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Review

Emulsion stabilised by yeast proteins and biomass: a mini review

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There has been a growing demand for affordable and environmentally sustainable, alternative sources of proteins to feed the growing human population, promoting planetary health. Among various single-cell proteins (SCPs), yeast biomass shows an untapped potential for food use. This review discusses our current understanding of technofunctional performance of yeast proteins either extracted from the cells or SCPs via exploiting whole cells, mainly focusing on their surface properties. We cover how yeast biomass, extracted yeast proteins, or cell wall–bound mannoproteins have been used to design molecularly adsorbed conventional emulsions or Pickering emulsions. In-depth characterisation of interfacial, rheological and tribological properties of yeast proteins is a necessary undertaking to allow rational design of yeast protein–derived colloidal food formulations for a sustainable future.

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Introduction

Food production and choice of diet have a huge impact on the environment and sustainability. Meat and animal products are a popular source of protein in the diet for much of the population on Earth. With the exponential growth in population, estimated to touch 10 billion by 2050, comes the challenge of providing human protein requirements [1] without putting undue strain on the environment [2]. Focusing on food sector, two major studies [3,4] in the last 5 years have quantified greenhouse gas (GHG) emissions with Poore and Nemecek [3] attributing 26% of anthropogenic GHG emissions to food systems, whilst Crippa et al. [4] place this figure at 34%. Production of meat and dairy proteins results in orders of magnitude higher GHG emissions compared with their plant counterparts [3]. To reduce carbon footprint, the 2019 EAT-Lancet Commission suggests a diet with alternative, sustainable sources of proteins, which should reduce reliance on animal-farmed proteins whilst maintaining a balance between human health and environmental sustainability [5]. Although food and feed sectors focusing on human and animal nutrition, respectively, are front runners in this area, use of microbial biomass is also not uncommon in nutraceutical and biodiesel sectors [6]. Grossmann and Weiss defined alternate proteins as those that are "*produced from sources that have low environmental impact to replace established protein sources*" [7].

Plant proteins mainly dominate the alternative protein space in literature. Although biotechnology has a long history of producing feeds and foods, fungi (including yeast), bacteria and microalgae themselves have rarely been reviewed with respect to their functional performance [8]. These single-celled micro-organisms (except for certain species of fungi that are filamentous) also referred to as single-cell proteins (SCPs) are used as whole cells as the protein source without extraction or purification in most cases. The production of SCPs was important during World War I and II where yeast SCPs were produced in Germany to combat food shortages. After that, SCPs further captured attention in 1977 when the Imperial Chemical Industries in the United Kingdom manufactured the microbial protein named Pruteen from *Methylophilus methylotrophus* bacteria that was used in animal feed [9]. However, it was only in 1985, when SCPs became a readily accessible, commercially available food product when Marlow Foods (UK) launched its microbial protein-based food product, Quorn^{TM}, made from the fungi *Fusarium venenatum* [10].

SCPs produced in controlled environment can be of great interests to provide protein for future. However, their functional performance such as their interfacial performance at oil–water interface, bulk rheological properties and tribological properties have been far less explored to date. Interfacial stabilisation of emulsions and foams has traditionally relied on the use of low-molecular-weight synthetic surfactants or emulsifiers derived from animal-based proteins, such as dairy (e.g. *β*-lactoglobulin, casein) and egg proteins (ovalbumin). In addition, rheological and tribological properties can offer a quantitative understanding of their mouthfeel performance [11], where alternative proteins tend to be inferior to animal proteins [12–14]. Hence, showing relevant functional performance is crucial before microbial proteins become a regular ingredient in food application. Recent studies on microalgae such as *Arthrospira platensis* (Spirulina) and *Chlorella protothecoides* have demonstrated the ability of the biomass (whole cells) and extracted protein fractions to stabilise oil-in-water emulsions at different protein concentrations [15]. Additionally, Firoozmand and Rousseau [16] have demonstrated that intact yeast (*Saccharomyces cerevisiae*) and bacterial (*Lactobacillus acidophilus* and *Streptococcus thermophilus*) cells act as Pickering particles to stabilise oil-in-water emulsions. Among the microbial community, yeasts are interesting source of inexpensive, microbial proteins because of their proficiency for vigorous growth and have been used as a food ingredient for baking and fermentation industries for centuries [17] but have not been reviewed with respect to their functional performance in food structure.

The aim of this review is to provide a concise understanding of the functional performance of yeast protein in food structure. We first discuss the structural features of yeast and question whether the intracellular proteins derived from the degradation of yeast cells or mannoproteins at the surface of yeast cells provide the emulsification properties in yeast protein-stabilised emulsions. Furthermore, we will compare the proteins, where relevant to alternative plant proteins. Noteworthy, any protein engineering done using yeast as a host organism or genetic manipulation is out of scope for this review [18]. We examine the very few studies on the functional performance of yeast biomass and yeast protein isolates (YPIs) that have surfaced in the literature in the last few years, clearly pinpointing the mechanisms of action, separating the molecularly adsorbed yeast protein at the droplet surface from the yeast cell-stabilised Pickering emulsions. For composition, nutritional properties and bioactivities of yeast proteins, readers may refer to other reviews [17,19]. Finally, we highlight perspectives for future studies for unlocking the full potential of yeast biomass as a functional ingredient in food applications.

Yeast structure, biomass production and protein extraction

For food applications, yeast is almost synonymous with *Saccharomyces cerevisiae* or *S. cerevisiae*, a unicellular, eukaryotic organism widely used in fermentation and brewing industries. In food research, other species have been utilised, such as *Saccharomyces sp.*, *Candida sp.*, *Kluveromyces sp.* etc., in creating model emulsion systems (see Table 1).

Yeast structure

S. cerevisiae cells are oval or ellipsoidal in shape (Figure 1a); their size can vary between 1 and 10 μm in diameter [20] and generally have a negative surface charge (ζ-potential ranging from −9 mV to −30 mV) [21,22] largely associated with the cell wall biopolymers.

A dynamic cell wall is being constantly remodelled [26] in response to various environmental stresses and cell growth. This wall is about 200 nm thick, composed of an elastic three-dimensional network of β-1,3 glucan dominating the cell wall (50–55% wall dry weight) followed by chitin (1–2% wall dry weight) in the inner layer of the wall and close to the cell membrane [27,28], whilst the outer layer of the cell wall consists of proteins (cell wall proteins) of wide range of molecular weights (Figure 1b). These proteins are of two types, glycosylphosphatidylinositol (GPI)–linked proteins and proteins with internal repeats (Pir). β-1,6-Glucans covalently link the GPI-anchored proteins to the inner layer of the cell wall proteins, that is, to the β-1,3-glucan-chitin network [29], whilst the Pir proteins are directly linked to the reducing end of β-1,3-glucan through an alkali-sensitive linkage [28]. Within the outer layer of the yeast cell wall, highly glycosylated mannoproteins are found to contain 15–90% glucans by weight (35–40% of the cell wall's dry weight). Overall, the collective volume of the whole yeast cells that have been propagated during fermentation, after removal of the growth media, is known as the biomass.

These yeast cells can then undergo further downstream processing to yield yeast protein powder or mannoproteins, both exhibiting intriguing functional characteristics (described later). Compared with alternative plant proteins, yeast proteins emerge as a favourable option due to their naturally higher overall protein content (ranging from 40% to 60% on a dry weight basis of yeast cells) and balanced essential amino acid profile that exhibits high digestibility, typically around 85% [19,25,30].

Production of yeast biomass and extracted yeast proteins

Yeast biomass can be produced by propagating cells in glucose-rich growth media, with a rich nitrogen source, under controlled temperature and oxygen conditions (2:1

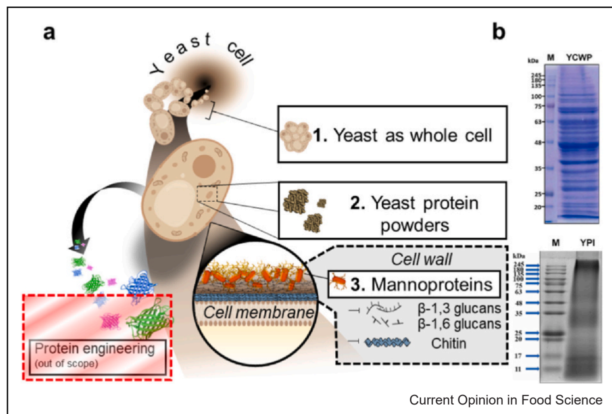
Table 1

Protein extracted from yeast (highlighted in blue) or whole yeast cells (biomass) used in literature to stabilise emulsions in the last few years.

Strain	Yeast protein type as the surface active agent	Oil type	Characterization	References
S. cerevisiae	Whole cell suspension	Kerosene (33.3 vol%)	Visual observation (pH, NaCl treatment), SDS-PAGE*	[41]
S. cerevisiae	Whole cell suspension (with defects in cell wall synthesis)	Kerosene (16.67 vol%)	Visual observation, optical microscopy	[40]
S. cerevisiae	Whole cell suspension (active or heat inactive or washed)	Hexadecane (30 vol%),	Visual observation, confocal laser scanning microscopy, static light scattering, viscosity, contact angle, interfacial tension, zeta-potential, rheology	[21, 35, 36]
S. cerevisiae, S. boulardii	Inactivated whole cell suspension	Medium chain triglyceride (MCT) oil (50 wt%)	Cell sphericity, optical microscopy, scanning electron microscopy, static light scattering, interfacial tension, zeta-potential, viscosity, viscoelasticity, EAI*	[22]
S. cerevisiae	Inactivated whole cell Suspension	Olive oil (60-70 wt%),	Visual observation, confocal laser scanning microscopy, viscosity, contact angle, static light scattering	[16]
S. cerevisiae, S. boulardii, Kluyveromyces marxianus	Yeast extract after cell lysis	Sunflower oil (33.3 vol%)	EAI*, ESI*, volatile analysis, sensory analysis, amino acid profile	[43]
Candida albicans	Cell wall protein extracted using enzymatic treatment preventing synthesis of GPI-anchor or β-1,3-glucan	Kerosene (33.3 vol%)	Visual observation, SDS-PAGE, mass spectrometry	[24, 40]
S. cerevisiae	Mannoprotein extracted from yeast cell wall by enzymatic treatment	Iso-octane, kerosene (60 vol%), Olive oil (50 wt%)	Capillary electrophoresis, protein assay, creaming height measurement (pH 2-11), electron microscopy, DSC, NMR, amino acid assay, viscosity, viscolelasticity, visual observation	[45-47]
Yarrowia lipolytica	Bioemulsifier extracted from yeast cells (lipid–carbohydrate–protein complex, Yansan)	Perfluoro-n-hexane, n-hexadecane and toluene (15-30 wt%)	EAI* (pH), interfacial tension, contact angle, zeta-potential, protein assay, static light scattering, FTIR*, XPS*	[48, 49]

Abbreviations: EAI: emulsifying activity index; DSC: differential scanning calorimetry; ESI: emulsion stability index (ESI); FT-IR: Fourier-transform infrared spectroscopy; NMR: nuclear magnetic resonance spectroscopy; SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; XPS: X-ray photoelectron spectroscopy.

Figure 1



Schematical overview **(a)** showcasing the approaches of using yeast as emulsifiers in food. (1) Yeast is used as a whole cell or processed to extract (2) intrinsic yeast protein as a powder or (3) mannoproteins are extracted from the yeast cell wall to emulsification. (1) results in Pickering emulsion, whereas (2) and (3) results in conventional molecularly adsorbed emulsions. The proteins that are metabolically engineered using yeast cells as host organisms (cell factories) for protein production [23] are out of scope for this review as indicated in the red box. SDS-polyacrylamide gel electrophoresis **(b)** of protein fraction comparisons are presented, featuring the yeast cell wall protein [24] and YPI [25] alongside corresponding reference marker (M) and molecular weight (kDa) indicators.
Figures in **(b)** are adapted with permission from Elsevier [24,25].

or 2.5:1 air/liquid ratio being commonly used; Figure 2a). The biomass can then be subsequently concentrated into functional yeast protein powders encompassing diverse protein fractions, such as actin, tubulin, histones (H2A, H2B, H3, H4, H1), ribosomal proteins, hormones, transporters, signalling receptors, motor proteins (myosins, kinesins, dynein), many functional enzymes (e.g. invertase) and mannoproteins located in the outer cell wall [20]. These mannoproteins have then been exclusively extracted through mechanical, chemical or biochemical means from the cell walls. Mechanical treatment includes bead mills, high-pressure homogenisation and ultrasonication [31] that mainly lyse the cell wall resulting in a mixture of β-glucan and mannoproteins, which may need further purification. In addition, such harsh treatment may denature the yeast proteins, which needs more investigation in the future.

Based on chemical extraction process, mannoproteins can be divided into two classes, which results in differences in the resultant molecular weight and properties: (1) sodium dodecyl sulphate (SDS)-extractable mannoproteins and (2) glucanase-extractable mannoproteins [31]. The molecular weights of mannoproteins may range between 20 and 450 kDa [32] (Figure 1b). To date, 36 members of mannoproteins have been identified in the yeast cell walls [33] with molecular weights mainly ranging in 34–59 kDa [34] independent of the extraction process (e.g. thermal treatment 90°C/4 hours), chemical treatment via addition of SDS and analysis by fermentation (room temperature/72 hours). This suggests that the mannoproteins of molecular weight range similar to plant-based glycoproteins, such as patatins, do exist in yeast cell walls [12]. Concerning mannoproteins, their association with the β-1,3-glucan-chitin complex and the diverse array of extraction methods pose challenges in achieving a reproducible variant with a specific, narrow molecular weight range. These are the bottlenecks hindering the development of a specific commercial mannoprotein-rich YPI ingredient and consequently limits research in the functional performance of extracted YPI unlike other alternative proteins.

Emulsification performance of yeast-derived proteins and yeast biomass

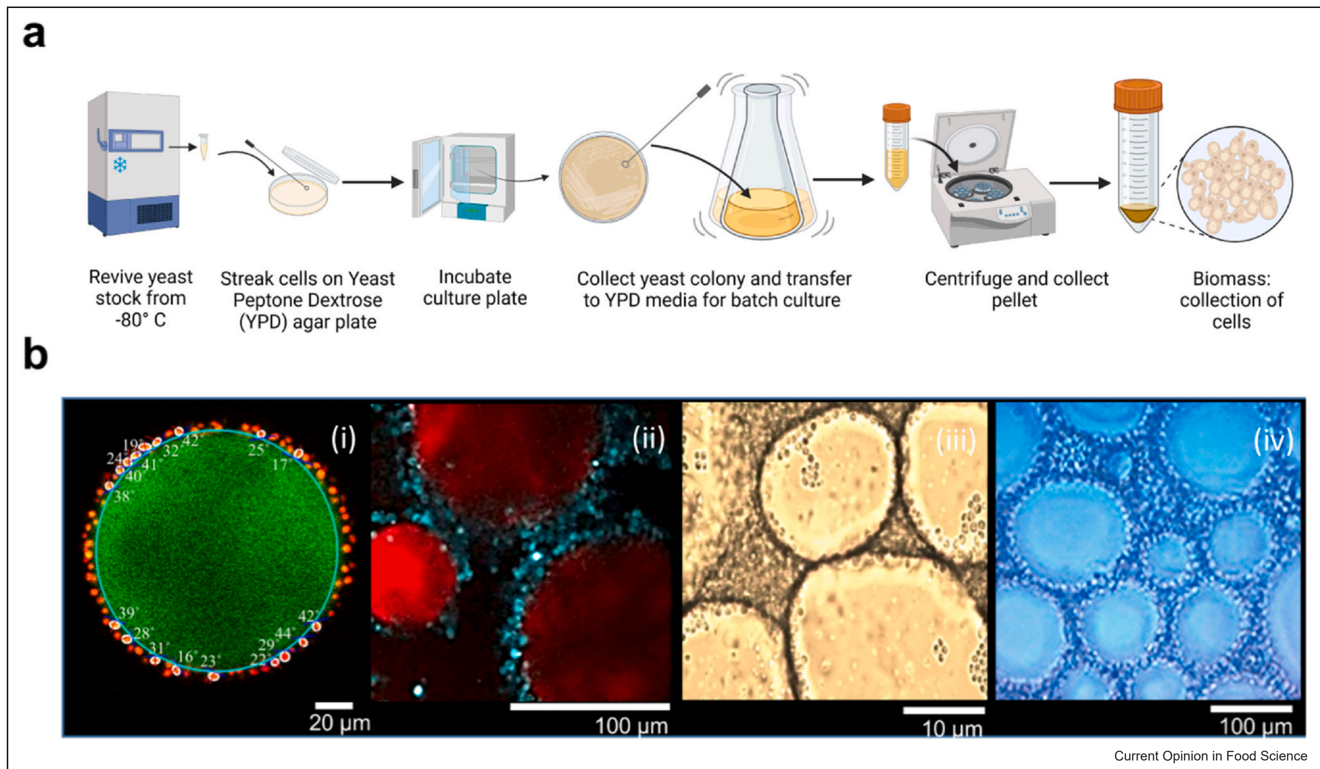
Yeast biomass and extracted yeast protein (Figure 1a) boast a number of emulsifying properties. These include Pickering-stabilised droplets from yeast as biomass [16] (Figure 2b) as well as utilisation of yeast proteins and mannoproteins, the latter of which have almost exclusively been exploited for their emulsification purposes [28,37,38]. Indeed, it could be these surface proteins or mannoproteins that facilitate the formation of such biomass-stabilised emulsions that emerge upon droplet homogenisation (discussed later) [35].

We discuss yeast in various forms that have been used to stabilise emulsions (Table 1). We categorised the discussion in this section, focusing on the whole cells/biomass that are used to prepare (1) Pickering emulsions with cells as particle stabilisers (Figure 2bi–iv), followed by the cell wall–extracted mannoproteins that are used to prepare (2) molecularly adsorbed conventional emulsions. Although there is a recent study using YPI and comparing with plant proteins (Figure 1b) [25], it includes surface hydrophobicity with no information of stability of emulsions prepared using it. It also remains unclear whether the protein was isolated from the whole cell or the YPI included only mannoproteins from the cell wall.

Pickering emulsions

Although the first evidence of yeast cells stabilising Pickering droplets dates back from 1970s from oil sector [39] and many studies thereafter involved using kerosene [40,41] and hexadecane [21,35,36] to showcase Pickering effects of yeast cells (Table 1), the first so-called food-grade Pickering emulsion using olive oil as the dispersed phase was fabricated in 2016 using inactivated whole yeast cells (Figure 2bi) [16]. The heat-inactivated *S. cerevisiae* cells of 2–15 μm formed droplets in the range of 50–350 μm, in line with the usual rule of thumb of Pickering droplets being 50–100 times larger than those of the Pickering stabilisers. The contact angle

Figure 2



Schematic of a typical batch process **(a)** for producing yeast biomass in laboratory scale (created with BioRender.com) and **(b)** micrographs of *S. cerevisiae* whole yeast cells used as Pickering stabilisers for oil-in-water emulsions using (i) olive oil [16], (ii) hexadecane [35], (iii) medium-chain triglycerides [22], (iv) hexadecane [36] dispersed phase, respectively. ii is stabilised by washed yeast cells.
Figures in **(b)** are adapted with permission from Elsevier [16,35,36] and Springer Nature [22].

was estimated to be ~30°, highlighting these hydrophilic particles were capable of forming oil-in-water emulsions that were stable. Noteworthy, the emulsions were highly shear thinning, had a self-supporting architecture highlighting a gel-like behaviour and showed high resilience to droplet coalescence for over 4 months [16]. Such weak gel-like behaviour with elastic modulus (G′) exceeding the viscous modulus (G″), even using lower droplet volume fraction (50 vol% medium-chain triglyceride oil) has been observed in another study [22] using whole yeast cells as Pickering stabilisers, highlighting the importance of cell-entangled network.

Questions may be raised whether such high storage stability was due to the *true* Pickering nature of these yeast cells adsorbing to the droplet surface and/ or the yeast cell–cell forming a jammed network in the continuous phase interlocking the high volume fraction of oil droplets (60–70 oil droplet vol%) in close vicinity, raising the continuous viscosity. In fact, bridging phenomena between particles attached to different droplets particularly in case of systems containing high volume fraction of droplets as well as particle–particle jamming in the continuous phase contributing to emulsion stability due to bulk stabilisation rather than *true* Pickering effects have also been commonly seen in ultrastable emulsions stabilised by plant-based aggregates and microgels [42].

Nevertheless, an elegant study [35] using washed inactive yeast cells with low volume fraction of hexadecane (30 vol%) confirmed that washed yeast cells without heat inactivation could reduce interfacial tension and readily adsorb to the oil droplets via a true Pickering phenomenon (Figure 2bii). Nevertheless, such surface adsorption did not contribute to more stability, whereas the presence of mannoproteins and other emulsifying molecules in the unwashed yeast cells offered longer stability. Also, it is worth pointing out that the type of organism and cell shape may also affect their interfacial properties. A recent study [22] has shown that *S. boulardii* does not reduce interfacial tension significantly unlike *S. cerevisiae*, which is likely attributed to the larger size, ellipsoid shape of the former and difference in cell wall composition between the two organisms. Nevertheless, this did not result in difference in their Pickering stabilisation property — both cell types adsorbed to the droplet surface (Figure 2biii). Overall,

there are speculations about the importance of mannoproteins or intrinsic cellular proteins that might leach out during either heat inactivation of the cells or homogenisation steps of the emulsion formation, which affect the adhesion of the cells to the droplet surface [16,21]. In summary, definitive studies about the role of mannoproteins in the cell surface contributing to the surface adsorbing capacity of the yeast cells in Pickering emulsion remain limited in the literature.

Molecularly adsorbed conventional emulsions

Frequently, yeast extracts exhibit emulsification capabilities post-cell lysis, underscoring the significance of extracted yeast proteins although the involvement of other biopolymers such as glucans, alongside cell wall mannoproteins, cannot be overlooked [43,45,48,49] (Table 1). However, recent studies have realised that defects in the formation of N-linked glycans or eliminating the synthesis of β-1,3 glucan can still generate emulsion stability highlighting the importance of proteinaceous molecules in the emulsification behaviour [24,40,44]. Mannoproteins are often hypothesised to be the key protein [46] in yeasts responsible for stabilising emulsions by molecularly adsorbing to the droplet surface, but systematic studies on pure mannoproteins extracted from yeast cells are fairly limited in literature (Table 1). A recent study [47] showed that enzymatic treatment of yeast extract with β-1,6-glucanase can result in pure mannoprotein (90.7% purity) of molecular weights 30–58 kDa with mannan-to-protein ratio of 14.5. Such mannoproteins were capable of producing molecularly adsorbed emulsions but with larger droplet sizes reported (> 50 μm) that without any microscopy data is difficult to comment on the kinetic stability of such emulsions. To date, limited information exists on the stability of emulsions stabilised by pure mannoprotein when subjected to different food processing–relevant environmental conditions (pH, temperature, ions, shear). Also, critical aspects of interfacial stabilisation by mannoproteins such as their adsorption kinetics and interfacial viscoelasticity at the oil–water interface compared with other alternative proteins are important future studies that need to be conducted.

Conclusions and future perspectives

Yeast protein has captured significant research attention as an alternative source of protein due to recent demands of exploring environmentally sustainable protein sources. Despite the literature showing promising directions in exploiting the whole yeast cells or extracted mannoproteins for emulsion stabilisation, many knowledge gaps still need to be filled in terms of the interfacial properties and stability of emulsions when subjected to environmental conditions. In addition, processing methods (e.g. extraction, heat treatment, enzyme treatment, degree of washing and removal of glucans) need to be standardised so that full practical potential of yeast mannoproteins as molecularly adsorbed emulsifiers or yeast biomass as Pickering stabilisers can be commercially realised.

Although Baker's yeast (*S. cerevisiae*) has been used in baking industries for millennia without any sensorial concerns, often their addition is limited to 1–2 wt% as processing aids. However, if used as an alternative source of protein and as Pickering stabilisers, larger cell concentrations (10–15 wt%) can be anticipated, which might result in rheological modification, gel-like property due to cell–cell interactions. Furthermore, potential cell-mediated gritty, rough and astringent mouthfeel issues might result in limited acceptability. Although not in food, tribological studies have been carried out in literature using non–food-grade yeasts, such as *Rhodotorula toruloides* and *Cutaneotrichosporon curvatus* [50], which show excellent lubrication qualities due to monounsaturated fatty acid content inherently present in those oleaginous yeasts. Nevertheless, detailed rheological and tribological studies using food-grade yeast cells as well as yeast mannoproteins and understanding their interaction with human saliva [11] are necessary for future studies. This may give indications about their *in vivo* oral processing behaviour and mouthfeel before they can be commercialised and used in mainstream food applications as technofunctional additives.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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- •• of outstanding interest

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