The 2nd International Electronic Conference on Foods

Future Foods and Food Technologies for a Sustainable World

University :

Agricultural University of Athens, School of Food and Nutritional Sciences, Department of Food Science and Human Nutrition, Laboratory of Microbiology and Biotechnology of Foods

Authors :

Georgios Tsekouras, Paschalitsa Tryfinopoulou and Efstathios Panagou

Detection and Identification of Lactic Acid Bacteria in Semi-Finished Beer Products Using Molecular Techniques

October 2021

1. Introduction

- Beer is an undistilled alcoholic beverage derived from a source of starch.

- Four main ingredients: water, malt, hops and yeast.
- Two main categories based on the yeast strain involved in processing:
    1. Lager beer - *Saccharomyces pastorianus*
    2. Ale beer - *Saccharomyces cerevisiae*

- Ale yeasts operate at room temperature (ca. 18-22 °C), ferment quickly and produce the characteristic "fruitiness" taste. (Top fermenters)
- Lager yeasts prefer lower temperatures (ca. 8-15 °C), ferment slowly and utilize more wort sugars, resulting in a cleaner, crisp taste. (Bottom fermenters)

Role of Lactic Acid Bacteria in Beer Processing (1/2)

- Deviations in the brewing process may occur due to the activity of LAB.
- The growth of LAB during the brewing process implies:
  1. Competition for nutrients with yeasts, causing decreased ethanol yields.
  2. Production of off-flavors (high indications of diacetyl and lactic acid).
  3. Changes in color.
  4. Excessive turbidity and viscosity alteration.

  } Quality degradation

- The metabolism of fermentation type could reveal that the spoilage is mainly caused by differences in amino acid and carbohydrate metabolism. According to the metabolic activity of LAB, they can be classified into three types:
  1. Obligately homofermentative.
  2. Obligately heterofermentative.
  3. Facultatively heterofermentative.

Role of Lactic Acid Bacteria in Beer Processing (2/2)

- Homofermentative LAB produce D-lactic acid while heterofermentative LAB produce D-lactic acid and acetic acid which are associated with spoilage due to the increase of acid content in the product.
  - acetic acid is perceived as vinegar and lactic acid as stale milk.

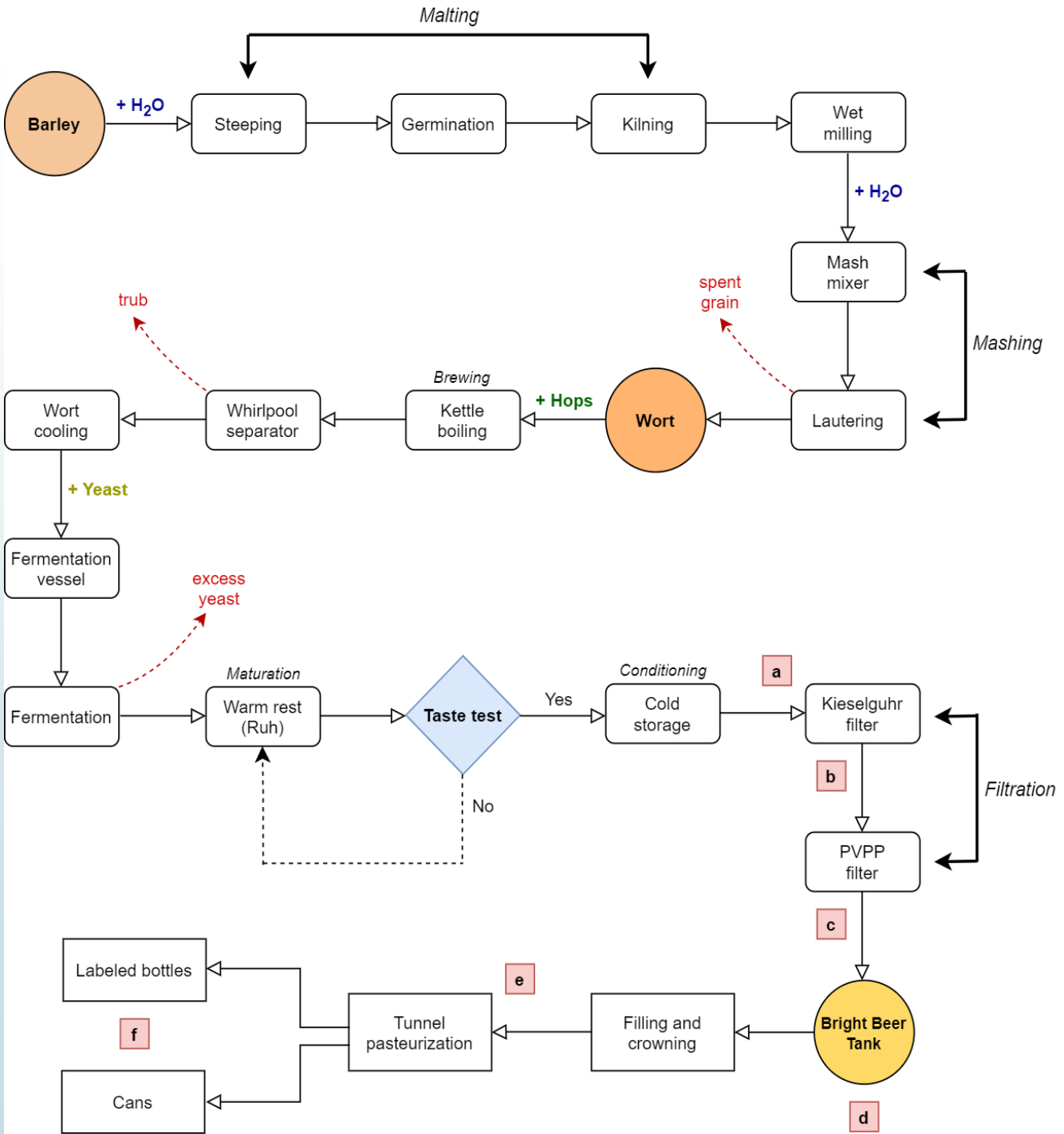
- Compounds produced from citrate metabolism, such as:
  1. Diketone.
  2. 2-3-butanedione.
  3. Diacetyl

  Low concentrations: Major source of pleasant flavor.

  High concentrations: Spoilage characteristic.

- Mannitol, which is produced by hef LAB (*Lb. brevis*) is not responsible for spoilage, but when it works together with acetic acid, D-lactic acid, n-propanol, 2-butanol and diacetyl, beer shows spoilage characteristics with viscous texture, sweet taste and vinegar-estery aroma.

Beer Production Pipeline and Sampling Points



2. *Materials and Methods*

2.1 Beer samples, transportation and fermentation procedures

- Samples were obtained from two different batch productions.

- Six sampling points: (a) pre-filtration
  (b) post-filtration
  (c) the buffer line
  (d) the filling tank
  (e) packaged non-pasteurized product
  (f) packaged pasteurized product

- Fermentation conditions: 10-14 °C for approximately 6 days.
- Maturation stages:
  1. Warm rest (Ruh) – Breakdown of unwanted volatile components.
  2. Cold storage (2-3 °C) – Yeast in suspension finalizes the flavor profile.

2. Materials and Methods

2.2 Microbiological analyses

- Determination of: i) total viable counts (TVCs)
  ii) yeasts
  iii) lactic acid bacteria (LAB)

Determination	Medium	Temp (°C)	Time (days)
TVCs	Plate Count Agar (PCA)	25	3
Yeasts	Rose Bengal Chloramphenicol agar (RBC)	25	3 - 5
LAB	de Man–Rogosa–Sharpe agar (MRS) *	30	5

*MRS added with cycloheximide 0.5%

- 20% of the LAB colonies from the proper dilution of the MRS agar plates were purified on the MRS (Cycloheximide-free) medium, followed by a second incubation phase at the same conditions.

2. *Materials and Methods*

2.3 Molecular analyses

- PCR technique: **Rep-PCR**
- Primer: **(GTG)5 –** (5-GTGGTGGTGGTGGTGGTG-3)
- Total number of LAB isolates: **80**

- Banding profiles of the rep-PCR products were undertaken by the electrophoresis, visualized after staining with ethidium bromide under ultraviolet light and were analyzed by the Bionumerics software.

- Finally, 20 representative isolates were selected for partial sequencing analysis of 16S rRNA region.

3. Results and Discussion

3.1 Microbiological results (Yeasts)

- Yeast presence was enumerated only before and after filtration.

	1st Batch	2nd Batch
Before filtration:	5.40 log CFU/mL	4.98 log CFU/mL
After filtration:	No enumerated (<1.0 log CFU/mL)	1.36 log CFU/mL

3. Results and Discussion

3.1 Microbiological results (LAB)

- Population of LAB was relatively low.
- LAB presence was detected only before filtration.

	1st Batch	**2nd Batch**
Before filtration:	1.52 log CFU/mL	3.44 log CFU/mL
After filtration:	No enumerated (<1.0 log CFU/mL)	No enumerated (<1.0 log CFU/mL)

3. Results and Discussion

3.2 Molecular identification of LAB

- Identified LAB species: i) *Lactobacillus brevis*
  ii) *Lactobacillus backii*
  iii) *Lactobacillus harbinensis*

Total Isolates	Species	Batch 1	Batch 2
80	*Lactobacillus brevis*	46.9 %	50.0 %
	Lactobacillus backii	6.2 %	39.6 %
	Lactobacillus harbinensis	46.9 %	10.4 %

4. Conclusion

- The present research elucidates the diversity of LAB isolated from industrially fermented and non-pasteurized Lager-type beer, one of the most economically important beer products in Greece.

- The relatively low population before the first sampling point (Kieselguhr filtration) indicates that hygienic conditions are partially successful.
- No enumerated population of LAB, after Kieselguhr filtration, advocates the production of a safe and high-quality final product.

- **Potential subject for further investigation:** Mechanisms that make it difficult for LAB cells to get over the Kieselguhr filter even though its pore diameter is multiple times wider than the dimensions of bacterial cells.

Thank you for your attention!