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Protein production from halophyte juice via lactic acid bacteria acidification and subsequent yeast fermentation

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BACKGROUND

Halophytes are **emerging supercrops** due to their large diversity and natural **high saline tolerance**. The main benefit lies in their suitability for **cultivation on marginal lands**, thus avoiding competition with traditional farm crops. The commercializing and cultivating of halophytes could significantly **improve the quality of areas affected by soil salinity, prevent further soil degradation**, and make use of their natural phytoremediation capabilities. However, the high salt content in halophyte biomass prevents them from being used directly for animal feed application, composting, incineration, and soil enhancement, resulting in halophytes being **treated as agricultural waste**. Nevertheless, in the scope of **green biorefinery**, halophytes can be fractionated and upcycled, as illustrated in **Figure 1**, thus maximizing their utilization and transforming them into **valuable bioresources**.

The present study explores the sustainable **production of fodder protein** through probiotic **lactic acid bacteria (LAB) and yeast cultivation** on **halophyte juice**, thereby overcoming its inherent salinity for future applications.

EXPERIMENTAL SET UP

Riasearch, Portugal, harvested the halophyte biomass on October 9th and 10th, 2023. The biomass was fractionated on October 12th, 2023, at the demonstration-scale grass processing facility, Aarhus University, Foulum, Denmark.

For microbial fermentation, 1 L of raw juice was supplemented with 20 g of glucose (VWR) and, if needed, autoclaved at 121°C for 15 minutes. Two methods were used to recover protein from sterile and raw juice with added glucose: 1) LAB fermentation and 2) LAB fermentation followed by yeast fermentation. In the case of LAB fermentation, 90 mL of juice in a 250 mL flask was inoculated with 10 mL of overnight pre-grown LAB culture in MRS broth and incubated under anaerobic conditions at 37°C and 130 rpm for 20 hours. For subsequent yeast cultivation, 50 mL of LAB-fermented juice in a 250 mL flask was inoculated with 0.10 g of *S. cerevisiae* (commercial dry yeast, Malteserkors tørgær, De Danske Spritfabrikker A/S, Denmark) and incubated under aerobic conditions at 30°C and 130 rpm for 20 hours.

Protein content was estimated from the total nitrogen content measured by the CHNS elemental analyzer (Pekin Elmer 2400 series CHNS/O). For the analysis of sugars, HPLC (1260 Infinity II, Agilent Technologies) was used with a Bio-Rad Aminex HPX-87H Column (Rio-Rad Laboratories Inc.) and a refractive index detector. The HPLC analysis of lactic acid was performed by (InfinityLab Poroshell 120 EC-C18, Agilent Technologies, Inc.), using a diode array as a detector.

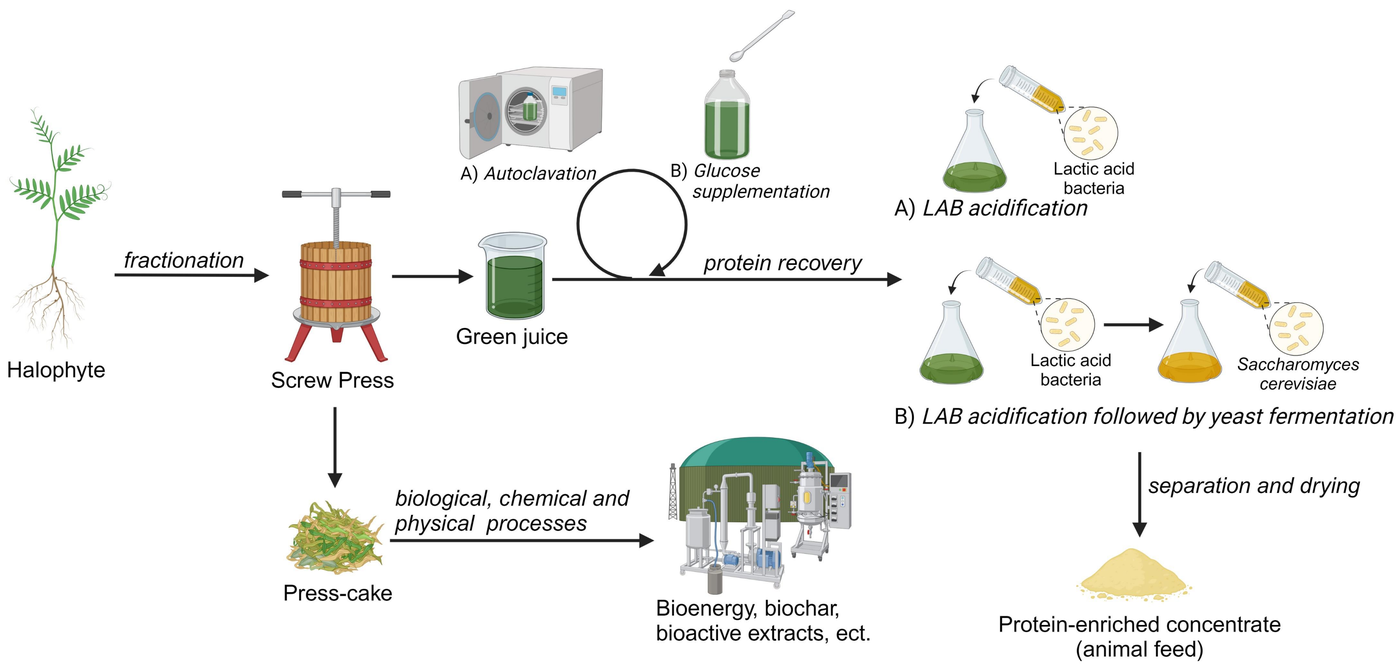

Figure 1. Proposed green biorefinery of halophyte plants

RESULTS

The compositional analysis revealed that green juice is high in **crude protein (CP)** but also in **ash (salts)**, in **Tab. 1**. Further investigation showed that **~70% of crude protein is solubilized** in green juice. The initial screening of protein precipitation via **LAB acidification did not result in significant protein yields**, in **Fig. 2A**. Moreover, **no correlation between pH reduction and protein precipitation** was observed, suggesting that other factors, such as salt and microbial proteases, may counter protein precipitation. The subjugation of LAB-fermented juices to *S. cerevisiae* cultivation resulted in considerable CP recovery rates of **92%, 95%, and 101%** after cultivation with *L. delbrueckii*, *L. paracasei* P 4155, and *L. lactis*, respectively, followed by yeast fermentation, as depicted in **Fig 2B**. The analysis showed that yeast was able to **consume all glucose and utilize lactic acid** to some extent, **increasing pH** as a result, as presented in **Fig. 3**. Furthermore, **sterilization** proved to be a significantly impactful step, **resulting in 22 to 58% higher CP recovery** compared to raw juice, shown in **Fig. 2B**. Additionally, the rapid yeast growth confirms that LAB-fermented juice is rich in easily **accessible protein**, likely **hydrolyzed and highly soluble**, thus **resistant to protein precipitation by LAB acidification**, but **easily assimilated by the yeast**.

Table 1. The compositional characteristics of halophyte juice

Parameter	Dry Matter (DM) (w/w %)	pH	Sugars (g/100g DM)	Protein (g/100g DM)	Ash (g/100g DM)
Halophyte juice	11.6	5.17	3.78±0.15	10.2±0.2	29.4±0.4

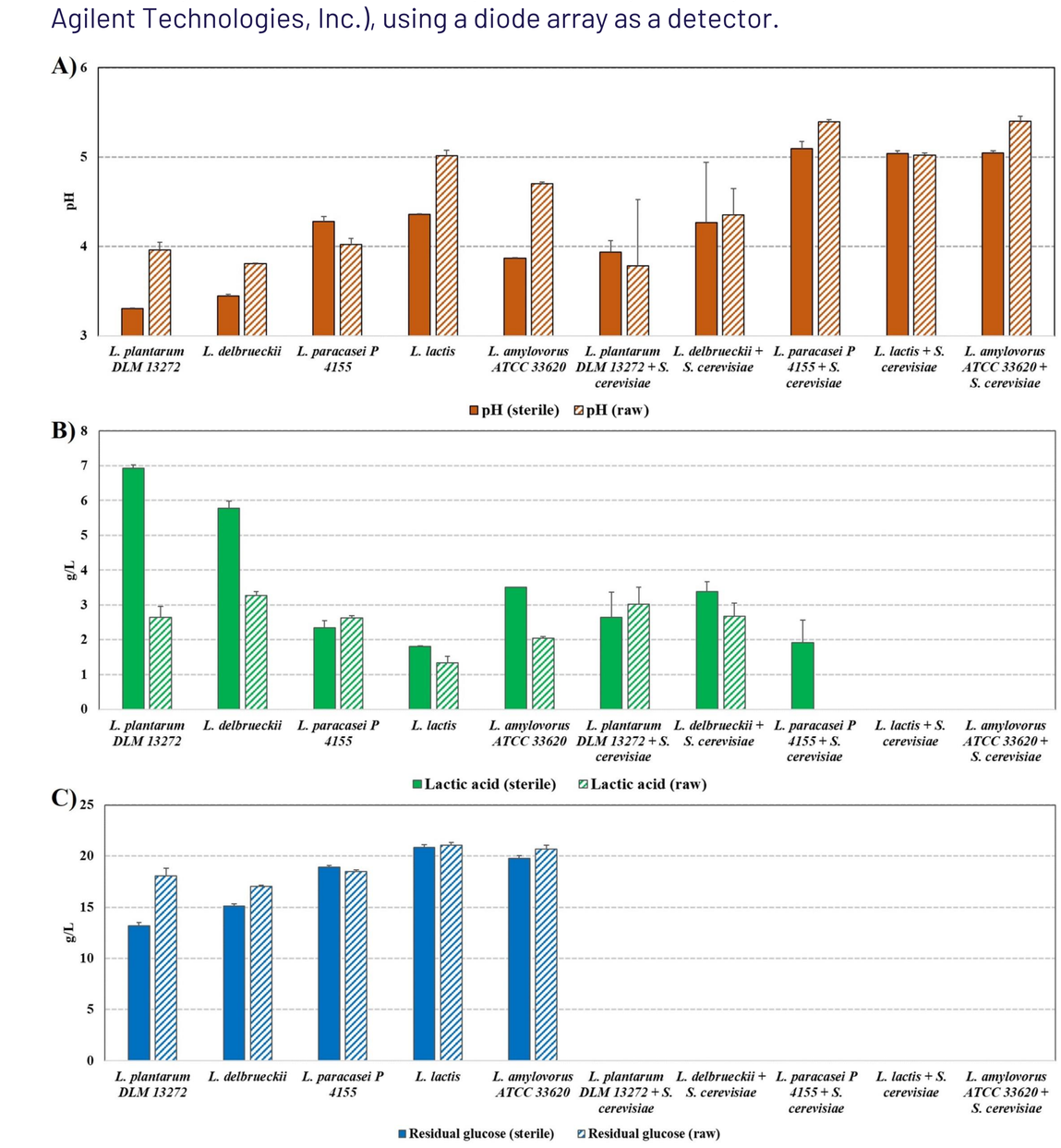

Figure 3. A) pH, B) Lactic acid, and C) Residual glucose in glucose-spiked halophyte juice after LAB acidification followed by *S. cerevisiae* fermentation

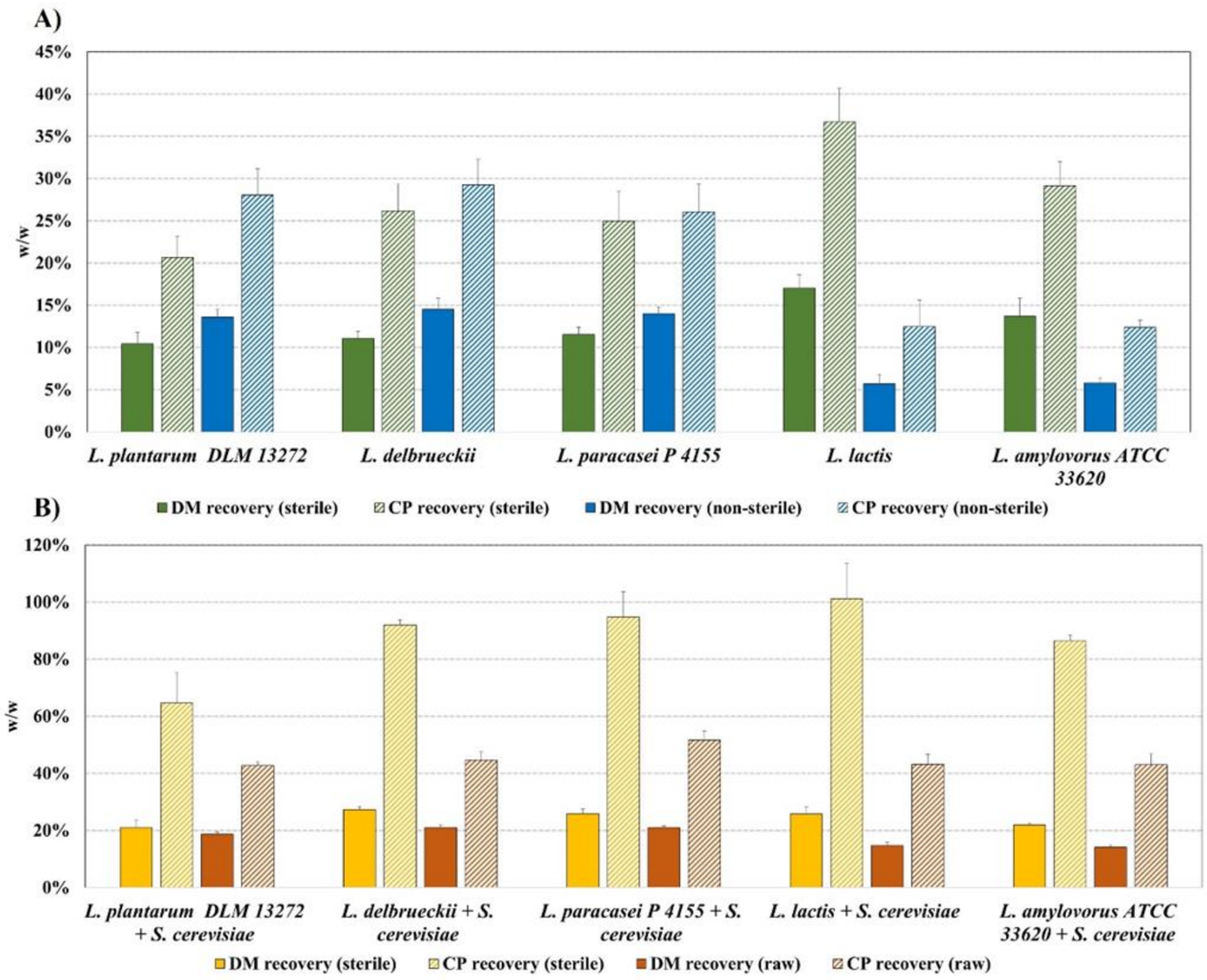

Figure 2. A) DM and CP recovery from glucose-spiked halophyte juice after LAB acidification and B) DM and CP recovery from glucose-spiked halophyte juice after LAB acidification followed by *S. cerevisiae* fermentation

CONCLUSION

To conclude, the presented findings underscore the potential of halophyte juice as a viable substrate for protein production through the synergistic advantages offered by the combined fermentation processes involving probiotic lactic acid bacteria and yeast, which could serve as an excellent animal feed additive, thus contributing to ecological farming and food security.

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