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# Cricket powder-based hydrolysates as high protein ingredients for sourdough formulation

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## Introduction

The first two years of research activities were focused on the production and characterization of cricket-based hydrolysates to be used as high protein ingredients in bakery products. For this reason, the technological potential of two strains of *Yarrowia lipolytica* (PO11, RO24) and two of *Debaryomyces hansenii* (DB, SP6L12) was evaluated in cricket powder mixed with water. Each strain produced hydrolysates with specific features in terms of free fatty acid (FFAs), amino acid (AA), volatile molecules profiles (VMP) and chitin content. Secondly, the hydrolysate with the best characteristics (*Y. lipolytica* RO25) was used to produce a sourdough for bread production. Sourdough was characterized for its microbiological features, FAs, protein and VMP.

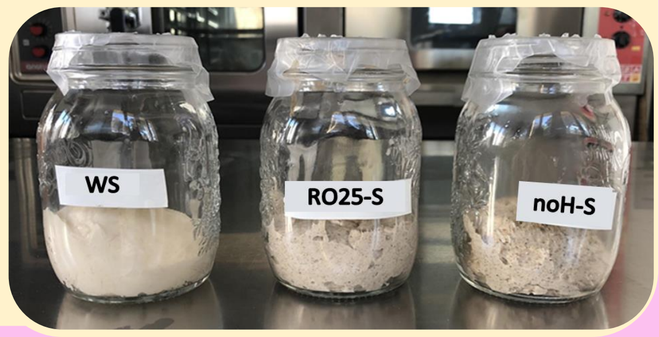
**Figure 1.** Hydrolysate of cricket powder inoculated with *Yarrowia lipolytica* RO25


## Materials and Methods

**Hydrolysates:** Commercial cricket powder + sterilized water (ratio of 1:3 "w/v") + selected strains (± 6 log CFU/mL). Incubation: 25 °C for 72 h in agitation (figure 1).
**Control sample:** Without yeasts inoculation (no-hydrolysed cricket powder "NoH-CP").

**Sourdough:** Two yeasts of *Kazachstania* strains + *Fructilactobacillus sanfranciscensis* + wheat flour + water. **Second refreshment:** 30% *Y. lipolytica* RO25 cricket hydrolysate + 23% sourdough on dry weight (RO25-S). **Two control samples: 1.** Without cricket powder (WS) **2.** With NoH-CP (noH-S) (figure 2).

**Figure 2. T**hree kinds of sourodugh analyzed

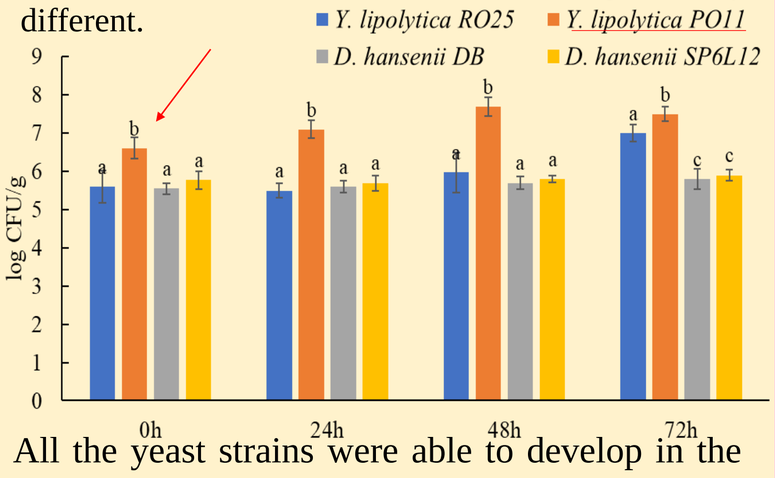



**Lipid fraction extraction:** [1] with modifications.
**Protein extraction:** [2] with modifications.
**Chitin quantification:** Filth test.
**Protein separation:** [3].
**Volatile compound detection:** GC/MS/SPME technique [4].
**Amino acids analysis:** AccQ-Tag protocol.

## Results and Discussion

### Characterization of cricket powder-based hydrolysates obtained by yeast biotechnological

**Figure 3.** Cell loads (log CFU/g) of yeasts inoculated in cricket powder at 0, 24, 48 and 72 h at 25 °C. The data indicated with different letters are significantly different.



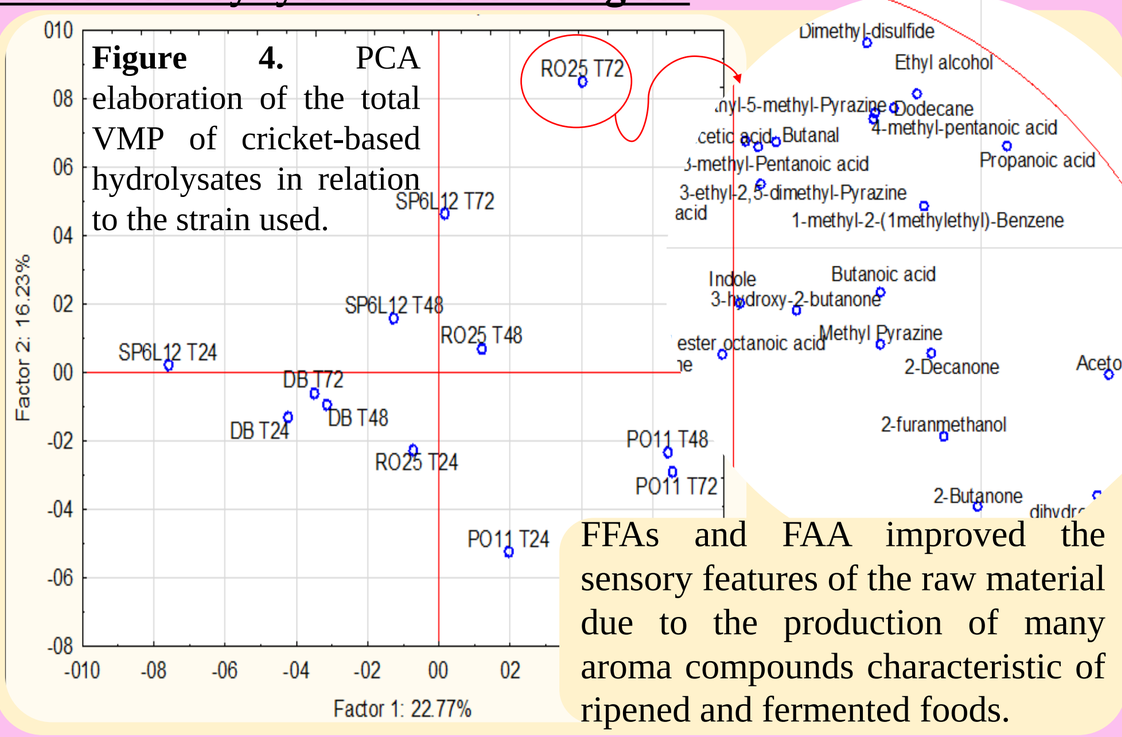

All the yeast strains were able to develop in the starting matrix.
***Y. lipolytica* PO11:** Cell load with values higher than 7 log CFU /g, compared to other strains.

**Table 1.** Chitin content (g/g flour) recorded in samples after 72 h of incubation. The data indicated with different letters are significantly different.

| Chitin content | g/g flour | | |
|---|---|---|---|
| **NoH-CP** | 0.823 | ± | 0.01 a |
| ***Y. lipolytica* PO11** | 0.617 | ± | 0.008 b |
| ***Y. lipolytica* RO25** | 0.59 | ± | 0.009 c |
| ***D. hansenii* DB** | 0.504 | ± | 0.005 d |
| ***D. hansenii* SP6L12** | 0.793 | ± | 0.009 e |

**PO11, RO25 and DB strains:** reduced chitin content after 72 hours.
***Y. lipolytica* RO25:** Greatest chitin content reduction of 28%.

**Figure 4.** PCA elaboration of the total VMP of cricket-based hydrolysates in relation to the strain used.



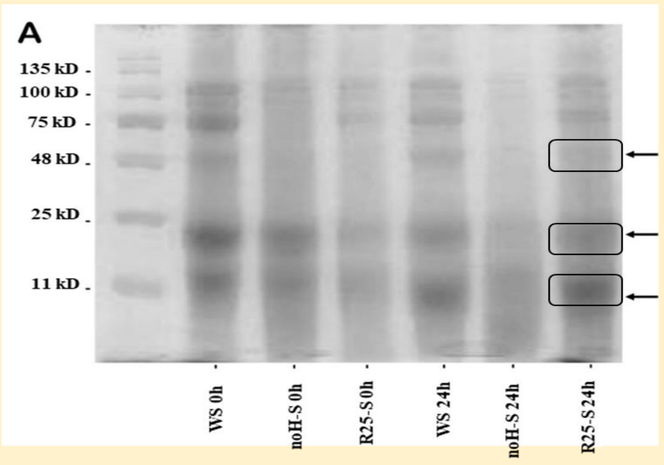

FFAs and FAA improved the sensory features of the raw material due to the production of many aroma compounds characteristic of ripened and fermented foods.

The **FA analysis** highlighted that all the considered strains enriched the total free fatty acid profiles of cricket hydrolysates with unsaturated fatty acids (data not sown).
The proteolytic activities of the strains increased the matrix digestibility and the release of **essential amino acids** such as Histidine, Threonine, Leucine and Ornithine (data not sown).

Each strain gave rise to a cricket-based hydrolysate characterised by a specific physicochemical, sensory and functional fingerprint.

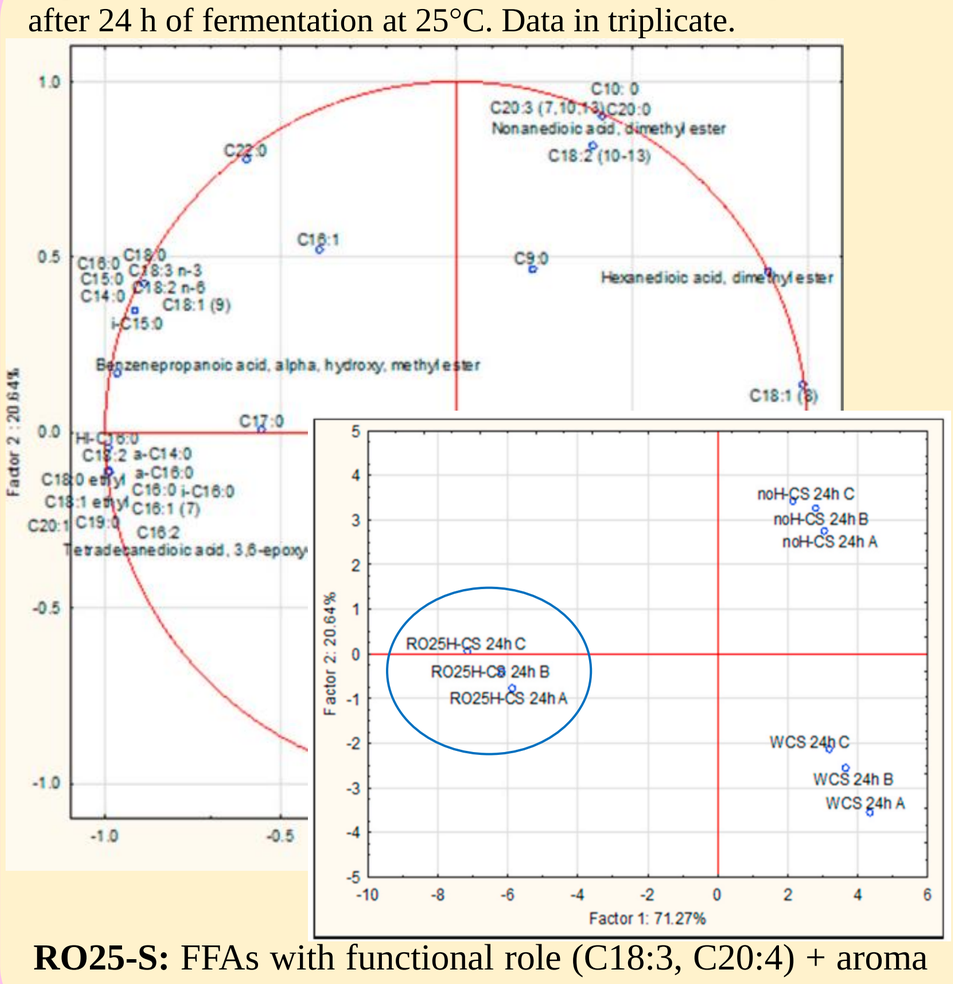
### Characterization of sourdough obtained using Y. lipolytica RO25 cricket-based hydrolysate as ingredient

**Figure 5.** SDS-PAGE containing extracted proteins in reducing conditions samples after second refreshment (0h) and after 24h of fermentation at 25°C.



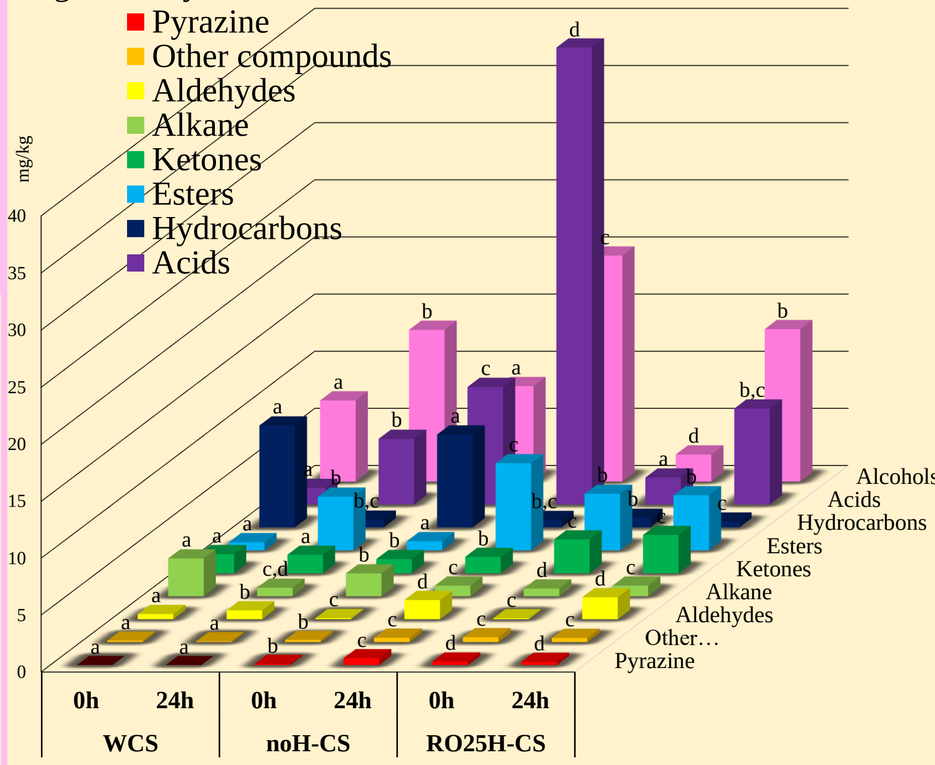

**RO25-S:** Marked and peculiar total protein profile attributed to the well-known proteolytic activities of *Y. lipolytica.*

**Figure 6.** FFAs' PCA elaboration of the three samples after 24 h of fermentation at 25°C. Data in triplicate.




**RO25-S:** FFAs with functional role (C18:3, C20:4) + aroma precursors (C18:2, C18:1,C16:1)

**Figure 7.** Principal classes of compounds (mg/kg) detected in the three samples, after second refreshment (0 h) and after 24 h of fermentation at 25°C. The data indicated with different letters are significantly different.




Identification of more than 60 molecules, underling a specific profile in the sample obtained by *Y. lipolytica* RO25 hydrolysed.

The results obtained showed the great potential of *Yarrowia lipolytica* RO25 to produce sourdough characterized by specific sensory and functional fingerprints that could be useful for innovative bread production with high nutritional and functional value.

**References**
[1]. Boselli E, Velazco V, Caboni MF, Lercker G (2001) Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. *J. Chromatogr. A*, **917:** 239-244.
[2]. Marco C, Pérez G, Ribotta P, Rosell CM (2007) Effect of microbial transglutaminase on the protein fractions of rice, pea and their blends, J. Sci. Food Agric. **87:** 2576-2582.
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