowrate. For separation of VC, a low polarity Rxi®-5MS column (Restek, USA) (length 30 m, coating thickness 0.25 μm, inner diameter of 0.25 mm) was used. The temperature gradient was programmed from starting at 40°C (3 min hold) to 220°C (5°C/min) up to 310°C (15°/min) (6 min hold).

The VC were identified according to mass spectrum libraries (NIST11, NIST11S, FFNSC2). For identification purposes, alkane mix (C8-C20) were analysed to obtain the retention indexes of unknown compounds.

Supplementary File S4 – Analysis of acrylamide concentration in biscuits

The acrylamide concentration was determined according to the method of Zhang et al. [24] with modifications, which are described below.

The biscuit samples were homogenized in blender (Ika A10, Germany). Two g of sample were weighed in 50 mL centrifuge tube and diluted with 20 mL of distilled/deionized water. Tube was vortexed (ZX3 Advanced VELP, Italy) briefly to mix the contents of tube for 10 min. The tube was centrifuged at 4,000 rpm for 10 min with a centrifuge (Hermle Z 306, Germany).

The 10 mL of the clarified aqueous layer solution in 15 mL centrifuge tubes was clarified with 100 μl Carrez I (85 mM K4[Fe(CN)6] × 3 H_2O) and 100 μl Carrez II (250 mM $ZnSO_4$ × 7 H_2O) solutions. The tubes were centrifuged at 4,000 rpm for 10 min. Acrylamide standard solution (30,4 μg/L). 15.2 mg of acrylamide analytical standard (99.8% purity) was weighed and dissolved in a 1000 mL volumetric flask and diluted with deionized water.

The obtained solution was diluted by pouring 2 mL of the obtained acrylamide solution into a 1000 mL measuring flask and diluted with deionized water. 3 mL of the sample supernatant (or standard solution) was derivatized in a glass tube by adding 1.5 g of potassium bromide (KBr), 1 mL of potassium bromate solution (0.1 M, $KBrO_3$) and 0.3 mL of sulfuric acid solution (50 %, H_2SO_4). The mixture was mixed in a shaker and kept for 2 h in a refrigerator (~4°C).

The derivative was neutralized by adding 250 μL of sodium thiosulphate solution (1 M, $Na_2S_2O_3$ × $5H_2O$) until the orange colour disappears. About 1.5 g of sodium chloride (NaCl) was added to the derivatization mixture and the mixture was extracted with ethyl acetate ($CH_3COOC_2H_5$) (2 × 5 mL).

The collected ethyl acetate was concentrated with a concentration system (Christ CT 02-50, Osterode, Germany) at a temperature of 40°C and reduced pressure. The solvent was evaporated and dissolved in 0.5 mL of ethyl acetate (for the standard, in a volume of 3 mL). The 100 mg of anhydrous sodium sulphate (Na_2SO_4), 20 μL of triethylamine ($(C_2H_5)_3N$) (20 μL of triethylamine in 0.5 mL of a concentrated derivatization solution) was added to the solution in 15 mL centrifuge tube, mixed and centrifuged for 10 minutes (4000 rpm).

The supernatant was analyzed by GC–ECD.A gas chromatograph (Shimadzu GC-17A, Japan) was equipped with an electron capture detector (ECD) and an integrator to measure peak areas, and a thermostatted column. Capillary column such as Rxi-5Sil MS (Restek, Germany): length 30 m; inner diameter 0.25 mm; stationary phase film thickness 0.25 μm. Working conditions: injection volume 1 μL; column temperature gradient 70°C (hold 1 min), 3°C/min to 140 (hold 0.5 min), 15°C/min to 280 (hold 4 min). Mobile phase nitrogen 18.0 cm/sec flow rate, split 3.0; injector temperature 250°C, detector temperature 260°C, detector current 2 nA.

Table S1. Volatile compounds (VC) in non-treated and fermented edible cricket flour (Cr) samples.

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
5.435	2,3-Butanediol	nd	1.40 ± 0.19 a	nd	1.66 ± 0.16 a	nd
5.926	Hexanal	15.7 ± 2.33 c	3.46 ± 0.60 a	nd	5.98 ± 0.92 b	nd
6.171	Butanoic acid	nd	nd	0.766 ± 0.181 a	nd	1.25 ± 0.23 b
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
8.048	2-methylbutanoic acid	nd	1.88 ± 0.29 c	1.11 ± 0.14 b	1.43 ± 0.26 c	0.634 ± 0.084 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
9.342	2,6-dimethylpyrazine	nd	0.310 ± 0.046 a	3.29 ± 0.45 c	1.25 ± 0.14 b	5.27 ± 1.04 d
10.512	2,2-Dimethyl-3-heptanone	nd	0.974 ± 0.137 a	0.817 ± 0.164 a	nd	1.26 ± 0.27 a
10.683	2,6-dimethyl-4-heptanol	nd	3.20 ± 0.41 d	1.17 ± 0.13 a	2.21 ± 0.29 c	1.65 ± 0.20 b
10.93	2-hydroxy-3-methylpentanoic acid methyl ester	nd	0.506 ± 0.071 a	0.532 ± 0.099 a	0.489 ± 0.041 a	1.05± 0.11 b
11.12	Benzaldehyde	nd	1.09 ± 0.11 a	1.17 ± 0.24 a	1.85 ± 0.25 b	6.05 ± 0.81 c
11.755	1-Octen-3-ol	2.29 ± 0.16 c	0.311 ± 0.051 a	0.388 ± 0.053 a	0.850 ± 0.133 b	0.389 ± 0.060 a
11.88	Phenol	nd	nd	1.53 ± 0.14 a	nd	10.4 ± 1.26 b
12.207	2-pentylfuran	2.95 ± 0.35 a	1.43 ± 0.18 a	1.52 ± 0.22 a	2.45 ± 0.24 a	1.97 ± 0.26 a
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
13.269	4-methyldecane	1.62 ± 0.31 b	0.511 ± 0.068 a	0.520 ± 0.084 a	0.572 ± 0.103 a	0.710 ± 0.069 a
13.896	Benzeneacetaldehyde	nd	0.577 ± 0.088 a	0.932 ± 0.183 b	1.41 ± 0.11 c	1.69 ± 0.14 d
14.42	3,6-dimethyldecane	2.92 ± 0.58 c	0.945 ± 0.075 a	1.37 ± 0.24 b	2.04 ± 0.43 c	1.96 ± 0.40 c
15.071	3-ethyl-2,5-dimethylpyrazine	nd	0.519 ± 0.064 a	1.45 ± 0.16 c	0.421 ± 0.053 a	0.844 ± 0.130 b
15.313	Tetramethylpyrazine	0.928 ± 0.107 c	0.405 ± 0.072 a	1.64 ± 0.24 d	0.700 ± 0.110 b	1.13 ± 0.13 c
15.518	2-Nonanone	1.56 ± 0.20 c	0.419 ± 0.077 a	6.18 ± 0.86 e	0.926 ± 0.168 b	4.29 ± 0.47 d
15.886	5-methylundecane	1.42 ± 0.25 b	nd	0.554 ± 0.066 a	nd	0.687 ± 0.063 a
15.94	Nonanal	nd	0.596 ± 0.052 a	nd	1.68 ± 0.16 b	nd
16.264	Phenylethyl Alcohol	nd	1.41 ± 0.27 a	nd	nd	1.68 ± 0.21 a
18.081	3-methylundecane	1.26 ± 0.15 c	0.323 ± 0.033 b	0.208 ± 0.040 a	0.416 ± 0.090 b	0.229 ± 0.028 a
18.909	Ethyl octanoate	1.43 ± 0.13 c	0.629 ± 0.099 b	0.573 ± 0.122 b	0.773 ± 0.060 b	0.405 ± 0.044 a
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
10.235	3-hydroxybutanoic acid ethyl ester	nd	0.427 ± 0.065 a	nd	0.457 ± 0.076 a	nd
12.927	Acetic acid hexyl ester	0.879 ± 0.131 b	0.299 ± 0.059 a	0.264 ± 0.030 a	0.457 ± 0.047 a	0.418 ± 0.081 a
15.225	(4E)-4-hexen-1-ol acetate	nd	nd	0.442 ± 0.054 a	nd	nd
15.79	Undecane	0.589 ± 0.111 d	0.070 ± 0.011 a	0.304 ± 0.025 c	0.309 ± 0.043 c	0.224 ± 0.030 b
17.726	2,3,5-Trimethyl-6-ethylpyrazine	nd	0.183 ± 0.033 a	0.902 ± 0.073 c	nd	0.496 ± 0.058 b
17.845	5-(2-methylpropyl)-nonane	0.515 ± 0.038 b	0.150 ± 0.029 a	0.176 ± 0.025 a	0.186 ± 0.016 a	0.152 ± 0.020 a
18.757	2-Decanone	0.710 ± 0.098 c	0.236 ± 0.030 a	0.245 ± 0.023 a	0.433 ± 0.046 b	0.285 ± 0.030 a
19.135	(E)-2-Decen-1-ol	0.459 ± 0.063 b	0.251 ± 0.033 a	0.260 ± 0.034 a	0.592± 0.111 b	0.226 ± 0.033 a
19.295	4,4-dimethylundecane	nd	nd	0.076 ± 0.008 a	nd	0.090 ± 0.008 a
19.397	2,5-dimethylundecane	0.619 ± 0.077 c	0.163 ± 0.025 a	0.265 ± 0.027 b	0.284 ± 0.042 b	0.260 ± 0.028 b
19.641	4-methyldodecane	0.344 ± 0.061 b	0.118 ± 0.018 a	0.174 ± 0.03 a	0.138 ± 0.011 a	0.197 ± 0.032 a
20.28	1-(2-butoxy-1-methylethoxy)-2-propanol	nd	0.228 ± 0.044 a	nd	0.212 ± 0.044 a	nd
20.66	1,3-bis(1,1-dimethylethyl)-benzene	0.782 ± 0.071 c	0.281 ± 0.057 a	0.503 ± 0.097 b	0.520 ± 0.071 b	0.692 ± 0.087 c
21.051	Nonanoic acid	0.401 ± 0.066 c	0.593 ± 0.071 d	0.203 ± 0.043 b	0.334 ± 0.077 c	0.116 ± 0.016 a
21.311	(Z)-3-Octen-1-ol acetate	nd	nd	0.936 ± 0.209 a	nd	0.686 ± 0.096 a

21.39	2,6,10-trimethyldodecane	nd	0.297 ± 0.026 a	0.480 ± 0.077 b	0.434 ± 0.068 b	0.467 ± 0.037 b
21.565	tetrahydro-6-pentyl-2H-pyran-2-one	nd	nd	0.167 ± 0.017 a	nd	0.174 ± 0.020 a
21.789	2-Undecanone	nd	nd	0.633 ± 0.060 b	nd	0.344 ± 0.039 a
23.518	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	0.789 ± 0.151 b	0.309 ± 0.043 a	0.293 ± 0.042 a	0.605 ± 0.092 b	0.315 ± 0.042 a

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