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Advances in osmoporation as a biotechnological tool for food ingredients production

Edilene S. da Silva[1], Camila D. F. de Medeiros[1], Eduardo Wagner V. de Andrade[1], Fábio G. M. de Medeiros[2], Roberta T. Hoskin[1,2] & Márcia Regina da S. Pedrini1[1]*

[1] *Laboratory of Food Engineering, Chemical Engineering Department, Universidade Federal do Rio Grande do Norte, Lagoa Nova, Natal, RN 59078-970, Brazil.*
[2] *Plants for Human Health Institute, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, North Carolina Research Campus, Kannapolis, NC 28081, USA.*
** Corresponding author's email address: marcia.pedrini@ufrn.br*

ABSTRACT

In the food industry, various techniques are used with the objective of producing food ingredients with preserved bioactive compounds. Osmoporation is an innovative approach to internalizing active substances into yeast cells. This technique is based on the yeast physical response to osmotic perturbations that allows for the internalization of target molecules. Thus, this review briefly presents the evolution of the research on osmoporation in recent years, as well as its mechanism of action, and prospects. It focuses on the scientific literature on osmoporation that demonstrates meaningful insights into the development of yeast-based release systems for the protection and delivery of sensitive phytoactive agents intended to produce food ingredients. Overall, here we show that in the last decade, relevant advances have been reported, regarding the optimization of key processing parameters and development of diversified protocols for expanded applications.

Keywords: yeast, biocapsules, microencapsulation, osmoregulation, phytochemicals.

1 INTRODUCTION

Natural phytochemicals are highly sought molecules with potential health-relevant applications for both the food and pharma industries. However, due to their excessive perishability, they normally require appropriate processing technologies that preserve their integrity. In this regard, encapsulation techniques have been widely used in the food industry due to their versatility and wide range of applications[1,2]. Spray drying[3], freeze drying[4] and liposome microencapsulation[5,6], for example, have been reported to provide enhanced protection to bioactive substances derived from fruits and vegetables that allow for their effective incorporation into complex food matrices, a promising strategy to deliver solutions that meet the increasing consumer demand for healthier products.

Yeast cell osmoporation is an innovative approach to take advantage of safe, readily available yeast cells for internalizing active substances. In this process, non-pathogenic eukaryotic microorganisms such as yeasts have been investigated as pre-formed, food-grade encapsulation systems to produce enhanced phytochemical-rich food ingredients. This technique is based on the physical response of the yeast cells to osmotic disturbances[7–9]. The internalization of target molecules into the yeast cell structure is possible due to temporary permeabilization of the plasma membrane that occur when yeast cells are dehydrated in a non-lethal osmotic pressure medium and rapidly rehydrated to an iso-osmotic medium. Intrinsic attributes of yeast cells such as envelope and cell wall strength and rigidity make them interesting structures as osmoporation encapsulating agents for phytochemicals of interest[10]. Moreover, osmoporation is considered a simple method because it does not require dedicated equipment and/or expensive materials[9]. Therefore, this review presents the development roadmap of osmoporation, its mechanism, recent advances, and prospects for its application to produce food ingredients.

2 HISTORICAL OVERVIEW

The osmoporation protocol for the internalization of bioactive substances into *Saccharomyces cerevisiae* cells was initially reported in 2014 by Pedrini et al. [9]. The authors used hydrophilic FITC-Dextran (20 kDa)[9] to demonstrate the material transfer from the extracellular medium to the intracellular medium due to the temporary plasma membrane permeability resulting from the stretching of the *S. cerevisiae* cell wall, and validate using *S. cerevisiae* cells as bioagents for encapsulating molecules of interest through non-lethal osmotic shocks.

Since the establishment of the method, osmoporation has continued to improve. Câmara et al. [11] developed an enhanced protocol for internalizing fisetin in *S. cerevisiae* by investigating the effects of fisetin concentration, osmotic pressure and operating temperature to maximize encapsulation efficiency. In this study, hydrophobic fisetin (286 Da; [11]) was used as model compound to study cell osmoporation to produce yeast cell biocapsules. The outcomes of this study opened new perspectives for the use of phytochemically enriched yeast cells for the food industry.

In a follow-up study carried out by Medeiros et al. [12], a modified, more efficient protocol was proposed that included sequential cell osmoporation stages. The impact of sequential osmporation stages on the internalization of fisetin and curcumin into *S. cerevisiae* cells and on cell viability was evaluated. As a result, the proposed improved protocol enabled remarkably higher

concentration of polyphenol compounds internalization into the cells. However, studies on the physicochemical characterization of the produced biocapsules were still lacking.

To address this knowledge gap, Medeiros et al. [13] investigated the thermal and photochemical stability of curcumin in osmoporated yeast biocapsules. The thermal stability results showed that the encapsulation into yeast cells raised the degradation temperature of curcumin to 207°C. Moreover, the antioxidant activity of the encapsulated curcumin into osmoporated yeast cells was kept over 80% after heat treatment (150 °C) and over 70% after exposure to artificial light (50 days). The photochemical stability of curcumin encapsulated in yeast increased 5.7 times and the half-life time reached 181 days under light exposure.

In view of these favorable results using yeast cells, the osmoporation studies were expanded to include other species of microorganisms such as probiotic bacteria. Thus, the performance of *Lactobacillus acidophilus* cells as encapsulating carriers for fisetin via osmoporation was evaluated by Andrade et al.[14] , considering the effects of osmotic pressure and the initial fisetin concentration. After encapsulation, the degradation temperature of fisetin increased by 40°C, according to differential scanning calorimeter thermograms. Furthermore, this was the first report in the literature on the *in vitro* bioaccessibility of fisetin into biocapsules (99.6%-gastric phase; 45.5%-intestinal phase).

Continuing their research into the use of probiotic bacteria, Andrade et al. [15] investigated the first application of fisetin-enriched biocapsules to produce one of the most popular fermented food products in the market today, yogurt. For this, osmoporated *L. acidophilus* containing fisetin were used as the starter culture and compared to non-osmoporated bacteria cells. It was observed that the milk acidifying process occurred at a slower rate and the antioxidant activity of yogurts produced with fisetin biocapsules was kept constant during refrigerated storage. On the other hand, yogurts made with non-encapsulated fisetin showed a 2.5-fold reduction in antioxidant activity after 28 days.

Further studies compared osmoporation with other food processing techniques. Andrade et al. [16] demonstrated that sonoprocessing coupled to drying can efficiently encapsulate fisetin into *S. cerevisiae* cells. Parameters such as the cell density, fisetin concentration and acoustic energy density were studied to maximize the internalized fisetin content. The use of spray drying and freeze drying led to improved fisetin encapsulation for both drying protocols. However, spray drying resulted in higher encapsulation efficiency (+ 11.5%), encapsulation yield (+ 11.1%) and antioxidant activity (+ 26.6%) compared to freeze drying.

3 OSMOPORATION MECHANISM IN *S. CEREVISIAE* YEAST CELLS

Osmoporation is based on the osmoregulatory mechanisms of non-pathogenic yeast cells. When yeast cells are subjected to high osmotic pressure environments, they dehydrate, promoting an outwards flow of water that causes shrinkage of the cell volume and wrinkles in the plasma membrane. Thus, the subsequent exposure of dehydrated cells to isotonic environments leads to a temporary permeabilization of the plasma membrane that allow for rapid cell rehydration, which is achieved through the mass transfer of significant amounts of water from the extracellular to the intracellular medium. When the cell rehydration occurs in the presence of the compound of interest (bioactive/phytochemical molecules), the mass transfer across the plasma membrane promotes a forced transfer of these substances to the interior of the cells [9,13], i.e., the osmoporation of target molecules.

Pedrini et al. [9] used FITC-Dextran, a fluorescent water-soluble polysaccharide of high molecular mass (20 kDa), to understand this transport phenomenon through the plasma membrane of *S. cerevisiae* yeasts. Results showed that shock dehydration with a 30 MPa water-glycerol solution, followed by shock rehydration with an isotonic water-glycerol solution (1.4 MPa) containing FITC-Dextran, was able to deliver the FITC-Dextran to the cell intracellular space. Objectively, it was observed that: i) the molecule not only penetrated the cell wall, but also the plasma membrane; ii) the process occurred through permeabilization of the entire length of the plasma membrane, rather than simple cell endocytosis, since, if this were the case, the content would have been trapped in vesicles within the cytoplasm.

Osmoporation is considered a promising method for incorporating bioactive compounds into *S. cerevisiae* cells. This is due to its ease, versatility, short processing time and use of food grade reagents. It has also been shown that cell osmoporation does not affect cell functionality or integrity [11]. According to Dardelle et al. [17], yeast cells allow for the controlled release of the encapsulated content and protect it against significant temperature changes, up to 200 °C. This shows that the use of this type of protocol is more effective in situations where the aim is to maintain these compounds in the cell structure compared to natural endocytosis.

4 RECENT ADVANCES

Recent advances include applications related to the osmoporation process and the application of biocapsules produced from osmoporation in food. Sonoprocessing has emerged as a gentle, non-thermal technology that can alter the permeability of the yeast wall membrane as a result of turbulence, cavitation, microstreaming, dynamic agitation and applied shear stresses. Specifically, as the cavitation energy disrupts the conformational matrices of yeast envelopes, pores are created on the cell surface and a thinner lipid membrane is formed. This facilitates the passage of phytoactive substances through the biotransporter [18]. The sonoproduced food ingredients were investigated for their ability to protect fisetin in the gastrointestinal tract and against harmful environmental factors such as heat, light, and humidity. The hypothesis raised by Andrade et al. [19] was that the composition of yeast cells (such as β-glucan, chitin, protein and cytoplasmic materials) would protect fisetin from environmental and gastrointestinal-induced degradation. These inclusion interactions resulting from sonoprocessing combined with drying mechanisms, such as internal body clustering and cell shrinkage, and osmoporation, would increase the encapsulation efficiency and provide better protection of the target compound.

Another advance is the application of biocapsules to produce yogurt enriched with fisetin using the osmoporation starter culture of *Lactobacillus acidophilus*-based bio-capsules [15]. This technique proved to be a versatile encapsulation bioprocess that allows preserved phytoactives to be effectively delivered to fermented foods such as yogurt. The use of lactic acid bacteria as both the encapsulation matrix and fermentation agent is an approach that can be expanded to other applications in the dairy sector.

5 FUTURE PERSPECTIVES

Future studies should be conducted to determine whether this method can effectively preserve phytoactives of varying sizes, polarity, and complexity utilizing probiotic strains and other cell-derived carriers. Further research should be conducted on the optimization of osmoporation parameters and the introduction of sonoproduced capsules for food ingredients production. More studies assessing the performance of other yeast strains as biocarriers on the efficiency of osmoporation coupled to sonoprocessing and spray drying (OSPSD process) must be carried out. In this sense, implementing the OSPSD protocol in a batch configuration or larger scale will reveal the industrial challenges that need to be addressed for other uses. Taken altogether, osmoporation is a promising biotechnology strategy to produce innovative and functional products using microorganisms-based materials.

6 CONCLUSION

This overview shows the most relevant osmoporation studies over a decade of research. Osmoporation is a simple method for internalizing phytochemical molecules into food-grade microorganisms such as viable yeasts or lactic acid bacteria, as it requires only widely available equipment and supplies. Sonoprocessing is a promising process to improve conventional osmoporation and widen its food applications using not only yeast, but other species of microorganisms. Overall, this report unveils new possibilities for the development of food ingredients and products derived from microorganisms (yeasts and lactic acid bacteria), as well as it shows realistic osmoporation applications directed to the protection and delivery of sensitive phytoactives using cell-based release systems.

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