Freeze drying of beads containing yeasts

1. Introduction

Next to fermentation processes or transformation of foods, microorganisms such as Saccharomyces cerevisiae or lactic acid bacteria are of economic importance in the field of probiotic dietary food and feed supplements. These applications however require the preservation of cell viability during storage[1]. By combining granulation and freeze drying, dust free particles, homogeneous in size and composition can be obtained. This will enable a good particle flowability, an easier dosage and a faster reconstitution of the product due to a higher surface area. Despite some challenges, freeze drying remains a convenient method of preserving yeasts, sporulating fungi and bacteria since the long term viability remains usually rather good and the requirements for storage and distribution of the strains are quite simple[2].

This Short Note therefore aims to produce Saccharomyces cerevisiae particles as a model microorganism using the Encapsulator B-390 as a granulator to drip the yeasts droplets into liquid nitrogen and form beads that will then be freeze dried using the Lyovapor™ L-200.

2. Experimental

The work described in this Note was performed under aseptic conditions. 84 g of commercially available baker's yeast were suspended in 50 mL of sterile YPD Medium (Sigma Aldrich). 50 mL of sterile lyoprotectant medium containing 5 g of trehalose (Sigma Aldrich) and 5 g of skimmed milk in deionized water was then added to the yeast suspension before extrusion with the Encapsulator B-390. The extruded droplets were collected and frozen in a liquid nitrogen bath before being transferred in stainless steel trays and stored in a -25°C freezer until freeze drying.

The freeze drying steps (primary and secondary drying) were programmed using the Lyovapor™ L-200 Software as listed in Table 2. The Lyovapor™ L-200 Pro was used with the bell drying chamber, heatable shelves and ambient air.

Table 2: Parameters of the primary and secondary drying

	Primary Drying	Secondary Drying
Duration [min]	1200	360
Shelf temperature [°C]	-15	30
Vacuum [mbar]	0.200	0.200

After freeze drying, 1 mL of sterile water was added to 1 mL of beads in order to reconstitute the sample. For the bead containing yeasts, serial dilution of 10x, 100x and 1000x were performed for each reconstituted solution. The reconstituted solution and the dilutions were then plated on YPD agar plate and incubated at 28°C for 24h to evaluate cell viability.

3. Results

Results showed that yeasts could be granulated by dripping it in liquid nitrogen with the Encapsulator B-390; beads of around 700 µm and 1500 µm were produced with the 300 µm nozzle and the 1 mm nozzle, respectively.

After lyophylisation the beads remained similar in shape and size as the wet frozen beads.

The beads containing yeasts exhibit a rough structure made of 5 µm agglomerated particles that can be assumed to be the microorganisms, while the beads containing only lyoprotectant have a smoother structure.

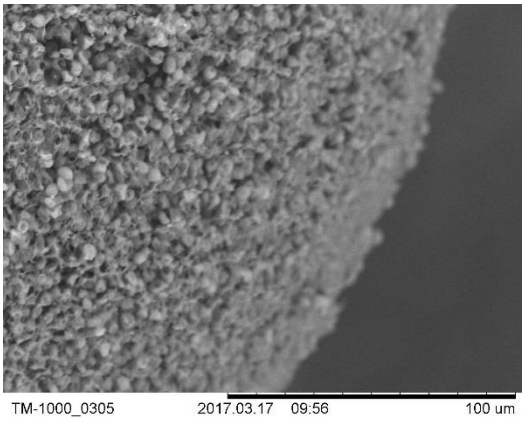

Figure 1: SEM picture of freeze dried beads containing yeasts

In order to verify yeast viability, the yeasts were rehydrated, diluted and incubated on YPD-agar plate at 28°C for 24 hours. Results confirm that despite a loss of viability, yeast can still grow after lyophylisation[3–6].

4. Conclusion

Yeast beads could easily be produced with the Encapsulator B-390 and freeze dryed using the Lyovapor™ L-200. Homogeneous beads of 700 µm and 1500 µm diameter were obtained using the B-390 with the 300 µm and 1000 µm nozzle, respectively. No change in size and shape of the beads were observed after freeze drying

References

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More information can be found in the BUCHI application note 294/2017