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Unravelling the potential of cricket-based hydrolysed sourdough on the quality of an innovative bakery product

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Abstract

The purpose of this research was to evaluate the potential of a sourdough containing cricket powder hydrolysate by the RO25 *Yarrowia lipolytica* strain to produce an innovative bakery product. RO25 hydrolysed cricket bread (RO25H-CB) was compared with control bread obtained from a traditional sourdough using wheat flour and with an additional bread control obtained from no-hydrolysed cricket powder sourdough. The results obtained showed that RO25H-CB had a highest amount of proteins and free fatty acids than wheat control bread, attributed to the well-known proteolytic and lipolytic activities of *Y. lipolytica.* Moreover, RO25H-CB sample was characterised by high content of health-promoting and aroma precursors lipids as well as a lowest biogenic amine index among samples analysed, suggesting for this sample a high overall quality respect to no hydrolysed cricket powder bread. Finally, the data relating the sensory analysis highlighted good application opportunities for RO25 cricket hydrolysate as ingredients for baking. In fact, RO25H-CB had received positive evaluations for almost all the parameters considered. These results demonstrated that hydrolysates from *Y. lipolytica*, compared to the no hydrolysed cricket, were able to impart specific sensory and qualitative characteristics to the final product, with positive feedback from the involved panellists.

Keywords: cricket based bread, sensory analysis, biogenic amine, protein profile, aroma profile

1. Introduction

Insects constitute a valid food resource since they are characterised by high nutritional values in terms of fats, proteins, vitamins and minerals (Patrignani *et al.*, 2020). The growing interest in this sector has led to a rapid expansion of industrial insect farms (especially domestic cricket) (Hanboonsong *et al.*, 2001). However, although insects represent an alternative protein source to animal proteins, their use as foods is still limited in the culture of western countries, because they are not considered part of our alimentary diet model (Caparros Megido *et al.*, 2016). In fact, although EU regulation (EC) No 2021/882 of 1 June 2021 authorised the placing on the market of dried *Tenebrio molitor* larva as a novel food (European Commission, 2021), recent studies proved that consumers find insects more appealing when used as ingredients, masking their appearance, for food formulations with familiar flavours and textures such as snacks, chips, shakes, pastry and bakery products (Nissen *et al.*, 2020). To mitigate their negative perception, it was suggested their usage in the form of flour or protein powder extracts (Schouteten *et al.*, 2016). The high nutritional value of edible insects and their potential use in the formulation of foods with functional nutritional characteristics have attracted the attention of food industry and researchers since they can be also good sources of

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polyunsaturated fatty acids endowed with health promoting features (Patel *et al.*, 2019).

Although according to the different food legislation insect powder can be used in food formulations in several countries. For example, insects and insect-based foods for human consumption, such as cricket croquettes and cricket burgers, are already available in the Netherlands and Belgium (Poma *et al.*, 2017; Stoops *et al.*, 2017). However, the use of insect powder involves limitations. In fact, the flavour is not always considered pleasant and, therefore, it is necessary to mask or limit its percentage inside the formulation. In addition, insect powder is generally subjected to different forms of hydrolytic, oxidative, microbial and enzymatic deterioration (Patrignani *et al.*, 2020; Rossi *et al.*, 2021). Also, short chain fatty acids can be easily accumulated during the storage and detected even in small quantities through bad smell or taste. Moreover, the presence of high content of chitin in the powder can represent a technological limit in food formulation.

Several authors have reported the use of cricket powder for bread production (Osimani *et al.*, 2018) and gluten-free bread (Kowalczewski *et al.*, 2021) in order to enhance the nutritional value of bakery products, to valorise the technological properties of cricket powder and raise consumer awareness toward this kind of products. However, for cricket powder, chitin content remains a hot topic due to its toxicological effect together with the presence of biogenic amines, not deeply investigated in insect-based products (Elias *et al.*, 2018). About chitin issue, recent studies, performed by Patrignani *et al.* (2020) and Rossi *et al.* (2021), have highlighted the use of *Yarrowia lipolytica* to reduce the presence of chitin in cricket powder due to the yeast hydrolytic activities. This biotechnological approach resulted in a high protein content hydrolysate characterised by strain specific free amino acids and fatty acid profiles, making the hydrolysate available as high quality and safe ingredient for food preparation, also able to impart specific features to the final product. Particularly interesting in bakery sector could be the use of the *Y. lipolytica* cricket-based hydrolysate to prepare a new kind of sourdough for innovative bakery product. In fact, Sourdough, a complex ecosystem constituted by yeasts and lactic acid bacteria, has been used to produce many leavened baked goods, exerting positive effects on their nutritional, sensory, rheological, and shelf-life properties (Corsetti and Settanni, 2007; Gobbetti *et al.*, 2019). Moreover, the large microbial biodiversity of common sourdough is an additional source of potential innovation. In particular, the main microorganisms responsible for the improved characteristics of bakery products are lactic acid bacteria (LAB) which own different metabolic capabilities and perform an intense activity in the raw matrix (Galle and Arendt, 2014; Gobbetti *et al.*, 2005) e.g. peptidase activities, acidification, exopolysaccharide production, and anti-nutritional compound reduction.

Thus, in this framework, the purpose of the present research was to evaluate the potential of a sourdough containing cricket powder hydrolysate by the RO25 *Y. lipolytica* strain, already characterised by Rossi *et al.* (2021), to produce an innovative bakery product. This baked product was compared with a bread obtained using a sourdough based on cricket powder not hydrolysed by *Y. lipolytica* RO25 and a control bread produced using a traditional sourdough, based exclusively on wheat flour. The final dough and the bread samples were characterised for the protein content, total and free fatty acids, volatile molecule, biogenic amine, texture and sensorial profiles.

2. Materials and methods

Sourdough and bread preparation

The different types of sourdough and bread were prepared according to the scheme shown in Figure 1. The sourdough was obtained according to the method reported by Rossi *et al.* (2021).

All the samples of sourdough obtained after 24 h from the second refreshment were used in the formulation of the doughs for baking. The formulation of the dough for bread preparation, shown in Table 1, was made with 75 g/100 ml dry weight sourdough and with the addition of 2 g of fresh brewer's yeast according to the procedures reported by Osimani *et al.* (2018).

The formed doughs were left to rise for 2 h at 25 °C. The samples were named wheat control dough (WCD), no hydrolysed cricket powder dough (noH-CD) and RO25 hydrolysed cricket dough (RO25H-CD), according to the sourdoughs described in Rossi *et al.* (2021).

The loaves obtained were baked in a professional oven at 200 °C by a local baker and the bread samples were named wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).

Microbiological analyses and pH

Microbiological analyses were performed on the three types of dough after rising at 25 °C. The lactic acid bacteria and yeast cell load of the different samples were detected, after decimal dilutions in physiologic solution (0.9 g/100 ml NaCl w/v), using maltose De Man, Rogosa, Sharpe and yeast extract peptone dextrose agar (Oxoid Ltd, Basingstoke, UK), respectively and incubated for 48 h at 30 °C.

The pH values of the doughs after fermentation were determined by a pH meter (BASIC 20, Crison, Modena, Italy).

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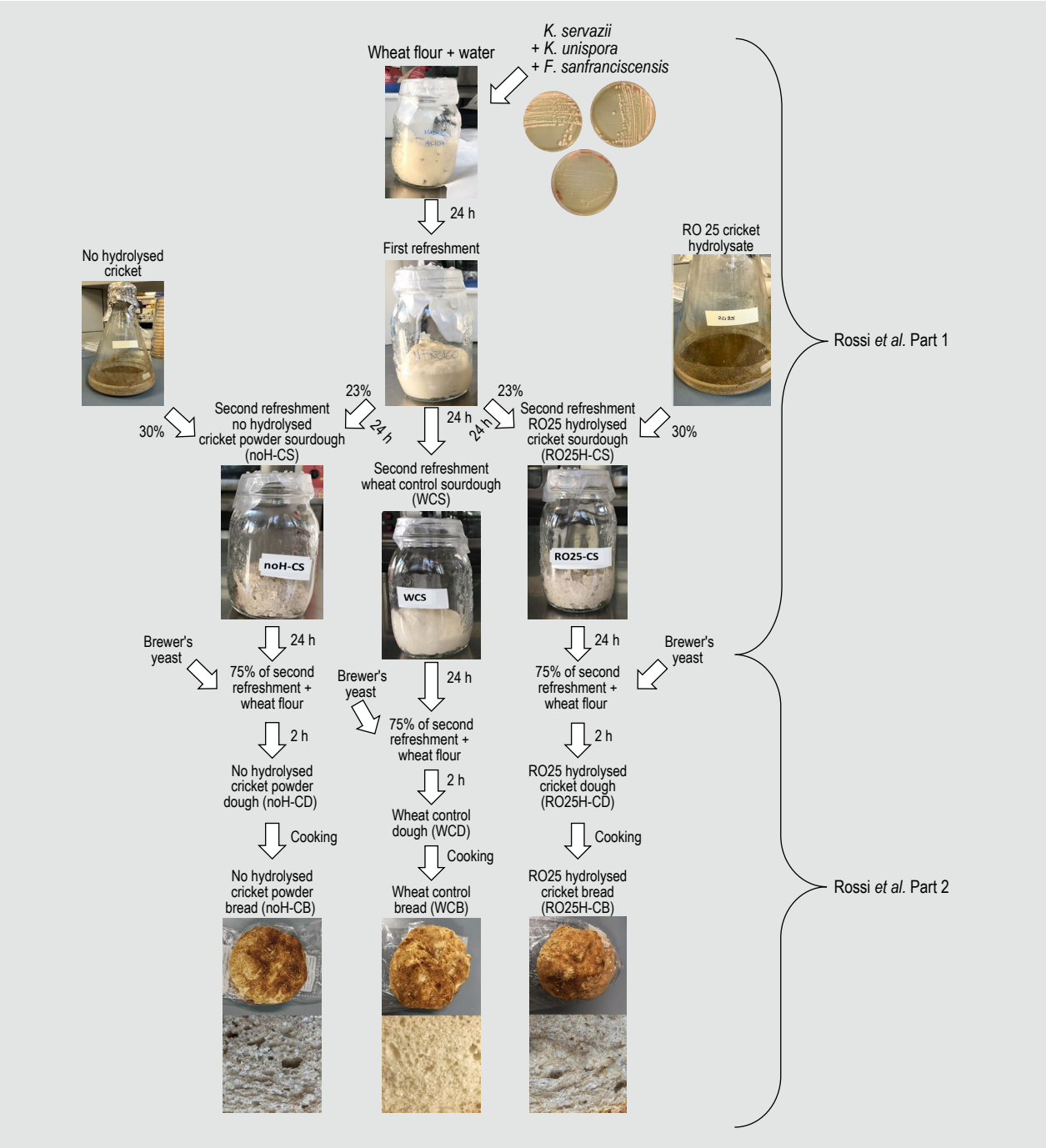

Figure 1. Sourdough and bread preparation scheme.

Protein profile analyses

Proteins were extracted from dough after rising at 25 °C and on the final bread, according to the method reported in Scarnato *et al.* (2016), and subsequently modified in Rossi *et al.* (2021). Briefly, total proteins were extracted under non-reducing conditions (by adding 5 ml of 100 mmol/l Tris-HCl pH 6.8, 4 g/100 ml (w/v), sodium dodecyl sulphate (SDS), 20 g/100 ml glycerol, 200 mmol/l β-mercaptoethanol to 1 g of sample) and reducing conditions (following a sequential extraction with following solvents: 5 g/100 ml of NaCl, 50 g/100 ml 1-propanol and 0.1 M NaOH, 0.5 g/100 ml SDS and 0.6 g/100 ml β-mercaptoethanol). Samples were centrifuged and supernatants were precipitated overnight. Precipitated proteins were washed and then resuspended in suitable buffers for 1-D electrophoresis. Separation of proteins by 1-D electrophoresis was described by Laemmli (1970).

Table 1. Formulations of first and second refreshment, and of the dough for bread preparation obtained with the use of wheat flour, sourdough, water and cricket-based ingredient.

Formulation[1]		Temperature (°C)	Ripening time (h)	Wheat flour (g)	Sourdough (g)	Water (g)	Cricket based ingredient (g)
First refreshment		25	24	221	67	112	–
Second refreshment	WCS	25	24	276	83.5	142	–
	noH-CS	25	24	168	83.5	35	108
	RO25H-CS	25	24	168	83.5	35	108
Dough for bread preparation	WCD	25	2	100	300	50	6
	noH-CD	25	2	100	300	50	6
	RO25H-CD	25	2	100	300	50	6

[1] noH-CD = no hydrolysed cricket powder dough; noH-CS = no hydrolysed cricket powder sourdough; RO25H-CD = RO25 hydrolysed cricket sourdough dough; RO25H-CS = RO25 hydrolysed cricket sourdough; WCD = wheat control dough; WCS = wheat control sourdough.

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Fatty acid analyses

The three different breads were subjected to fatty acids extraction, according to the method reported in Boselli *et al.* (2001) with some modifications. Specifically, 75 ml of 1:1 (v/v) chloroform: methanol solution was added to 6.0 g of sample and incubated at 60 °C for 20 min. 30 ml of chloroform were added and the solution was filtered using medium flow filtering papers. In order to remove polar solutes, 30 ml of 1 N KCl were added in each sample and incubated at 25 °C for 16 h. The organic lower phase (containing fatty acids) was recovered by filtration and with 5 g of Na_2SO_4 anhydrous and evaporation at 40 °C using a Rotavapor (IKA RV8; Staufen, Germany). The lipidic extracts were resuspended in n-hexane and stored at -80 °C. 2 N methanolic KOH was used to methylate total fatty acids contained in 20 mg of lipidic extract. For each sample, free fatty acids were obtained from the total lipid extracts using aminopropyl bonded sorbent columns (SPE-NH_2) ISOLUTE (Biotage, Uppsala, Sweden). Free fatty acids methyl esters were obtained by directly adding to each sample 50 µl of diazomethane. The fatty acid composition was determined as fatty acid methyl esters (FAMEs). Methyl tridecanoate (Sigma, Milan, Italy) (13:0, 0.02 mg/ml was used as internal standard and while Supelco FAME MIX 37 (Sigma) was used as external reference. The total and free fatty acids methyl esters profiles analyses were carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5970 mass selective detector operating in electron impact mode (ionisation voltage, 70 eV). A Chrompack CP-Wax 52 CB capillary column (50 m length, 0.32 mm i. d., 1.2 µm df) was used (Chrompack, Middelburg, the Netherlands). The temperature program was described in Rossi *et al.* 2021. Injections were performed with a split ratio of 1:10 and helium (1 ml/min) as the carrier gas. National Institute of Standards and Technology-United States Environmental Protection Agency-National Institute of Health (1998) and the Registry of Mass Spectral Data (1998) mass spectra libraries were used to identify the compounds.

Volatile profile analyses

Volatile molecule profiles were observed on the doughs after 2 h of rising at 25 °C and on the final bread. The analyses were conducted using a GC-MS coupled with a solid phase microextraction technique, according to Burns *et al.* (2008) with some modifications. The samples (5 g), placed in sterile vials, were added with 10 µl of standard 4-methyl-2-pentanol at 10,000 mg/kg and heated for 10 min at 45 °C. The absorption phase was carried out by a fibre (SPME Carboxen/PDMS, 85 µm, Stalleflex Supelco, Bellefonte, PA, USA) kept in the headspace for 40 min. Adsorbed molecules were desorbed for 10 min during the running in the gas-chromatograph column Chrompack CP-Wax 52 CB (Chrompack). The analysis was performed with an Agilent Technology 7890 N gas chromatograph, Network GC System combined with a Network Mass Selective detector HP 5975C mass spectrometer (Agilent Technologies). The characteristics of the column and the conditions used were described in Rossi *et al.* 2021. Volatile peak identification was carried out by computer matching of mass spectral data with those of the compounds contained in the NIST library (NIST/EPA/NIH Mass spectral Library, Version 1.6, Gaithersburg, MD, USA) of 2011 and WILEY (sixth edition, New York, NY, USA) of 1995. The quantification of volatile compounds in equivalent mg/kg was performed on the basis of the internal standard used (4-methyl-2-penthanol).

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Biogenic amine analyses

The biogenic amines (BA) in bread samples were determined by extraction with 10 g/100 ml trichloroacetic acid followed by pH adjustment before pre-derivatisation with ortho-phthalaldehyde. BA was then analysed according to Gardini *et al.* (2013), using a PU-2089 Intelligent HPLC quaternary pump (Jasco Corporation, Tokio, Japan), Intelligent UV-VIS multiwavelength detector UV 2070 Plus (Jasco Corporation, Tokyo, Japan) and a manual Rheodyne injector equipped with a 20 µl loop (Rheodyne, Rohnert Park, CA, USA). For the chromatographic separation, an analytical Cartridge Waters Spherisorb 3 µm ODS-2 4.6 mm 150 mm column (Waters Corporation, Milford, MA, USA), coupled with Guard Cartridge Waters Spherisorb S5 ODS2 column, 4.6 10 mm (Waters Corporation), was used with the following gradient elution: 0-5 min phosphate buffer (pH 7)/acetonitrile 35:65, 5-6 min water/acetonitrile 20/80, 6-15 min water/acetonitrile 10/90, 15-25 min phosphate buffer (pH 7)/acetonitrile 35:65; flow rate 0.8 ml/min. The amount of each amine was expressed as mg amine/kg by reference to a calibration curve obtained through aqueous dansyl-chloride derivatised histamine standards. The detection limit for all the amines was 1 mg/kg of samples, under the adopted conditions.

The biogenic amine index (BAI) was calculated as follows according to Sánchez and Ruiz-Capillas (2012):

$$BAI = \frac{\text{putrescine} + \text{cadaverine} + \text{histamine}}{1 + \text{spermidine} + \text{spermine}}$$

Textural analyses

Rheological analyses were performed on the three types of breads: WCB (bread from wheat flour), noH-CB (bread from no hydrolysed cricket,) and RO25H-CB (bread from RO25 cricket hydrolysate), by using a texture analyser mod. TA.HDi 500 (Stable Micro System, Godalming, Surrey, UK) equipped with a 50 mm diameter aluminium cylinder probe and a 25 kg load cell.

A texture profile analysis was performed by applying a double compression cycle with 40% penetration depth, 3.0 mm/s test speed and a 5 s gap between compressions (Gámbaro *et al.*, 2002). The considered textural parameters were hardness (kg), cohesiveness, gumminess (kg) and chewiness (kg). Tests were carried out on breads cooked and cooled at room temperature.

Sensory analyses

The sensory analysis of the three experimental breads was carried out the same day of the baking as already described by Osimani *et al.* (2018). The small-scale acceptance test was performed after the bread samples were left to cool at room temperature (Svensson, 2012). The analysis was conducted by 25 untrained panellists (58% men and 42% women). Initially, the sensory analysis was carried out only with the two samples of bread containing cricket (RO25H-CB and noH-CB). For each sample, the panellists were asked to evaluate: Visual, Olfactory, Taste and texture characteristics, on a scale from 1 (not much intense) to 5 (very intense). In specific, the attributes rated were the following: colour of the crumb, colour uniformity, presence of bubbles, general appearance of the samples, aroma features, bitterness, taster pleasantness crunchiness, gumminess, friability and hardness. Finally, the panellists were asked to express preference between the two bread samples containing cricket and the WCB sample.

Statistical analysis

All the results are represented as the mean of three different replica from three repeated experiments on different days. Fatty acid raw data were statistically analysed using the one-way ANOVA (version 8.0; StatSoft, Tulsa, OK, USA) and Tukey's HSD (honestly significant difference) test was used to highlight significant differences ($P \leq 0.05$). Statistica software (version 8.0; StatSoft) was used to perform principal component analysis (PCA) on raw data to underline the statistical variance among the samples for volatile and free fatty acids profiles.

Statistical analysis (T-test) was performed using GraphPad (Prism Inc., San Diego, CA, USA) to analyse protein profile raw data. Differences among sample sets were defined by analysis of variance with two-way ANOVA, performed in RStudio with 'anova' function, followed by a post-hoc 'pairwise.t.test' function, with a threshold $P=0.05$ and $P=0.01$. The two-way ANOVA was performed assuming two factorial variables: the origin of the flour (WC = wheat control; CP = cricket powder; CH = cricket hydrolysate) and the final product (sourdough or bread). Differences between the samples were analysed based on the total protein subfraction content, the albumins/globulins subfraction content, the prolamins subfraction content, and the glutelins subfraction content, separately.

3. Results

Microbiological analyses and pH of the final doughs

The wheat control sourdough (WCS), the no hydrolysed cricket powder sourdough (noH-CS) and the RO25 hydrolysed cricket sourdough (RO25H-CS), characterised by yeast and LAB cell loads of 6.85,8.79, and 7.5 and 9.25, 7.12, and 9.09 log10 cfu/g, respectively, were used at 75 g/100 ml to prepare the three final doughs according to the formulations reported in Table 1. In these also 2 g of yeast brewing was added and left to ferment for 2 h at 25 °C. In all the samples, *Saccharomyces cerevisiae* reached value

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higher than 8 log10 cfu/g. The results obtained showed a good growth capability also for LAB showing values of 8.89, 9.38 and 8.95 log10 cfu/g for WCD, noH-CD and RO25H-CD, respectively. The final pH values recorded were 4.06 for WCD, 4.25 for the noH-CD, and 4.15 for RO25H-CD.

Protein quantification and electrophoretic profile

In Table 2, the protein content of the different doughs, after fermentation, and breads are reported. The total subfraction protein content was generally higher both in RO25H-CD and deriving bread than in wheat control and no hydrolysed cricket powder ones. Specifically, in RO25H-CD, albumins/globulins fraction showed the higher value of protein quantity, while noH-CD contained a higher concentration of prolamins. Also, in breads, the samples containing cricket powder, hydrolysed or not, had a higher amount of proteins, but the differences between samples were not significant.

Electrophoretic analysis was conducted on dough and bread samples. First analysis (Figure 2) reported separation of total protein extracted in reducing condition of WCB, noH-CB and RO25H-CB and WCD, noH-CD and RO25H-CD samples. As evidenced, the sample of dough deriving from the use of RO25H-CS was characterised by highest accumulation of low molecular weight peptides at 11 and 25 kDa and of a band having molecular weight ranging between 25 and 48 kDa. Moreover, in this sample also the presence of bands corresponding to a molecular weight of 75 and 100 kDa were highlighted. The proteolysis was less relevant in the remaining dough samples. About the bread, the protein profile of RO25H-CB showed a minor presence of protein having molecular weight ranging between 11 and 100 kDa.

ANOVA results indicated that: (1) For albumin/globulin fraction WCD samples and noH-CD ones were significantly different (P=0.003928); significant differences were recorded also between the two types of bread based on WCD and noH-CD (P=2.166^{e-05}); (2) in prolamin fraction data were significantly different only between bread samples (P=1.183^{e-07}); (3) in glutelin fraction data were statistically different only between bread samples (P=5.293^{e-06}); and (4) indeed, in total protein fraction all data were statistically different both between samples (P=5.330^{e-05}) and between bread samples (P=6.982^{e-05}).

Fatty acid and free fatty acid profiles

Table 3 shows the concentrations, expressed as mg/kg, of total fatty acids contained in the three bread typologies. As evidenced by the Table 3, the bread characterised by the highest amount of total fatty acids was the noH-CB followed by WCB and RO25H-CB. In particular, WCB was characterised by the presence of C16:1, C16:0, C18:2 n-6, C18:1 (9), C18:3 n-3 and C18:0. This last fatty acid was present in higher concentration in the RO25H-CB which contained also the highest concentration of C18:2 and C18:1 trans. The noH-CB, in addition to the fatty acids detected for the other samples, was specifically characterised by the presence of C20:0.

In Table 4, the free fatty acids in relation to the bread considered are reported as mg/kg. The highest amount of free fatty acids was recorded in RO25H-CB. In particular this product was characterised for the highest presence of C10:0, C12:0, C14:0, a-C14:0, tetradecanedioic acid, 3,6-epoxy-, dimethyl ester, C15:0, i-C15:0, C16:1 Δ7, C16:1 Δ9, C16:0, a-C16:0, i-C16:0, C16:0 ethyl, cyclopropaneoctanoic acid, 2-hexyl-, methyl ester, C17:0, C18:2 n-6, C18:1 Δ9, C18:1

Table 2. Protein and subfraction content of wheat control dough (WCD), no hydrolysed cricket powder dough (noH-CD) and RO25 hydrolysed cricket dough (RO25H-CD), immediately after fermentation with brewer's yeast at 25 °C, and wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB). Data are means of three independent measurements. Standard deviation are also reported.[1]

		Total of protein subfraction (mg/g)	Albumins/globulins (mg/g)	Prolamins (mg/g)	Glutelins (mg/g)
Dough for bread preparation after fermentation with brewer's yeast	WCD	35.86±1.07^{a}	2.61±0.38^{b}	16.44±0.44^{d}	16.82±0.26^{d}
	noH-CD	34.65±0.19^{a}	6.44±0.44^{b}	18.04±0.16^{d}	10.17±0.52^{d}
	RO25H-CD	40.06±0.12^{a}	8.90±0.34^{b}	15.84±0.20^{d}	15.32±0.59^{d}
Bread	WCB	25.43±0.57^{a}	1.27±0.24^{a}	4.60±0.40^{a}	19.56±0.40^{a}
	noH-CB	32.09±0.22^{a}	2.44±0.40^{a}	6.53±0.73^{a}	23.12±0.41^{a}
	RO25H-CB	36.73±0.54^{a}	1.52±0.35^{a}	11.47±0.52^{a}	23.75±0.33^{a}

[1] For each column considered, the protein and subfraction content indicated with different letters are significantly different. Letters indicated a = 0.001, b = 0.01, c = 0.05, d = 0.1.

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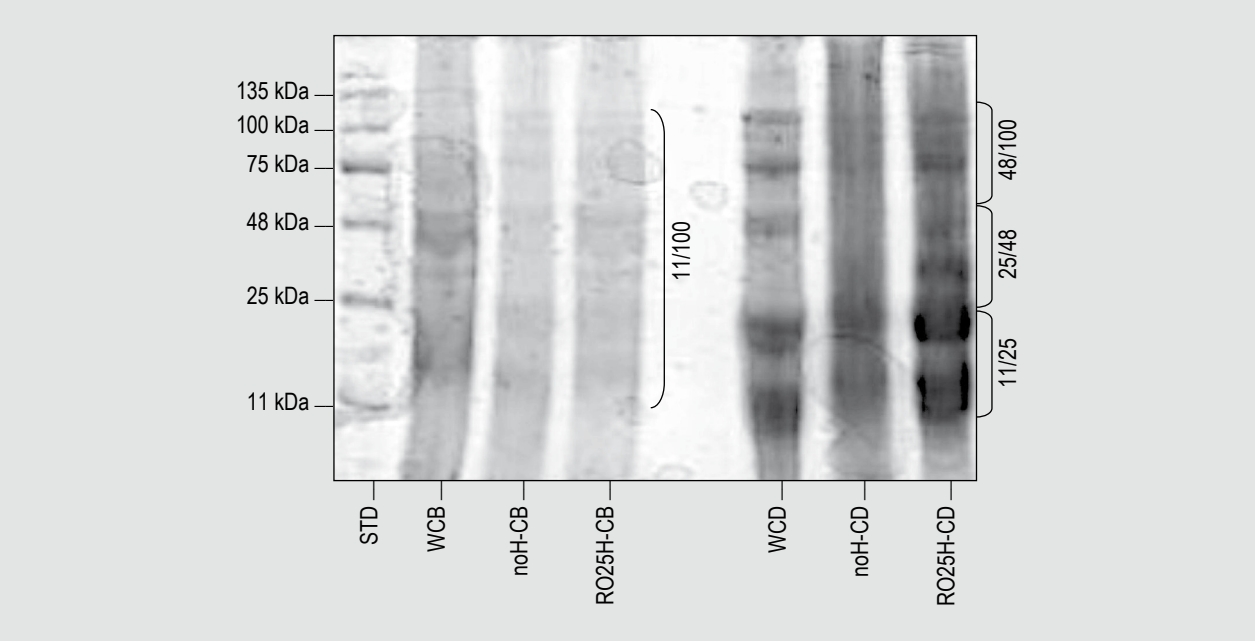

Figure 2. Coomassie coloured SDS-PAGE containing a mix of three independent extraction analysis of proteins in reducing conditions of wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB) and wheat control dough (WCD), no hydrolysed cricket powder dough (noH-CD) and RO25 hydrolysed cricket dough (RO25H-CD), immediately after fermentation with brewer's yeast at 25 °C. Molecular weights markers in the first lane and their values in kDa on the left.

Table 3. Total fatty acids (FA; expressed as mg/kg) detected in wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).[1]

Total FA	WCB (mg/kg)	noH-CB (mg/kg)	RO25H-CB (mg/kg)
C10:0	0.6±0.2	*	nd
C12:0	0.7±0.1^{b}	1.6±0.1^{a}	*
C14:0	1.9±0.2^{c}	7.9±0.2^{a}	2.3±0.1^{b}
C15:0	1.6±0.4^{a}	1.8±0.2^{a}	1.0±0.1^{b}
C16:1	6.7±1.1^{b}	15.8±0.3^{a}	4.4±0.1^{c}
C16:0	266.6±58.0^{b}	408.3±2.2^{a}	192.1±13.2^{c}
C16:0 Ethyl	nd	nd	1.8±0.1
C17:0	2.3±0.8^{b}	3.8±0.3^{a}	1.4±0.0^{a}
C18:2 n-6	676.7±114.8^{a}	779.5±10.0^{a}	380.1±87.2^{b}
C18:1 (9)	170.9±57.0^{b}	369.6±8.3^{a}	115.8±32.6^{b}
C18:3 n-3	53.4±18.7^{a}	48.8±4.4^{a}	22.4±7.9^{b}
C18:0	16.0±5.2^{b}	127.1±2.6^{a}	28.8±9.7^{b}
C18:2	nd	nd	9.9±4.0
C18:1 T	nd	nd	6.1±2.8
C20:0	nd	3.8±0.6	nd
Unsaturated FA	907.7±291.6^{ab}	1,213.6±23.2^{a}	538.7±134.4^{b}
Saturated FA	289.7±64.1^{b}	554.5±1.9^{a}	227.4±22.8^{b}
Total	1,197.4±355.7^{b}	1,768.1±21.4^{a}	766.1±157.1^{c}

[1] For each line considered, the fatty acids indicated with different letters are significantly different. * = detected in trace: under 0.5 mg/kg; nd = not detected.

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Table 4. Free fatty acids (FA; expressed as mg/kg) detected in wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).[1]

Free FA	WCB (mg/kg)	noH-CB (mg/kg)	RO25H-CB (mg/kg)
C9:0	nd	*	*
Hexanedioic acid, dimethyl ester	*	0.9±0.1[a]	0.8±0.0[a]
C10: 0	*	1.3±0.0[b]	1.5±0.0[a]
Benzenepropanoic acid, alpha, hydroxy, methyl ester	nd	1.6±0.8[a]	1.1±0.0[a]
C12:0	0.7±0.0[b]	0.7±0.2[b]	2.1±0.1[a]
Nonanedioic acid, dimethyl ester	trace	1.8±0.1[a]	1.4±0.1[b]
C14:0	1.3±0.2[c]	4.0±0.2[b]	25.8±0.0[a]
a-C14:0	nd	nd	1.9±0.1
Tetradecanedioic acid, 3,6-epoxy-, dimethyl ester	nd	nd	1.7±0.1
C15:0	0.5±0.0[c]	1.8±0.2[b]	5.8±0.1[a]
i-C15:0	nd	nd	1.2±0.0
C16:2	nd	nd	2.9±0.1
C16:1 (7)	nd	nd	22.8±0.8
C16:1	9.8±0.8[c]	30.1±0.9[b]	61.6±1.0[a]
C16:0	55.9±8.8[c]	313.6±14.9[b]	952.9±56.2[a]
a-C16:0	nd	nd	1.9±0.4
C16:0 ethyl	nd	nd	1.4±0.0
i-C16:0	nd	nd	3.6±0.0
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	nd	nd	5.6±0.2
C17:0	nd	3.2±0.3[b]	12.9±0.0[a]
C18:2 n-6	61.6±19.3[c]	452.8±28.0[b]	1,872.6±58.6[a]
C18:1 (9)	18.0±5.3[c]	118.1±6.2[b]	993.7±33.3[a]
C18:3 n-3	1.7±2.5[b]	21.6±2.5[a]	nd
C18:1 (8)	nd	8.6±0.0[b]	116.8±9.9[a]
C18:0	16.2±1.2[c]	154.1±8.1[b]	466.4±1.1[a]
C19:0	nd	nd	1.0±1.5
C18:2 (10-13)	nd	nd	5.1±1.1
C20:2	nd	nd	4.4±0.8
C20:1	nd	nd	10.2±1.1
HI-C16:0	nd	13.1±0.5[b]	55.8±3.6[a]
C20:0	nd	nd	23.1±2.6
C22:0	nd	5.5±0.4[b]	6.8±0.1[a]
Total	166.7±43.3[c]	1,133.1±60.7[b]	4,665.2±220.2[a]

[1] For each considered fatty acid, the statistical analysis was performed among the data resulting from the three different samples. Samples with different letters are significantly different (P<0.05). * = detected in trace: under 0.5 mg/kg; nd = not detected.

Δ8, C18:0, C19:0, C18:2 (10-13), C20:2, C20:1, HI-C16:0, C20:0, C22:0. The product containing the lowest amount of free fatty acids was the bread obtained by WCD.

To better highlight difference among the samples, fatty acid and free fatty acid raw data were analysed by using PCA. Figure 3A reports the projection of bread samples in the factorial space in relation to fatty acids. In particular, the samples grouped according to sourdough type (WCS, noH-CS and RO25H-CS). In particular, the RO25H-CB A, B and C, were separated by noH-CB A, B and C along PCA 1 explaining the 76.58% of variance among samples. The grouping of these samples were affected by C18:1 trans, C18:2 and C16:0 ethyl characterising RO25H-CS ones while C18:0, C14:0, C20:0, C18:1 Δ9 and C16:1 characterising bread from noH-CS (Figure 3B). These samples were separated along PC2, counting for 18.33% of variance, from WCB. The projection of the samples in the factorial space in relation to free fatty acids is reported in Figure 3C described by PC1 (72.71% of variance among the samples) and PC2 (18.62% of variance among the sample). Also in this case the samples grouped according to the

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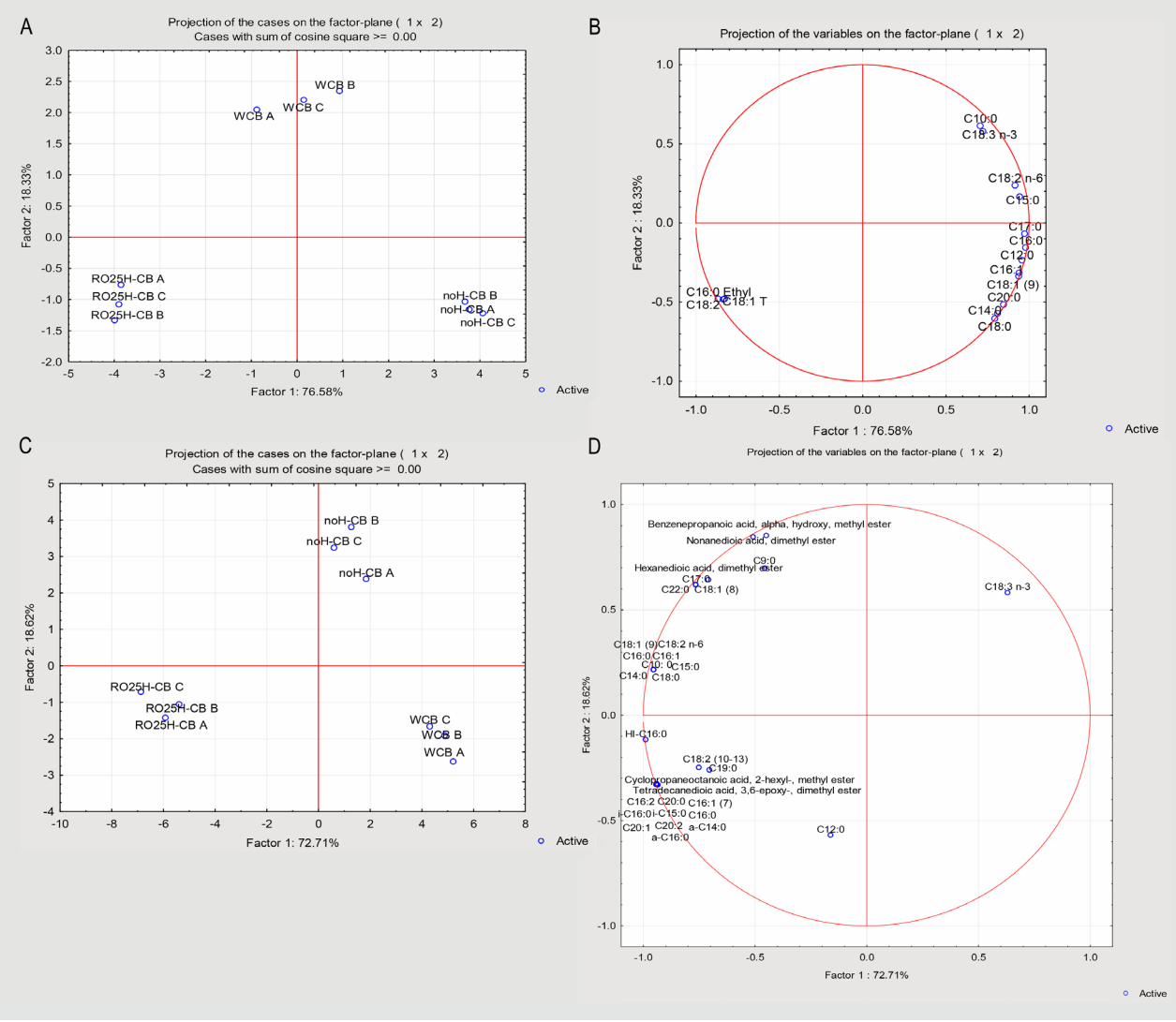

Figure 3. Projection of cases (A and C) and variables (B and D) obtained by principal component analysis elaboration of total and free fatty acids, respectively, wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB). The data are shown in triplicate (A, B, C).

initial sourdough used from bread making (from WCS, noH-CS and from RO25H-CS), in particular, RO25H-CB was characterised by tetradecanedioic acid, 3,6-epoxy-, dimethyl ester, cyclopropaneoctanoic acid, 2-hexyl-, methyl ester, C16:1 Δ7, i-C15:0, C16:0, C16:2, C18:2 (10,13), C19:0, a-C14:0, a-C16;0, i-C16:0, HI-C16:0 and C20:1 (Figure 3D).

Volatile molecules profiles in the final doughs and bread samples

The final doughs and the bread samples were analysed for their molecule profiles in relation to the sourdough used. The detected molecules, belong to different classes of compounds, included aldehydes, alcohols, ketones, acids, esters, hydrocarbons and pyrazine. More than 120 molecules were identified in relation to the different samples, highlighting specific peculiar aromas. In particular, the bread samples showed a higher total molecule amount than the doughs. The total aldehyde amounts in the dough were 0.19 mg/kg for WCD, 0.27 mg/kg for noH-CD, 0.77 for RO25H-CD, while, in the bread samples were 14.57 mg/kg for WCB, 32.25 mg/kg for noH-CB and 15.62 mg/kg for RO25H-CB. The total alcohol amounts were 56.89 mg/kg for WCD, 61.51 mg/kg for the noH-CD and 40.26 mg/kg for RO25H-CD, while smaller quantities were detected in the bread samples: specifically, 37.96 mg/kg for WCB, 30.27 mg/kg for noH-CB and 33.58 mg/kg for RO25H-CB. Ketones in the dough were detected at levels of 0.54 mg/kg, 1.32 mg/kg and 1.49 mg/kg for WCD, noH-CD and RO25H-CD, respectively, while in the bread samples they amounted to 34.41 mg/kg, 46.71 mg/kg and 26.72 mg/kg for WCB, noH-CB and RO25H-CB, respectively. The dough from WCS was characterised by 5.25 mg/kg of acids while amounts of 17.42 and 3.88 mg/kg characterised the dough from

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noH-CS and RO25H-CS, respectively; a similar trend was observed also in the bread samples with 15.91 mg/kg, 39.00 mg/kg and 15.98 mg/kg for WCB, noH-CB and RO25H-CB, respectively. The highest concentration of esters was found in RO25H-CB bread. This can be explained with the highest amount of precursors (free fatty acids) released by the hydrolytic activity of *Y. lipolytica* RO25. Alkanes were mainly present in the bread samples with amounts of 27.18 mg/kg for WCB, 43.34 mg/kg for noH-CB and 45.34 mg/kg for RO25H-CB. Pyrazine characterised mainly noH-CB which contains an amount of 30.37 mg/kg.

To highlight the effect of the used sourdough on the aroma profiles of the samples, the results of volatile molecule analysis were studied by PCA. Figure 4A and 4B represent the projections of the samples and variables in the spaces encased by the first two main components PC1 and PC2, which account for 23.63 and 55.75%, respectively of the total variance between the different samples. The three bread types were differentiated from each other in the factorial space along PC2, while they were well differentiated in the factorial space along PC1 from the three types of dough which were grouped due to the effect of brewer's yeast. Specifically, the molecules affecting the differentiation of noH-CB mainly belonged to pyrazines category, while, the RO25H-CB was characterised by 2,4-dimethyl-heptane, ethyl formate, 2,4-dimethyl-undecane, eicosane, 4-methyl-octane, phenol, 2-nonenal, 3-methyl-decane, 2,6,11-trimethyl-dodecane and nonanoic acid.

Biogenic amines in cricket bread samples

Bread samples were characterised for the presence of several biogenic amines (Table 5). In particular, the noH-CB was characterised by the higher levels of cadaverine (20 mg/kg) and tyramine (28 mg/kg) with respect the sample from RO25H-CB (cadaverine 8 mg/kg, tyramine 17 mg/kg). No significant difference between the samples was found for spermidine, spermine and putrescine while histamine was under the detection limit (1 mg/kg) in both the samples. The WCB was characterised by the lower content of biogenic amines with exception of tyramine that was present at level of 30 mg/kg. The biogenic amine index was 2.9 for RO25H-CB and 5 for noH-CB.

Textural analyses of bread samples

Table 6 shows the hardness, gumminess, chewiness and cohesiveness values obtained in the three different bread types.

The noH-CB sample was characterised by a highest significant value of hardness when compared to WCB and RO25H-CB, both showing no significant difference between them. On the other hand, no significant differences were found among samples in relation to gumminess, cohesiveness and chewiness parameters.

Sensory analysis of the cricket bread samples

In Figure 5, results obtained from sensory analysis are reported. Graph shows that the evaluation of the visual appearance and textures were very similar for both samples, except for the colour of the crumb which was darker for the cricket powder bread. The olfactory analysis shows that the samples had very different results from each other. The flavour analysis followed the trend observed for olfactory analysis. In fact, the RO25H-CB sample had a greater aroma attributable to bread and a better gustatory pleasantness, differently to noH-CB sample which has higher intensity and anomalous flavour and bitterness.

In addition, panellists were asked to highlight the presence of any anomalous compounds which resulted mainly in noH-CB samples, which were recognised for unpleasant flavours of cheese and rancid butter. Moreover, the panellists were asked to express the overall acceptance, in a scale from 0 to 10, on the 3 different kinds of breads. The results showed a final score of 7.2 for WCB, 6.2 for bread from RO25H-CD and 3.8 for bread from noH-CD confirming the results obtained by Osimani *et al.* (2018), regarding this type of product.

4. Discussion

Novel foods represent sustainable alternatives to traditional farming and conventional foodstuffs and the house cricket (*Acheta domesticus*) is considered as one of the most promising reared insect due to its attractive nutritional profile and lower feed conversion ratio compared to other animals. According to the literature data, many attempts have been made to incorporate cricket powder into several food formulations (Bawa *et al.*, 2020; Osimani *et al.*, 2018). In this research, an innovative bread product produced from a sourdough containing cricket powder hydrolysate by the yeast *Y. lipolytica* RO25 was investigated as cricket powder carrier. The bread formulation was performed including a sourdough since it is considered as a complex biological ecosystem in which characterising microorganisms (yeasts and lactic acid bacteria) are able to improve nutritional quality of the final bread and antimicrobial compounds able to stabilise the shelf-life of the final product (Corsetti and Settanni, 2007). Moreover, the use of hydrolysed sourdough by mean of a biotechnological approach based on *Y. lipolytica* RO25 was performed in order to increase the proteolytic and lipolytic pattern of the final product and to solve the chitin issue linked to the use of cricket powder. According to the literature data, there is a lack of work regarding insect protein hydrolysates incorporation into food products (Luna *et al.*, 2021) and the present research is

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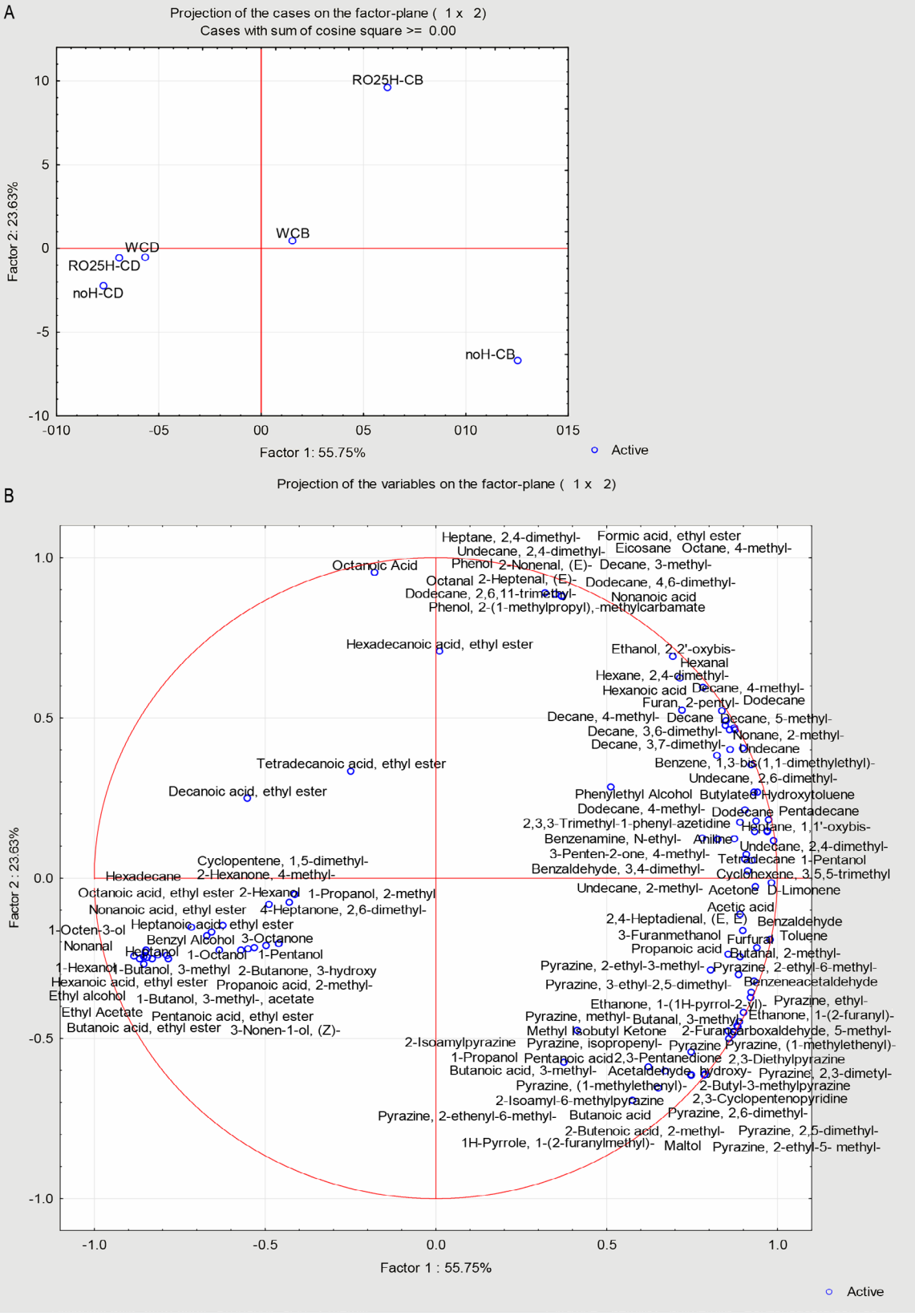

Figure 4. Projection of cases (A) and variables (B) obtained by principal component analysis elaboration of volatile molecule profile characterising wheat control dough (WCD), no hydrolysed cricket powder dough (noH-CD) and RO25 hydrolysed cricket dough (RO25H-CD), immediately after fermentation with brewer's yeast at 25 °C, and wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).

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Table 5. Biogenic amines content (mg/kg) in control bread (WCB), RO25 hydrolysed cricket bread (RO25H-CB) and no hydrolysed cricket powder bread (noH-CB).[1]

Biogenic amines	WCB	RO25H-CB	noH-CB
Cadaverine	7.0±0.1[c]	8.0±0.4[b]	20±1.00[a]
Histamine	<1[*]	<1	<1
Putrescine	11.0±0.2[b]	15.0±0.8[a]	15.0±1.8[a]
Spermidine	3.0±0.1[b]	4.0±0.2[a]	4.0±0.2[a]
Spermine	2.7±0.1[b]	3.0±0.1[a]	2.0±0.1[c]
Tyramine	30.0±0.2[a]	17.0±0.9[b]	28.0±1.4[c]
B.A.I. index	2.7±0.2[b]	2.9±0.1[b]	5.0±0.3[a]

[1] For each line considered, the biogenic amines indicated with different letters are significantly different. * = under the detection limit.

Table 6. Hardness, cohesiveness, chewiness, gumminess, parameters measured in wheat control bread (WCB), no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).[1]

	Hardness (kg)	Cohesiveness	Gumminess (kg)	Chewiness (kg)
WCB	7.4±0.2[b]	0.9±0.1[a]	7.0±1.2[a]	123.7±27.3[a]
noH-CB	7.9±0.1[a]	0.9±0.1[a]	7.0±1.2[a]	114.0±24.1[a]
RO25H-CB	7.5±0.2[b]	0.9±0.1[a]	7.0±0.6[a]	106.1±11.0[a]

[1] For each column considered, the data indicated with different letters are significantly different.

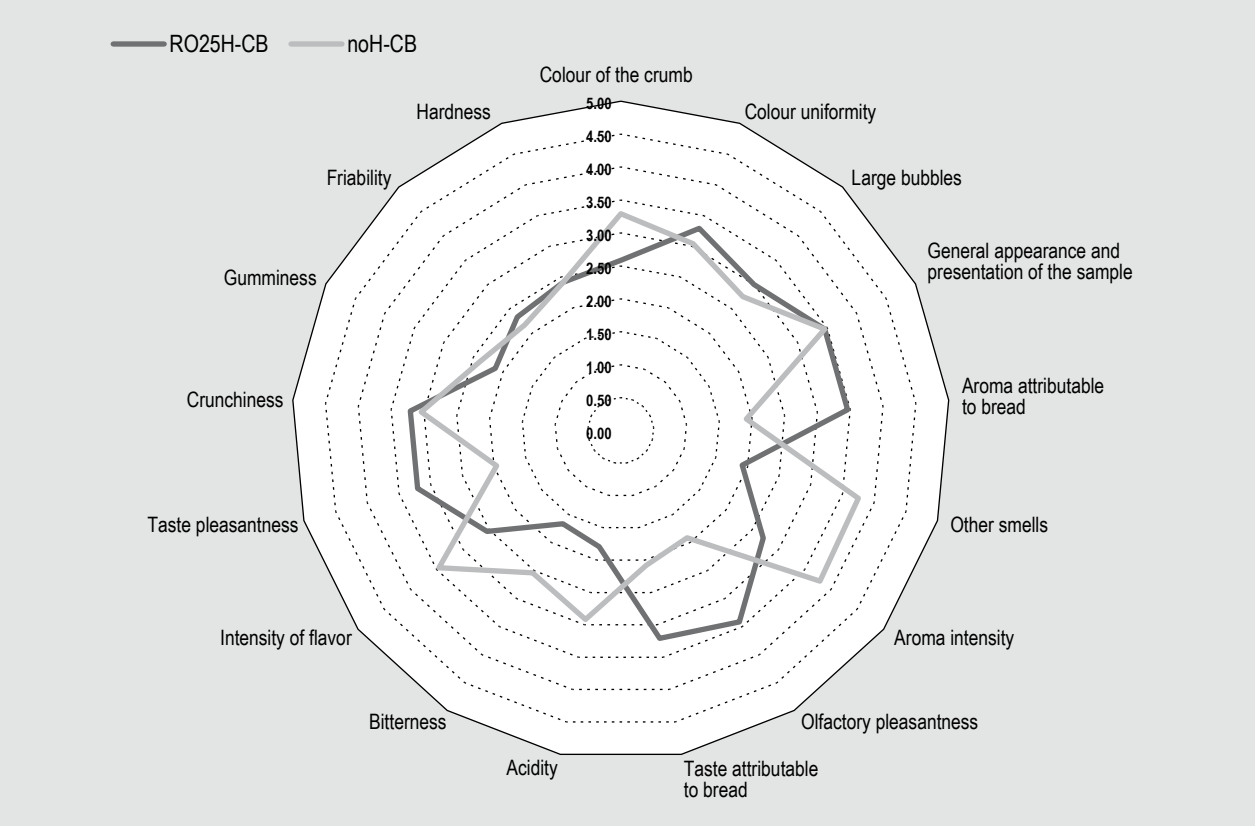

Figure 5. Sensory analysis of no hydrolysed cricket powder bread (noH-CB) and RO25 hydrolysed cricket bread (RO25H-CB).

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an important tassel using a friendly approach with respect the enzymatic approach proposed by the literature.

The difference found in proteolytic and lipolytic patterns in dough and bread from RO25 hydrolysed cricket sourdough can be attributed to the well-known hydrolytic activity of *Y. lipolytica* whose high technological potential is well documented and recognised also in the food sector where this yeast was used also for lipase production (Darvishi *et al.*, 2019; Gálvez-López *et al.*, 2021; Iucci *et al.*, 2007). In fact, the RO25H-CB sample was characterised by the highest releases of C16:1, C18:1 and C18:2, which are considered aroma precursors (Maire *et al.*, 2013; Patrignani *et al.*, 2008). Furthermore, the samples containing cricket powder, hydrolysed or not, were composed of a high content of C18:2 and C18:3 (omega-3 and omega-6 fatty acid, respectively), which are healthy lipids, important for children and infants' development (Lands, 2014). Moreover, the breads and doughs obtained in the present research were characterised by specific volatile profiles coming from raw materials and processes. Many of these compounds were mainly due to the fermentation process, lipid oxidation and Maillard caramelisation reactions (Prost *et al.*, 2020). The production of volatile compounds in doughs was mainly due to the metabolism of yeasts and LAB. This explains the greater presence of alcohols and esters as result of fermentation. By contrast the absence of pyrazines in the WCD samples can be attributed to the limited Maillard reaction and the absence of precursor deriving from the protein hydrolysis of *Y. lipolytica* RO25. The noH-CB sample showed higher concentration of aldehydes and ketones, as a result of a marked lipid oxidation activity (Pétel *et al.*, 2017). Also, the sensory panel performed demonstrated that the RO25H-CB sample has a greater flavour attributable to bread and a better olfactory pleasantness compared to the noH-CB sample which instead shows a strong flavour and abnormal smell.

Also textural properties of the innovative bread were tested. In particular, hardness is an important characteristic commonly used as index of bread quality (Wang *et al.*, 2007) and the noH-CB sample showed higher values and, consequently, a reduced quality compared to the samples WCB and RO25H-CB. Instead, the data relating to the gumminess, cohesiveness and chewiness parameters did not show significant differences between the samples. Similar trends were found by (González *et al.*, 2019) for wheat bread and cricket powder-based bread prepared with brewer's yeast for hardness and chewiness while cohesiveness, differently from our research, was affected by the presence of cricket powder. Of course, the inclusion of insects' flours can affect the rheological behaviour of the final product since insect flours do not contain starch and despite their high proteins content, generally water adsorption is reduced. Also, the protein composition, in terms of amino acids, can affect these textural parameters and water adsorption (Rumpold and Schlüter, 2013). However, the adopted biotechnological process in breadmaking (sourdough approach and the quantities of cricket powders hydrolysed or not) has mitigated the rheological features of the final cricket flour-based products, obtaining breads with textural properties similar to the standard bread. Also, the reduction of chitin content by *Y. lipolytica* RO25, demonstrated by Patrignani *et al.* (2020), contributed to attain in RO25H-CB rheological properties very closed to that of the control bread for hardness.

In this research, also the biogenic amines of the final products were investigated. In fact, health hazards associated with consumption of crickets have not been deeply investigated (Fernandez-Cassi *et al.*, 2019). In fact, although the biogenic amine content of foods has been widely studied because of their potential toxicity, few evidences are available for insect powder-based food products. Intake of exogenous biogenic amines at elevated amounts may result in toxicological effects with various degrees of severity (Del Rio *et al.*, 2019). In healthy persons, dietary biogenic amines can be rapidly detoxified by amine oxidases, whereas persons with low amine oxidase activity are at risk of their toxicity (Maintz and Novak, 2007). From a physiologically point of view, histamine and tyramine are the most important biogenic amines. Although histamine was not detected, tyramine was found at the highest level in noH-CB and its role is generally linked to vasoactive effects responsible for the immediate and short-lived responses in inflammation including vasodilation, increased vascular permeability and smooth muscle contraction. However, the occurrence of biogenic amines is not only a risk factor for intoxications but is also an indicator of food quality. Di- and polyamines (cadaverine and putrescine) are considered as indicators of the freshness and quality of foods. A quality index (BAI) based on the increases in putrescine, cadaverine and histamine, and decreases in spermine and spermidine is generally used to indicate food quality (Cheng *et al.*, 2016). As evident, RO25H-CB was characterised by the lowest BAI among samples analysed, suggesting, for this sample a high overall quality with respect noH-CB (Sánchez and Ruiz-Capillas, 2012).

5. Conclusions

The data obtained in the present research highlighted the good features of the cricket-based bread obtained from a cricket hydrolysed sourdough from *Y. lipolytica* RO25. In fact, this bread was characterised by the highest concentration of polyunsaturated free fatty acids, protein content subfraction (albumins/globulin, prolamins and glutelins) and the lowest level of biogenic amines when compared to the bread sample obtained from noH-CB. In addition, the textural data showed hardness values for RO25H-CB no significantly different from the WCB. The data relating the sensory analysis

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highlighted good application opportunities for RO25 cricket hydrolysate sourdough as ingredients for baking. In fact, the bread samples obtained from RO25 cricket hydrolysate received positive evaluations for almost all the parameters considered. These results demonstrated that the hydrolysates from *Y. lipolytica*, compared to the no hydrolysed cricket, were able to impart specific sensory and qualitative characteristics to the final product. On the other hand, reinforcing positive eating experiences may be crucial for repeat consumption and require a gradual familiarisation with the unique sensory properties of the cricket hydrolysed ingredient and the final baked product.

Conflict of interest

The authors declare no conflict of interest.

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