Valorisation of protein-rich extracts from spent brewer's yeast (*Saccharomyces cerevisiae*): an overview

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Abstract

As one of the main brewing by-products, *Saccharomyces cerevisiae* extracts (from spent yeast) have been commercialized as food supplement for years. Among their several claims, the application as protein source is highlighted. In fact, their high protein content (about 45-60%) including essential amino acids with high biological value, safety and low cost are primarily responsible for their spreading in agri-food sector. Meanwhile, cosmetic and health sectors have been working on yeast bioactive peptides because of their antihypertensive, antioxidant and antimicrobial properties, among others. Several studies related to valorisation of *S. cerevisiae* are currently ongoing, aiming to create novel products and optimize production processes. The present review aims to provide an overview from production of protein-rich extracts from *S. cerevisiae* to their chemical characterization, detailing protein extraction, isolation and purification processes, as well as characterization methods for the final extracts.

Keywords Peptides, protein extraction, protein isolation, protein purification, protein characterization techniques

Contents

1. Introduction

Nowadays, the world is facing the need to develop new strategies to provide resources due to the global population growth and socio-demographic changes [1]. Several competitive, sustainable and economically viable production processes have emerged in last years, exploring different food sources (plants, animals, and microorganisms) in order to minimize planet resource depletion and environmental impact [2]. Regarding the global demand for food, protein demand was estimated to be approximately 202 million tonnes when the world population was 7.3 billion (in 2017). Estimations using a population growth of 2.3 billion and similar protein consumption levels, resulted in an expected increase in protein demand for the next years in the range of 30 to 40% [3].

Peptides, as protein hydrolysis products, have been described as bio-functional ingredients in the nutraceutical and functional food market; due to fewer side-effects when compared with synthetic drugs, they are becoming an option in health sector as well [4]. In fact, peptide therapeutics market was valued at $25 billion in 2018 and it is anticipated to expand at a compound annual growth rate (CAGR) of 7.9% from 2019 to 2027 due to the high incidence and prevalence of metabolic disorders [5]. Several biological activities of peptides have been reported over the years such as antioxidant [6], antimicrobial [7], anti-diabetic [8], anti-obesity [9], anticoagulant [10], ACE inhibitors [11] and chelating effects [12]. Nowadays they are also commercialized as food supplements or incorporated in some foods [4, 13].

The analysis of current protein supply sources show that agri-food sector has been the one where more alternative protein sources have being evaluated (**Fig. 1**). As observed in **Fig. 1**, recent data from Food Agricultural Organization of the United Nations (FAO) revealed that 59% of protein supply globally arises from vegetal sources (141 453 675 ton), followed by meat (42 156 770 ton), dairy (24 971 110 ton), fish (16 349 445 ton) and other sources (14 792 355 ton) [14]. Legumes, pseudocereals, seeds, almonds and nuts are examples of protein plant sources that have been widely studied and used as protein supplements [15]. The dairy based ingredients have been established in the protein market mainly because of the global milk industry and the diversity of milk by-products. [3]. The high protein content in fish has also been explored, being its hydrolysates a significant source of bioactive peptides [16]. Furthermore, edible insects have currently emerged as a potential protein source as well, since they are an environmentally friendly choice with high nutritional value, vitamins and minerals [17].

Apart from the abovementioned protein and bioactive peptide sources, microorganisms have also become an important natural matrix since they may contain more than 30% of crude protein in their biomass [18]. Microbial protein or "single cell protein" (SCP), as it is generally referred, are crude protein that can be obtained from microorganisms such as microalgae, yeast and other fungi, and bacteria. High growth rates, the ability of some microorganisms to use single substrates, such as carbon dioxide or methane, the wide variety of microorganisms and the independence from seasonal factors when grown in bioreactors, make the extraction of microbial protein a potentially high efficient and sustainable scalable process [18, 19]. Some microalgae species have a protein content of about 60-70%, although their omega-3 polyunsaturated fatty acids and carotenoid contents attract more interest in the scientific community [20, 21]. Bacteria strains, mainly those used as fodder, have a protein content of 50–80% (dry weight basis) with an equal or higher content in essential amino acids than conventional sources, as recommended by FAO [22]. However, as occurs in other low nutrient plants, palatability issues related with non-pleasant sensorial properties can be observed in several SCP bacterial ingredients [18].

From the current microorganisms used for SCP production, yeast and fungi continue to dominate the traditional agri-food market with well-established processing methods [18]. In fact, the microbial protein products in market contain more than 30% of protein content, being higher than 65% for bacteria, 40-55% for yeasts, 35-55% for fungi and 30-40% from microalgal [23], providing a healthy balance of essential amino acids and vitamins from B-complex group as well [18, 20]. Since the 19th century, yeast has been a commonly-used organism in the production of biomass for human consumption due to the high acceptability of fermented foods [23, 24]. Apart from its acceptability and availability, the ability to grow at low pH (4.5-5.5), which reduces the need to work at strict aseptic conditions, is pointed as one of yeast advantages compared to other microorganisms employed for SCP production [23]. Furthermore, they are rarely associated with the occurrence of gastroenteritis intoxications or other infections related with food intake [25], since they are capable to produce antimicrobial compounds, thus inactivating the growth of other microorganisms and providing a safe application in food area [26].

In addition to several *Aspergillus*, *Candida* and *Fusarium* strains, *S. cerevisiae* is a protein source increasingly explored by the food industry, since it is one of the main brewing by-products (2 to 4 kg of spent yeast per 100 L of beer [27]). In fact, spent brewer's yeast have been commercialized for years in yeast extracts, but the increase of protein rate in the final product is still a challenge [18].

Considering the importance of valorisation of spent brewer's yeast and their bio-functional ingredients, a considerable number of research works is currently ongoing, creating novel products and production processes. Therefore, the purpose of the present review is to update and summarize the advances on processes to obtain protein-rich extracts from *S. cerevisiae*, describing the protein extraction, isolation and purification methods reported so far. In order to understand their chemical and biological performance for application into different economic sectors, protein-rich extracts characterization methodologies are also analysed.

2. Spent yeast: an emerging source for SCP extracts

SCP is considered a generic term for crude or refined protein originated from microorganisms [28]. Currently, cheap wastes such as carbohydrates materials (molasses, vinasse, wood hydrolysates, sulphite liquors, starch, lignin–cellulose, etc.) can be used as substrates to supply carbon and nitrogen for yeast growth in SCP production [29]. Yeast inactivated biomass has been suitable as SCP source at commercial scale because of their high nutritional quality. In 2018, many processed products were launched using yeast extract as a major ingredient, such as snacks, soups, sauces and seasonings [30]. In fact, the expected grow at a CAGR of 7.0% of the Global Specialty Yeast Market from 2019 to reach $4.8 billion by 2025, is mainly attributed to increasing demand for processed foods which contain specialty yeast as a main ingredient (yeast extracts, autolysates and beta-glucans) [30]. Low production costs, the larger size when compared with other microorganisms (easier to harvest) and the ability to grow at acidic pH, are other advantages pointed for the use of yeast as a food source. However, poor digestibility can be a constraint for protein extraction, because of yeast's complex and thick cell wall [19]. Furthermore the high nucleic acid content in yeast is still a problem in food industry since their excess in diet has been related with uric acid increase, that can lead to diseases like gout [31]. For this reason, the reduction of nucleic acid content in yeast products for use in food industry continues to be a challenge since it exceeds the limit dose for dietary

supplements manufactured with yeast [32]. Yeast market is currently leaded by *S. cerevisiae* although there are thousands of yeast species [30, 33]. The growing launch of new products in beverage industry using yeast as ingredient is pointed as one of the major factors driving the growth of yeast market [30]. *S. cerevisiae* and *S. pastorianus* are the main two species of *Saccharomyces sensu stricto* used for beer production [34]. Considering the annual world beer production of 1.82 billion hectolitres in 2020 [35] and 1.7 to 2.3 g of spent yeast per litre [13], it was estimated the generation of 309,400 to 418,600 tonnes of brewer's spent yeast worldwide. Furthermore, brewer's spent grains is other by-product of beer production that can also be used for medium supplementation to grow new yeast for beer making, since the spent grain has high nutritional levels, mainly in terms of protein and fibre [36]. Brewer's yeast is described as a Generally Recognized as Safe (GRAS) microorganism [32]. Proteins (structural, functional and hormones) are their main constituent, representing 45-60% of dry weight basis, and including essential amino acids in amounts similar to those recommended by FAO/WHO [37]. Other constituents are polysaccharides (25-35%) (mainly capsular and cell wall glucans, mannans and chitin), followed by glycoproteins (5-10%) which correspond to mannoproteins from cell wall and functional enzymes. Small amounts of nucleic acids (4-8%), lipids (4-7%) and polyphosphates (1-3%) are also present in yeast structure [38].

Proteins, as other cellular compounds, are found in the yeast cell wall, plasma membrane and periplasm (**Fig. 2**). In cell wall, most proteins are bound to polysaccharides, as mannoproteins. While mannoproteins may represent up to 40% of the dry weight of the cell wall, when isolated, proteins *per se* represent only a small fraction (13%) [39, 40]. Other cell wall proteins can be linked to the β-1,6-glucan-chitin network as well, which provide elasticity and yet rigidity against cell disruption processes [41, 42]. The thin semi-permeable lipid bilayer of plasma membrane is also formed by proteins in addition to lipids. These proteins play a vital role at controlling the permeability of the cell, in cell wall biosynthesis and overall protection. Secreted proteins that are unable to permeate the cell wall and the plasma membrane constitute the periplasm structure [43].

3. Production of protein-rich extracts from *S. cerevisiae*

3.1. Protein extraction

In order to access *S. cerevisiae*'s protein, the extraction processing starts with cell disruption, since most proteins are found within the cell. The choice of disruption method

can significantly impact the yield and quality of the final product, as well as both fixed and variable costs on industrial processes [44, 45]. An efficient breakage of cell wall strength-providing components, namely mannoproteins and glucans, is necessary to effectively extract protein, since their release is determined by the functionality of the plasma membrane and the porosity of the yeast cell wall [46]. Depending on the cell disruption method and subsequent purification and isolation processing steps, different amounts of proteins, peptides and free amino acids are found in the yeast extract product [44].

A way to classify the protein present in *S. cerevisiae* is according to their molecular weight (MW), since it is a relevant factor on the bioactivity of peptides (usually ranging from 3 to 20 amino acids) [11]. Oligopeptides with 2000-3000 Da of MW usually represent the main group of total protein after yeast autolysis process, followed by di-, tri- and tetra-peptides (MW < 600 Da). Although only 2-5% of the total protein are oligo-peptides with a MW higher than 3000 Da [32], the ratio between di-, tri-, tetra, and oligo-peptides is strongly related with cell wall degradation during yeast lysis process [41].

The *S. cerevisiae* extraction methods for protein release described in literature can be classified, according to their main operation mode, in physical methods, either using pressure or waves, and chemical or enzymatic methods (autolysis and hydrolysis), supported by additional chemical substances or enzymes, respectively (**Fig. 3**). However, a combination of methods is also possible and often desirable. These treatments present a broad variation in their process conditions, which can be explored using the "One Factor At a Time" strategy, where one factor is changed and evaluated independently, or applying a factorial design in order to take all factors into account at the same time [47]. Over the years, many extraction processes have been developed attempting to achieve efficient and cost-effective release of proteins from their yeast cells.

3.1.1. Physical methods

Physical methods are described as non-specific and their extraction efficiency is highly dependent on the nature of the substrate of interest, the cell or tissue itself, like the extent of the cell's fragility [48, 49]. However, there are some relevant scale-up and operation cost differences to be discussed at industry level processes.

Several studies using bead milling, high-pressure homogenization (HPH), ultrasonication, supercritical carbon dioxide (SCO_2) and pulsed electric field (PEF) have been described as physical extraction methods to release *S. cerevisiae*'s protein (**Table 1**). In general, the former methods damage the yeast cell envelope with the breaking of cell wall due to stress produced by abrasion, pressure (with or without combination of temperature) or cavitation. On the other hand, PEF allows a permeabilization of yeast cell membrane that can be reversible or irreversible according to the electric parameters used. Temperature is not usually employed for protein extraction due to its irreversible effect on conformational modifications in protein structure (denaturation).

3.1.1.1. *Driven by pressure*

3.1.1.1.1. Bead milling

For decades, bead milling has allowed to achieve high recovery of intracellular compounds from yeast in a single-step operation with reasonable temperature control, easy to scale-up and low requirements in terms of sophisticated equipment or trained personnel [43, 50]. The various designs of bead mills are based on the principle of a cell suspension agitation with glass or zirconium beads performed in batch or in a continuous recycling mode where the yeast cell wall damage occurs by a mechanical disruption effect [49]. Currie, Dunnill and Lilly [51] started to study the protein release from *S. cerevisiae* and established a first-order kinetics for disruption in a high-speed bead mill. Bead size, agitation, concentration of cell suspension, temperature, time and bead volume are the most studied variables, however, the authors seemed to disagree about the most significant factor to obtain the maximum of protein release [49, 51]. In another study, Currie, Dunnill and Lilly [51] concluded that temperature was not a determinant factor for protein release, whereas Gaver and Huyghebaert [49] hypothesized the denaturation of certain proteins with temperature increase for a long disruption time (more than 7 passes). In fact, Gaver and Huyghebaert [49] observed differences in excretion of two enzymes (glucose-6-phosphate dehydrogenase and invertase) according to the number of disruption passes. This means that the full opening of the cell wall may not be a requirement to release cell wall-bounded molecules, such as invertase. Concentration of yeast suspension seemed to have no effect on disruption efficiency in this study [49]. Jacob et al. [52] investigated three industrially applicable cell disruption methods for yeast extracts production and they found bead milling (321.56 mg/g), followed by

ultrasonication (285.40 mg/g), as the method that released the highest protein content in comparison with autolysis (52.90 and 102.00 mg/g). Moreover, these extracts presented low degradation rates of polyphenols and glutathione, showing their potential in antioxidant and reduction properties. On the other hand, the free amino acids amount present in autolysis extracts (433.21 mg/g) was higher than the one found in mechanical methods (115.68 and 155.38 mg/g), raising questions about sub estimation of protein in these extracts, quantified by Bradford method. Hedenskog and Mogren [53] showed that other processes could be coupled to bead milling for increased protein in the final extract, namely the selective alkaline precipitation of protein. Bead milling is frequently used for the extraction of specific cell wall components such as β-glucan [46].

3.1.1.1.2. High pressure homogenization

HPH is based on forcing a cell suspension to pass at high pressure (several hundred bars) through a narrow gap called a homogenizing nozzle or a high-pressure valve [54]. It is currently the most widely accepted disruption cell method by the biotechnology and pharmaceutical industries [55]. Cells experience multiple actions of cavitation effect and high speed impact, disrupting through their interactions with valve and impact ring [43]. Concerning its application for yeast protein extraction, Ekpeni *et al.* [56] and Balasundaram and Harrison [57] described that pressure plays an important role on protein extraction, since this variable induced alteration in yeast pH and viscosity related to the micronisation of the cell debris. However, Siddiqi, Titchener-Hooker and Shamlou [58] observed that cell debris particle size distribution and the extent of the protein release were independent of the flow rate through the system (scale operation) or the design of HPH valve geometry. Most of the cells were disrupted by the end of fifth pass and, above that number, HPH caused a further degree of the debris formation [58]. Balasundaram and Harrison [57] compared two cell disruption techniques where they discovered that HPH (1.7 mg/mL) allowed a higher protein release than hydrodynamic cavitation (0.1 mg/mL), maintaining a low biomass concentration. In combination with HPH, Liu, Lebovka and Vorobiev [59] coupled an electrical treatment to yeast suspension aiming to maximize the protein yield. It must be noticed that, by applying only pulsed electrical field (PEF) or high-voltage electrical discharges (HVED), a complete rupture of yeast cells was not observed (40 kV/cm, 500 pulses)[59].

3.1.1.1.3. Supercritical carbon dioxide

A different physical method applied to disrupt and extract proteins from *S. cerevisiae* yeast cells is the use of SCO_2, which involves the application of SCO_2 followed by a sudden pressure drop. The expansion of SCO_2 within the cells forces cell wall breakage, releasing intracellular proteins [60, 61]. While using this technique, Lin *et al.* [60] observed an efficient cell disruption at 1000 and 5000 psi of SCO2 injection (15h or 5h, respectively) with simultaneous preservation of the protein functional properties, as indicated by enzymatic activities (alcohol dehydrogenase, invertase, glucose-6-phosphate dehydrogenase and fumarase). The addition of β-glucuronidase to the process decreased the extraction time (90 min at 5000 psi) since the combination of enzymatic hydrolysis with SCO_2 allowed the deactivation of the released enzymes, suggesting it may be used to reduce the cost of protein isolation [60].

3.1.1.2. *Driven by waves*

3.1.1.2.1. Ultrasonication

Ultrasound has also been extensively reported for extraction of proteins and peptides from yeast, facilitating higher yields and rates of extraction [62]. Ultrasound principally acts by generating bubble cavitation in the biological matrix through the conversion of sonic into mechanical energy, in the form of intense elastic shockwaves; cavitation is assumed to be the main mechanism of cell disruption [43, 62]. Zhang *et al.* [63] and Wu *et al.* [64] suggested that ultrasound disruption mechanism starts with the breakdown of cell wall before continuing to the cell membrane, since a significant larger amount of polysaccharide was released at early stages of sonication. James, Coakley and Hughes [65] established the kinetics of protein release from an ultrasound batch and flow system, where a good agreement between the theoretical prediction and experimental results was observed. High acoustic power was pointed by several authors as the essential condition to increase protein release from *S. cerevisiae*, although this increment would not be feasible in terms of industrial energy consumption [64, 66–68]. The increase of temperature, processed volumes or cell suspension concentration are other variables that can lead to a decrease in protein yield [63, 64, 67, 68]. As in other physical disruption methods, protein could be denatured or suffer thermal coagulation due to temperature, duration of ultrasound treatment or even the sonicator type used (horn or bath) [63, 67, 68]. James, Coakley and Hughes [65] suggested the use of a more efficient cooling system

to minimize the activity loss of the released enzymes. The decrease of protein yield with high cell suspension concentration and high processed volume could be related with the decrease of number of cavitation bubbles available for each cell [63]. Agrawal and Pandit [69] observed that soft alkaline conditions (pH 8) also allowed for higher protein release. At higher pH, however, a decrease in yield is found, likely due to proteases activity, which deactivate other enzymes or proteins.

3.1.1.2.2. Pulsed electric field

Electrical methods are those where yeast cells are treated with high intensity electric field pulses. One of them is PEF, which is based on the electro permeabilization phenomenon, where the applied electric field provokes an electroporation of yeast cell membrane, being this permeability reversible or irreversible according to the electric parameters used. Nevertheless, this treatment can cause leakage of cytoplasmic content, leading to cell breakdown [70]. The variation of electric field strength, as well as the time of treatment, are the main factors responsible for the released protein yield obtained in the process, and need to be adjusted to the used cell suspension concentration [70–72]. Ganeva, Galutzov and Teissié [70] observed a electroextraction of proteins at 3.2 kV/cm (15 pulses, 2 ms, 6 Hz), in agreement with Ohshima, Sato and Saito [72], which experienced an increase of protein release below 10 kV/cm with few cell deaths, suggesting that the disruption of cell membrane with PEF occurs without cell breakdown. Ganeva and Galutzov [71] observed the release of cytoplasmic enzymes such as glutathione reductase, 3-phosphoglycerate kinase and alcohol dehydrogenase, while the yeast cell wall remained intact. Other factors also considerably affect protein yield in PEF treatment, such as the cell growth phase, presence of monovalent ions in the medium, or the incubation of a reducing agent capable of break disulphide bonds, such as dithiothreitol (DTT) [70, 71].

In conclusion, among the abovementioned physical methods for protein extraction in *S. cerevisiae*, ultrasonication seems to be quite effective, since it allows the recovery of periplasmic, membrane-bound and insoluble recombinant proteins [69]. However, due to operational and economical limitations of ultrasonication methods, such as amplitude and energy consumption, bead milling and HPH are widely favoured at industrial scale [55]. On the other hand, bead milling and HPH have the downside of poor selectivity, with micronization of the cell debris which can substantially increase the costs of subsequent

downstream operations of protein purification or isolation [63]. HPH and bead milling also require frequent and costly maintenance requirements since they easily get clogged [73]. In order to overcome these methods limitations, Bystryak, Santockyte and Peshkovsky [55] explored a pilot scale device of ultrasonic technology, namely Barbell Horn Ultrasonic Technology (BHUT), that achieved an productivity increase with respect to laboratory-scale results.

Table 1. Physical extraction methods for protein release from *S. cerevisiae*.

Physical methods		Cell suspension concentration	Quantification method	Maximum protein	Main conclusions	Reference
Driven by pressure	*Bead milling*	30%	Lowry	5.32 kg/h	Temperature was not a determinant factor for protein release. Bead size, agitation and yeast concentration had a considerable effect on total protein.	[51]
		10%	Kjeldahl	60% of yield (DW)	Bead milling allowed the highest protein released compared to lyophilized, spray-dried or drum dried material. In these processes, no cell wall disruption or cell fragmentation could be observed.	[53]
		30%	Kjeldahl	80 mg/g yeast	A long disruption time and a high beads volume created a cumulative effect of the disruption forces which may cause denaturation of certain proteins. The yeast cell concentration seemed to have no effect on disruption efficiency.	[49]
		7%	Bradford	321 mg/g yeast (DW)	Bead milling, followed by ultrasonication, increased the amount of protein in yeast extract produced in comparison with autolysis. However, the autolysis process allowed a higher release of free amino acids from yeast than mechanical methods.	[52]
	HPH	NM	Bradford	96 mg/g yeast	The cell debris particle size distribution and the total protein release are independent of the scale of operation and HPH valve geometry.	[58]

Physical methods		Cell suspension concentration	Quantification method	Maximum protein	Main conclusions	Reference
		5%	Bradford	1.7 mg/mL yeast suspension	HPH showed the higher amount of protein release in relation to hydrodynamic cavitation. Alteration in the viscosity and pH of the disrupted cell suspension was obtained as a function of disruption intensity.	[57]
		5%	Bradford	50 µg/g dry yeast	The combination of electrical and HPH treatments allowed to obtain a good protein yield with low content of nucleic acids. Incomplete damage of yeast cells under PEF or HVED treatment.	[59]
		30:70	Bradford	1.4 mg/mL yeast suspension	Protein yield showed an increment rate as the ratio yeast: buffer increases from 10:90 to 30:70. The pressure rise induced an alteration in yeast viscosity, influencing the protein release.	[56]
	SCO_2	NM	Lowry	33 mg/g wet yeast	High-pressure CO_2 fluid could prevent the deactivation of the released enzymes. The use of lytic enzymes, as β-glucuronidase, decreased the extraction time which may reduce the cost of protein isolation.	[60]
Driven by waves	*Ultrasonication*	20%	Lowry	9 mg/mL yeast suspension	Good agreement between the theoretical prediction of protein release and experimental results. A more efficient cooling system would be desirable to minimize the activity loss of the released enzymes.	[65]
		2%	Lowry	1.27 mg/mL yeast suspension	At weak alkaline pH was observed the maximum of protein release under ultrasonication (11.62W or 20% amplitude) for 60 min. Very alkaline media seemed to increase proteases or	[69]

Physical methods	Cell suspension concentration	Quantification method	Maximum protein	Main conclusions	Reference
				other enzymes activity which may deactivate other enzymes or proteins.	
	9%	Lowry	85% of release	High acoustic power, duty cycle and the addition of glass beads to the process increase the protein release. No influence of different cell concentration. However, the increase of acoustic power was not feasible in terms of energy consumption due to economic costs.	[66]
	1%	UV/BCA	80 mg/g yeast	Maximal protein release was observed at high power conditions (80W). The increase of cell concentration decreased the final protein level. Higher protein recovery using a 20 kHz-horn compared with 130kHz-bath sonicator.	[67]
	1%	Bradford	0.6 mg/mL yeast suspension	High acoustic power increased the protein release. However, the increase of sonication time may cause significant protein degradation due to high temperature.	[68]
	10%	BCA	25% of release (DW)	Temperature showed to be the most important parameter to selective release of polysaccharide and protein. The increase of cell concentration, processed volumes and temperature decreased the final protein.	[63]
	20%	Bradford	16.6 mg/mL yeast suspension	The maximum protein released obtained in a pilot scale device of ultrasonic technology (BHUT) compared with conventional ultrasonication.	[55]

Physical methods		Cell suspension concentration	Quantification method	Maximum protein	Main conclusions	Reference
		10%	BCA	92% of release (DW)	High acoustic intensity (24 and 39W/cm^2) allowed the increase of protein release. The increase of cell concentration, processed volumes and temperature decreased the final protein.	[64]
	PEF	10^8-10^{10} cells/mL	Lowry	40 µg/mL yeast suspension	Released protein increased with the electric field strength, more rapidly below 10 kV/cm. Protein can be released together with few cell deaths.	[72]
		NM	Bradford	29% in final extract	Some cytoplasmic proteins were extracted with intact cell walls. The electro-induced protein release showed a strong dependence on the cell growth phase and the presence of monovalent ions in the medium. The pre-incubation with DTT provoked a faster and exponential protein efflux.	[71]
		4.5%	Commercial kit	85% in final extract	The parameter of field intensity is the core of this process and must be adjusted to the yeast concentration. A high protein yield required long extraction with DTT after PEF (> 4 h at 30 °C).	[70]

351 DW- dry weight basis, NM - not mentioned, UV - protein absorbance, BCA - bicinchoninic acid kit. * - Free amino acids release

3.1.2. Chemical methods

Chemical treatments using chelating agents, detergents and solvents can lead to permeabilization or lysis of yeast cells, triggering the subsequent release of intracellular molecules. These procedures rely on the relative selective interaction of the chemicals with specific components of the membrane, allowing proteins to seep through the cell wall [74]. In **Table 2**, the main chemical procedures for protein extraction from *S. cerevisiae* are listed.

Alkaline precipitation is one of the most used chemical procedures to extract *S. cerevisiae* protein, although the involved mechanism is not clear [75, 76]. Kushnirov [75] hypothesized that under alkaline conditions (0.2 M NaOH) the O-chains of O-glycosylated proteins (covalently linked to other cell wall components, such as β-1,3-glucans), are cleaved off in a process called beta-elimination, which allow the release of O-glycosylated proteins. In order to increase the protein yield, Zhang *et al.* [76] introduced a pre-treatment with lithium acetate (LiAc), described as an enhancer of cell wall permeabilization. Mukherjee [77] tested several protein extraction methods already described in literature aiming to study *S. cerevisiae* at different growth phases. The modified protocol of Kushnirov [75] with alkaline and SDS-buffer treatment showed the maximum protein release at exponential and late stationary phase cells, in comparison with physical methods (glass beads, sonication or both) or individual SDS-buffer or alkaline treatment, displaying sharp and distinct bands in SDS-PAGE. However, the loss of enzymatic activity may be a concern when applying this protocol [77]. Ionic liquids, namely 3-(dimethylamino)-1-propylaminium formate ([DMAPA]FA), were also tested for cell wall breakage, and the extracted target proteins maintained their properties unchanged [78].

Generally, the chemical approaches are followed by separation and purification techniques, such as sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [75–78].

Table 2. Chemical extraction methods for protein release from *S. cerevisiae*.

Cell suspension concentration	Quantification method	Maximum protein release	Main conclusions	Reference
NM	NQ	NQ	Pre-treatment with 0.2M NaOH, followed by 3 min of boiling in SDS-PAGE buffer, significantly increased the yield of extracted proteins.	[75]
NM	NQ	NQ	[DMAPA]FA was the ionic liquid solution capable of the higher efficiency on protein extraction. Chemical properties of target proteins remained unchanged during the extraction process.	[78]
NM	NQ	NQ	The combined pre-treatment with LiAc, followed by NaOH and SDS-PAGE buffer gave the best results in yeast whole protein extraction. Using LiAc or NaOH individually showed a lesser extent of protein extraction.	[76]
NM	Lowry	70 µg/g yeast	The modified method from [75] revealed the maximum protein yield in comparison with physical (glass beads and sonication) and alkaline or SDS-buffer individual treatment.	[77]

NM - not mentioned, NQ – not quantified, NaOH – Sodium hydroxide, SDS-PAGE - Sodium dodecylsulfate-polyacrylamide gel electrophoresis, [DMAPA]FA - 3-(dimethylamino)-1-propylaminium formate, LiAc - Lithium acetate

3.1.3. Enzymatic methods

Enzymatic methods for protein release involve attacking the mannoprotein complex and glucan backbone of the yeast cell by endogenous (autolysis) or exogenous lytic enzymes [43].

3.1.3.1. *Autolysis*

Autolysis is an endogenous process that represents the degradation of the cell components from inside out by action of yeast own enzymes. It occurs when the cell growth cycle is completed and death phase is initiated [79]. Intracellular enzymes are activated by appropriate process conditions, such as temperature, time and pH, which results in a partial degradation of the cell wall structures [80]. It was observed that a long autolysis time increase the amino acid content from 11.2% (2h) to 77.5% (48h), with a consequent decrease of peptide amounts and size, as they were decomposed into free amino acids [80]. The same observations were conducted by Jacob, Hutzler and Methner [44], who concluded that a strong enzymatic degradation of protein takes place during the autolysis process; indeed, autolysis is biased to hydrolyse proteins to the greatest possible extent, in contrast with bead milling and ultrasound, thus obtaining an increase in free amino acids content and peptides with low MW (below 4 kDa). The precise control of autolysis extension leads to different fractions of free amino acids and peptides with distinct MW, which can be explored for the production of different protein-rich extracts in a single process [80].

3.1.3.2. *Enzymatic hydrolysis*

If additional exogenous enzymes are added to the yeast, the process is named enzymatic hydrolysis. Hydrolysis is the most efficient method of solubilizing yeast, where proteolytic or cell wall lysis enzymes firstly hydrolyse the compounds in cell walls to promote cell lysis. These enzymes can also enhance the activities of endogenous enzymes, thereby accelerating the leakage of intracellular substances [81, 82]. The effect of enzymatic hydrolysis on proteins is mild, changing their MW, charge and exposure of hydrophobic groups and reactive amino acid side chains, but does not destroy them. The specificity of the enzymatic complex used determines which peptides are produced [83]. The endopeptidases Alcalase®, Corolase®, Papain® and Brauzyn®, an aminopeptidase (Flavourzyme®) and other proteases (Protamex® and Promod®), as well as combinations thereof, are the enzymatic complexes most widely used for production of protein-rich

extracts from *S. cerevisiae* (**Table 3**). Chae, Joo and In [82] concluded that protein recovery is strongly dependent of the enzyme dosages, increasing with the hydrolysis length and being more responsive to Flavourzyme than Protamex. High solid concentrations of yeast cells (28%) also seemed to exert a positive influence in protein release [81]. The maximum protein recovery reported was about 53-76%, regardless of the enzyme complex used or time treatment [81, 82, 84, 85]. In such conditions, the final profile of protein extracts presented a high percentage of low MW peptides (90% below 3 kDa) and free amino acid content, also independent of the protease that was used for hydrolysis [81, 82, 84, 85]. The advantages of applying enzymes for protein extraction are mainly the gentle conditions employed and their specificity. However, the price of enzymatic complex may be a deterring factor on a large scale operation [74]. Nevertheless, some patents have been issued for the production of yeast protein extracts for foodstuffs using hydrolytic enzymes [86–89], with a final protein content about 60-80% [86, 87, 89]. A yeast peptide hydrolysate for cosmetic industry was also patented by Farra [90], where enzymatic hydrolysis was applied to degrade protein into peptides with low MW.

Among the abovementioned protein extraction methods, enzymatic hydrolysis is the most specific process; chemical methods are also relatively specific, although the violent experimental conditions and strong reagents may provoke alteration on final protein content or peptide and amino acid profile [63]. On the other hand, autolysis has the main advantage of only using yeast's own enzymes for the process, but the temperature and long-time treatment can lead to bacterial contamination on yeast suspension turning the final product unfeasible.

In general, process cost and selectively are inversely related, although it must be in mind that a clean extract (i.e., without significant amounts of contaminants) will facilitate the downstream isolation and purification processes, thus decreasing the final cost of the overall process. Nevertheless, there are no general rules since the selection of the extraction method will be dictated by the final use of the protein extract.

442 **Table 3.** Enzymatic extraction methods for protein release from *S. cerevisiae*.

Enzymatic methods	Cell suspension concentration	Quantification method	Maximum protein	Main conclusions	Reference
Hydrolysis	20%	Kjeldahl	53.6% recovery	Protein recovery was more responsive to Flavourzyme than Protamex, being strongly depended on the enzyme dosages and time treatment. After 12h, Protamex (0.6%) and Flavourzyme (2%) exhibited the highest protein release. The optimized conditions produced yeast extract contained mostly low MW peptides and free amino acids.	[82]
	NM	Kjeldahl	60% in final extract (w/w)	Papain was used to perform hydrolysis during 24h (50ºC-60ºC), resulting in a final extract with high content of free amino acids and only trace amounts of peptides (MW > 1740 Da).	[84]
	28%	Kjeldahl	67.7% recovery	High solid concentrations of yeast cells leaded to an increase of protein recovery. The hydrolysis degree was higher with Alcalase (0.1%, 48h, 55ºC) than Papain but the peptides MW were similar (< 3 kDa reached 90%). The addition of sodium chloride (1-3%) to cell suspension accelerated the hydrolysis process.	[81]
	NM	Far-UV (214 nm); Lowry	76% in final extract (w/w)	Brauzyn®, Protamex™ and Alcalase™ (2000 U/ g protein) hydrolysis at 50ºC during 2h (pH 7.0) produced a yeast hydrolysate rich in peptides from 7000 to 1000 g/mol (43%) with a small amount of short peptides and amino acids (25% of 1000-100 g/mol). The sequential membrane filtration process applied to the hydrolysate increase protein purity regarding RNA and total sugars up to 1.7 and 2.7-fold, respectively.	[85]
Autolysis	NM	HPLC-UV/VIS	324 mg/g in final extract (w/w)*	A longer autolysis (48h) allowed a substantial increase of free amino acids content with the presence of peptides from 1000 to 2000 Da (about 10-20 amino acids). The precise control of autolysis time process leaded to obtain autolysates with varying free amino acid content and peptides of different MW tailored to the specific nutritional needs.	[80]

	NM	HPLC	350 mg/g final extract (w/w) *	The cleavage of protein (MW peptides < 4 kDa) and release of free amino acids was higher in autolysis (24h, 50ºC) than bead milling or ultrasonication.	[44]

443 NM - not mentioned, UV - protein absorbance, RNA - ribonucleic acid, MW – molecular weight.* - Sum of free amino acids

3.2. Protein isolation and purification

Following protein extraction from *S. cerevisiae* cells, purification and/or isolation steps are generally required to obtain protein isolates and concentrates. Depending on the physicochemical properties of proteins present and the final goal, different isolation and purification methods are applied to the "crude protein extract". The aim of a purification process is not only the removal of unwanted contaminants, but also the concentration of the desired protein, preferably on a stable environment and in a form adequate for the intended application [91]. Cell debris with different size are contaminants resulting from the physical extraction, which can be easily removed by high-speed centrifugation, traditionally used for the primary recovery of soluble protein [92]. On the other hand, chemical and enzymatic extraction methods create less contamination due to their selectivity, at the cost of lower protein recovery. As previously mentioned, such selectivity may have a positive impact on economic assessment of the large-scale process [44].

Several procedures hereby described and discussed for *S. cerevisiae* protein separation and purification were applied in order to isolate specific molecules and obtain a final product with the desired performance.

3.2.1. Selective precipitation

As previously described, alkaline precipitation is considered one of the chemical methods for protein extraction from *S. cerevisiae* [75–77]. In fact, protein precipitation is one of the most used methodologies to concentrate and purify yeast protein extract [77, 91]. Generally, this reaction is applied after disruption of yeast cell [53, 90, 93–95] and can be followed by other purification processes such as dialysis and enzymatic hydrolysis, as patented by Farra [90] for production of a cosmetic peptide hydrolysate. Simple alkaline precipitation was used by Hedenskog and Mogren [53] and Butylina *et al.* [93], with the latter performing acidic precipitation for nucleoprotein complexes production. On the other hand, *S. cerevisiae* protein concentrates were produced by Caballero-Córdoba and Sgarbieri [94] using a salting out technique, with sodium perchlorate coupled to isoelectric precipitation at pH close to 4. Protein phosphorylation with sodium trimetaphosphate at alkaline pH for modifying protein structure was explored by Yamada and Sgarbieri [95] with the same intent. Organic solvents can also be used for protein precipitation as shown by Farra [90], who proposed the use of a saline solution and an

alcohol medium for protein precipitation. Since these yeast protein precipitations involved several steps and are difficult to scale-up, Akardere *et al.* [96] developed a scalable three-phase partitioning (TPP) to purify a *S. cerevisiae* glycoprotein, namely invertase, in a single step, after sonication treatment. This technique combines salting out, use of organic solvents and precipitation pH techniques into one-step system where the crude protein extract is mixed with solid ammonium sulphate and t-butanol in order to obtain the desired proteins selectively partitioned and concentrated to one phase. Hydrophilicity and protein MW affected the partitioning process outcome [96].

3.2.2. Membrane filtration

Membrane filtration is another alternative for separation of soluble intracellular proteins from cell lysates which allows filtrate recovery and cell debris removal with high selectivity and satisfactory efficiency both in cross-flow and dead-end systems and low energy consumption [92, 97]. In fact, this process is of great interest for increase of peptide fractions purity since it may promote the separation through sieving and charge-based mechanisms [98].Food processing industry has been using this technique over the last decades since peptides and proteins need a gentle product treatment (low temperatures and pH value close to neutrality) to maintain their structural and physicochemical characteristics since their bioactive properties depend on peptides and proteins sequence and structure [13, 97, 98]. Membrane selectivity and properties (material, structure and pore size), permeate flux (which is dependent on operating conditions such as temperature, pressure, process configuration, module characteristics, cleaning procedure) and feed characteristics (physicochemical of components, pH and concentration) are the main parameters that influence the separation performance [98]. However, the membrane fouling control and their cleaning processes are still a challenge [97].

Membrane filtration has been reported for the isolation of *S. cerevisiae* bioactive peptides [7, 99–102], specific proteins [103], nucleoprotein complexes [93] and separation of yeast hydrolysates from sugars and ribonucleic acids (RNA) [85], being the intended application of separation an important aspect of process design [98]. Many times, this technique was preceded by protein precipitation [93], enzymatic hydrolysis [85, 99, 100, 102], autolysis [102] and physical disruption methods, such as sonication [102] or bead milling [103]. Adsorption [93, 101] and chromatography [84,86-89] are other protein purification and fractionation methods used prior to membrane filtration.

Ultrafiltration with 50 kDa [85], 15 kDa [85], 10 kDa [7, 100–102], 8 kDa [85], 5 kDa [99, 102], 3 kDa [102], 2 kDa [7, 100, 101] and 1 kDa [85, 103] cut-off membranes have been reported on yeast protein separation and concentration. In fact, several authors applied a sequential ultrafiltration process using a 10 kDa cut-off membrane followed by 2 kDa for production of antibacterial peptides [7, 100, 101]. *Marson et al.* [85] applied two serial fractionation of 50, 8 and 1 kDa and 15, 8 and 1 kDa for separation of yeast hydrolysate from sugars and RNA, demonstrated that 15 kDa retained higher MW compounds, increasing performance of the next separation steps at 8 and 1 kDa . This fractionation was important for improvement of peptide purity of fractions from RNA and total sugars (1.7 and 2.7fold, respectively). Potential antihypertensive and antioxidant peptides were also produced through ultrafiltration processes [99, 102] as well as β-secretase [103]. Butylina *et al.* [93] applied a cross-flow microfiltration system through track-etched membranes 0.3 µm to separate the high and low molecular mass fractions, concluding that the nucleoprotein complexes were retained by membrane and were found to activate repair pathways in oxidative stress cells of *S. cerevisiae*.

3.2.3. Dialysis

Dialysis is a conventional lab-scale technique to reduce or remove salt from protein extracts by osmotic phenomena using a semi-permeable membrane [104]. However, this procedure can take up several days, requires large volumes of water and can lead to low protein yields, since significant losses of low MW compounds can occur through the membrane system [105], turning this a non-scalable process for industries. Nevertheless, Farra [90] patented a yeast peptide hydrolysate production process for cosmetic industry where a variant of the method includes a dialysis technique.

3.2.4. Chromatography

Chromatographic separation of protein mixtures has become one of the most effective and widely used techniques for purifying individual proteins. Depending on protein properties, such as size, charge, hydrophobicity, and bio specific interactions, different versions of liquid chromatography, with several types of stationary phases, are used for protein and peptide isolation from *S. cerevisiae.* As in the other brewer's yeast protein purification techniques, chromatographic methods are generally used after cell disruption methods [57, 69, 93, 103] and/or fractions separation by ultrafiltration [7, 93, 99, 101, 103].

Size-exclusion chromatography (SEC), also known as gel-filtration chromatography, is one of the widely used chromatographic techniques for isolation of antimicrobial, antihypertensive and antidementia peptides from *S. cerevisiae*. SEC is a reference technique for the qualitative and quantitative analysis of protein aggregates in protein biotherapeutics because of its speed and reproducibility [106]. It is based on the sieving properties of the stationary phase matrix, which is constituted by porous particles, with separation depending on protein's size and shape [91]. Preparative columns composed by gel matrices of dextran polymers and highly cross-linked agarose (Superdex) [7, 101] or epichlorohydrin (Sephadex) with different bead size are the most used in these studies. The surface of these supports contains predominantly hydroxyl groups and provides a good environment for hydrophilic proteins [91]. Gddoa Al-sahlany *et al.* [7] obtained three different peptide fractions after employing gel filtration chromatography using a purifying system (ÄKTA™) with a Superdex column. As SEC allows to separate proteins according to their size, Butylina *et al.* [93] used this method to estimate the molar mass (MM) distribution of yeast nucleoprotein complexes. The authors described the high MM fraction with tightly bound proteins in the first peak eluted since no dissociated protein molecules were detected.

Several studies described the application of ion-exchange chromatography (IEC) coupled with SEC in order to eliminate matrix contaminants or to concentrate the peptide fractions [93, 101, 107]. In fact, IEC is one of the most commonly used industrial chromatographic processes for purification of pharmaceutical proteins and peptides, since their mild conditions allows to maintain the native molecule structures and their resins have high binding capacities, offering a good and controllable selectivity [108]. IEC is based on electrostatic interactions, being the protein separation accomplished by competition between proteins with different surface charges for oppositely charged groups on an ion exchanger adsorbent [91]. Protein binding to the stationary phase, as well as their desorption, can be modulated by changes in ionic strength and pH, through ionic competition or change in protein charge, respectively [108]. Branco *et al.* [101] pooled the most bioactive peptide fraction resulted from SEC separation at 2-10 kDa ultrafiltration process into two different strong cation and anion-exchange columns. A similar approach was used by Butylina *et al.* [93] in order to remove protein from nucleoprotein complexes obtained after microfiltration ("retentate"). Although the aforementioned studies described combination of different chromatographic techniques,

IEC has also been described as the unique chromatographic method for the separations of *S. cerevisiae* enzymes. Lothe *et al.* [109] evaluated Amberlite XAD-16 and Indion NPA-1 resins performance to isolate α-glucosidase and invertase, trying to activate the adsorbent surface by ultrasound pre-treatment ("surface grafting") in order to reduce the hydrophobicity and nonspecific adsorption of proteins. Non-specific adsorption of proteins has considerably been reduced in grafting exchangers with an enhanced of adsorption enzymes selectively. Agrawal and Pandit [69] also optimized the batch adsorption process by native and grafted XAD-16 for α-glucosidase isolation on *S. cerevisiae* cell extract. Based on principle of IEC, expanded bed adsorption (EBA) was also proposed as a preliminary purification technique to capture *S. cerevisiae* total soluble protein and α-glucosidase after HPH or hydrodynamic cavitation yeast treatment [57]. This technique is capable to replace 3-4 unit operations in a typical downstream process, since the unclarified yeast suspension (biomass and extracellular medium) can be directly applied to the adsorbent, eliminating the need for previous solid–liquid separation and concentration steps. A higher adsorption of α-glucosidase was observed in anionic Streamline Diethylaminoethyl (DEAE) adsorbent than cationic Streamline Sulphopropyl (SP), being the yeast disruption extent an influence of dynamic binding capacity. In fact, the increase of disruption cell degree allowed a high protein release and dynamic binding capacity for total protein and α-glucosidase. However, the cell debris resulted from strong disruption seemed to have a negative impact on α-glucosidase selective adsorption [57].

Reversed-phase high-performance liquid chromatography (RP-HPLC) has also been used after SEC fractionation for isolation of bioactive peptides from *S. cerevisiae* [99, 103]. RP-HPLC is a separation method based on hydrophobicity characteristics of the protein where the stationary phase, as well of hydrophobic nature, is based on silica gel or a synthetic polymer [110]. The strong hydrophobic interaction in RP-HPLC is almost enough to adsorb proteins in pure water [91], although an acid (formic, acetic or trifluoroacetic acid) is generally added to the mobile phase to render the proteins and peptides positively charged and to reduce undesirable interactions with the stationary phase [110]. However, acids may cause the protein to denature. Pharmaceutically important globular proteins, peptides and small polypeptides are purified by RP-HPLC [104]. Nevertheless, the use of RP-HPLC is limited for large-scale processes since low mass yields and loss of biological activity of larger polypeptides can be found due to acidic buffering systems and hydrophobicity of silica columns [104]. The purification of

several *S. cerevisiae* peptides, separation and concentration in preparative µBondapak C18 column [99, 103] followed by analytical Protein & Peptide C18 column [99] was studied: Kim *et al.* [99] purified a novel decapeptide with antihypertensive properties through ultrafiltration, SEC and RP-HPLC separation with a yield of 3.5%. Lee *et al.* [103] characterized a new antidementia peptide obtained by yeast bead milling disintegration, ultrafiltration, SEC and two RP-HPLC separation and concentration processes with a yield of 0.6%. In both uses of µBondapak C18 column, a linear gradient with 0.1% of trifluoroacetic acid in water was used as mobile phase [99, 103].

3.3. Nucleic acids extraction

One of the challenges of using *S. cerevisiae* protein-rich extracts as food supplement for humans is the high content of nucleic acid, mainly RNA, since their high intake may result in health issues [94]. Several authors have already described processes of *S. cerevisiae* protein-rich extract production attending to obtain a low RNA content. However, the first proposed methods to reduce nucleic acid content from yeast protein involved strong chemical and enzymatic treatments which led to several harmful effects on the nutritional and functional qualities of the isolated protein. Potentially toxic compounds resulting from alkali treatment [111, 112], protein degradation by nucleic acid enzymatic hydrolysis [113] and the nutritional safety of proteins produced by their acid anhydride modification [111, 112, 114] were pointed.

On the other hand, chemical phosphorylation has been described for decades as one of the best RNA removal processes, since the addition of phosphorus oxychloride [115, 116] or sodium trimetaphosphate [95] to the disrupted cell extract at alkaline pH caused dissociation of nucleoprotein complexes. Kinsella and Damodaran [115] showed a maximum RNA reduction of 80% applying a minimum of phosphorus oxychloride/protein ratio. The proposed mechanism states that the net negative charges on the protein introduces a strong electrostatic repulsion within the nucleoprotein complexes. As the dissociated nucleic acids have an isoelectric pH around 1.5-2.0, they remain soluble during protein precipitation at pH 4.2 [115]. Huang and Kinsella [116] removed more than 85% of RNA by protein phosphorylation while no change in the amino acid composition of yeast proteins was observed. Despite modification of protein by phosphorylation may be more acceptable than other chemical methods with good percentages of RNA reduction, protein yield is pointed as one of the main issues since the reaction is depending on the pH of the protein precipitation [95, 115, 116]. Yamada and

Sgarbieri [95] observe a 10.4% increase in RNA content in the final extract by tuning pH to 3.2 in order to raise the protein yield.

Adding a step of sodium perchlorate treatment to the disrupted cell extract is another method for RNA reduction, leading to its decrease from 7.04% (in biomass) for 2.26% (in protein final extract) [94]. In fact, this reagent is used in experimental protocol for RNA determination in yeast extracts [94, 95].

Liu, Lebovka and Vorobiev [59] proposed a selective extraction of intracellular yeast components by electrical treatment (HVED and PEF) aiming to extract initially ionic and nucleic acid components and then proteins using HPH treatment, which can be useful for nucleic acid reduction in protein-rich extracts production. Besides, Chae, Joo and In [82] used nuclease treatment to dissociate nucleotides from yeast protein previously hydrolysed in order to produce flavour ingredients, which can be potentially used for RNA reduction as well. After treatments using optimal combination of enzyme, enzyme dosages and treatment sequence, low MW peptides and free amino acids were obtained in final yeast extract with a yield of 3.67% of 5'-nucloetides content [82].

In another approach, some authors aim to produce protein-rich extracts with a high RNA content in order to be used as flavouring ingredients. Oliveira et al. [117] optimized an autolysis process on spent yeast in order to obtain a RNA yield of 89.% that results a yeast extract with 57.9% protein as well (55.2 ºC, pH 5.1 with 9.8% NaCl for 24h), being the heat treatment (60ºC, 15 min) prior to autolysis an essential step to increase the RNA content for 91.4%. Sombutyanuchit *et al.* [118] also studied a similar autolysis process of for disodium guanosine-5'-monophosphate (5´-GMP)-rich extracts production using yeast pre-heat treatment and 5'-phosphodiesterase. A yeast extract was produced with a 5'-GMP maximum of 0.93% (w/w).

4. Protein-rich extracts characterization

Characterization plays an important role throughout the entire process of protein-rich extracts production, since it helps to understand the chemical and biological potential of extracts, adapting the application to different sectors according to their performance. Furthermore, characterization techniques are able to evaluate the efficiency of protein extraction and the subsequent purification and isolation processes. Regarding protein analysis, protein and amino acids determination and MW evaluation are the main parameters assessed. Since *S. cerevisiae* protein extracts are mainly used as food

supplement [80, 84, 94, 95] with potential low MW bioactive peptides [7, 99–101, 103], these parameters are the most relevant to monitor. Besides these, total sugars, lipids and fibre, fatty acids, ashes and RNA content are other nutritional and toxicological analysis included in evaluation of protein yeast extract composition [94, 95].

4.1. Protein content

The quantification of protein is a routine procedure in many research laboratories, since it is required to calculate and monitor the protein yield after various enrichment or purification processes, as well as to optimize and standardize downstream experiments [119]. As listed in previous tables, several methods have been used for protein determination in *S. cerevisiae* extracts. Overall, most methods overestimate protein content since they use indirect readings, which may suffer interference from other chemical substances. Furthermore, it is described that the reported protein content depends on the protocol used for determination and from the initial matrix, making a direct comparison between studies difficult [120]. A direct and precise protein determination is obtained when the amino acid residues are quantified (**Section 4.2**).

Regarding indirect protein determination methods used in *S. cerevisiae* extracts, the Kjeldahl method has been applied by different authors [49, 52, 53, 81, 82, 84]. In this method, the protein content is determined by the measurement of total nitrogen, which is multiplied by a conversion factor based on amino acid characterization of samples (spectrum, number of amino groups and MW) [52, 121].The main disadvantage of this method is the detection of other nitrogen containing non-protein compounds, such as nucleic acids, which can result in protein overestimation, besides the problem of unknown samples, where conversion factor cannot be accurately calculated [44]. Due to its speed and simplicity, many authors have used spectrophotometric assays such as the Bradford, Lowry and bicinchoninic acid (BCA) protocols. The Bradford protocol is not capable of detecting low MW peptides or amino acids [44]. On the other hand, Lowry and BCA assays are based on the identification of peptide bonds by protein-cooper chelation between Folin-Ciocalteu reagent and the ring structure on aromatic amino acids [122, 123]. The main advantage of BCA is that can be included in the copper solution to allow a one-step procedure, being stable at alkaline conditions. However, some single amino acids, such as cysteine, tyrosine and tryptophan will also produce colour and can interfere in BCA results [123]. Several examples of protein determination in yeast extracts

performed by Lowry and BCA are present in **Table 1** and **Table 2**. Both of these methods respond more uniformly to different proteins than the Bradford protocol [44].

4.2. Amino acid determination

A constant amount of amino acids needs to be maintained for ensuring a balanced level of nitrogen in human cells. The human body is responsible for synthesize some of proteinogenic amino acids, namely the non-essential, while others have to be absorbed via protein dietary intake (essential amino acids - EAA) [44]. Since spent brewer's yeast has been described as a potential source of EAA, its protein-rich extracts are widely used for food supplementation [124], and thus amino acid determination is one of the most important characterization analysis to be performed. The determination of amino acid content is generally preceded by acid hydrolysis, in order to cleave peptide bonds [44, 52, 80, 84, 94, 95]. Specific amino acid analysers with post column ninhydrin reaction [94, 95] and HPLC-UV/VIS with dansyl chloride derivatization [80, 84] were used for amino acid quantification. On the other hand, tryptophan is determined in the alkaline hydrolysate [80, 84, 94, 95] by HPLC-fluorescence detection [80, 84] or amino acid analyser equipment [94, 95].

In fact, yeasts themselves contain different types of proteases and peptidases responsible for the breakdown of the proteins into small peptides and then further into free amino acid [125]. Furthermore, many extraction processes on *S. cerevisiae* are also capable of breaking the protein into free amino acid, depending on cell disruption method and subsequent processing steps (**Table 4**). Jacob *et al.* [44] observed that the amino acid profile of yeast extract was dependent upon the disruption methods applied; autolysis (24h, 50ºC) allowed for the higher amount of amino acid release, followed by sonotrode and bead milling (307, 155 and 115 mg protein/g yeast extract, respectively). In another study, the same authors confirmed this conclusion since they observed a free amino content of 433.21 mg/g in autolysates in comparison with mechanical methods (115.68 and 155.38 mg/g) [52] . In relation to EAA content, with the exception of glutamic acid, no differences were observed between the different disruption methods [44]. Podpora and Swiderski [80] also observed an increase of free amino acids during autolysis process from 11.2% to 77.5% (2h from 48h). In agreement, when performing enzymatic hydrolysis (Papain during 24h; 50-60ºC), Podpora *et al.* [84] also observed the breakdown of protein into free amino acids, establishing yeast extract as valuable source of EAA such as isoleucine, lysine, valine, threonine and phenylalanine+tyrosine. High

concentrations of glutamic acid in the final extracts (3.84 and 2.07%) were also observed, leading to strong flavour-enhancing properties. Overall, the total EAA obtained by Podpora and Swiderski [80], Podpora *et al.* [84] and Jacob, Hutzler and Methner [44] were above the FAO/WHO protein reference (**Table 4**) [22] which turns yeast extracts an valuable components of several products from the group of functional foods and dietary supplements.

EAA profile of protein-rich *S. cerevisiae* extracts to be potentially used as food supplements are presented in **Table 4**. For phosphorylated yeast protein concentrates, sulphur amino acids were described as the limiting factor to the nutritive value of yeast protein [94, 95]. Caballero-Córdoba and Sgarbieri [94] obtained an EAA level of 87.2% in protein concentrate based on available lysine (limiting amino acid) and comparable with the FAO/WHO reference standard. However, no pattern is clear: while Caballero-Córdoba and Sgarbieri [94] observed a loss of lysine bioavailability in protein concentrate in comparison with yeast biomass, possibly explained by the reaction of the sodium perchlorate with the protein or by protein fractionation and/or precipitation with lysine loss, Yamada and Sgarbieri [95] have observed a slightly increase of all EAA in protein concentrate which suggest higher degradation of amino acids in yeast biomass due to acid hydrolysis or selective precipitation of proteins in the protein concentrate. Yamada and Sgarbieri [95] obtained a high content of lysine and tryptophan in the protein yeast concentrate, becoming a good candidate to enrich cereal proteins.

Regarding the amino acid sequencing of bioactive peptides, it is usually performed by mass spectrometry (MS). This technique has been proven as a robust and reliable tool for identification of amino acid sequence and protein post-translational modifications in proteomics [126]. Branco *et al.* [101] identified two main antimicrobial peptides with the amino acid residues VSWYDNEYGYSTR and ISWYDNEYGYSAR in extracted fractions, and Kim *et al.* [99] observed an amino acid sequence of YDGGVFRVYT for an antihypertensive peptide. Using protein sequencer equipment, Lee *et al.* [103] also obtained the amino acid identification of a purified antidementia peptide (GPLGPIGS).

765 **Table 4.** Essential amino acids content in protein-rich *S. cerevisiae* extracts to be potentially used as food supplements.

Extraction and purification [a]	**EAA (mg/g protein)**										**Total EAA (mg/g)**	**Reference**
	Thr	*Met + Cys*	*Val*	*Ile*	*Leu*	*Leu + Nva*	*Tyr + Phe*	*Lys*	*His*	*Trp*		
Bead milling followed by sodium perchlorate treatment and protein precipitation at isoelectric pH	40.7	23.0	59.1	50.9	86.2	NQ	87.9	87.8	27.7	13.9	NM	[94]
Bead milling followed by protein phosphorylation at alkaline pH	50.0	23.0	60.0	51.0	85.0	NQ	92.0	92.0	24.0	18.0	NM	[95]
Autolysis (48h, 47ºC)	61.3	23.8	69.7	23.7	NQ	65.1	90.6	47.2	NE	11.9	303	[80]
Enzymatic hydrolysis - Papain® (24h, 50-60ºC)	38.0	24	50.0	41.0	60.0	NQ	72.0	60.0	NE	11.0	356	[84]
Autolysis (24h, 50ºC)	46.9	46.8	55.9	42.7	76.3	NQ	48.8	66.1	25.5	NQ	409	[44]
FAO/WHO reference	11.0	20.0	15.0	15.0	21.0	NM	21.0	18.0	15.0	15.0	136	[22]

766 [a] Most efficient method for amino acids release. EAA – Essential amino acids; NQ – Not quantified; NM – Not mentioned; Thr – Threonine; Met – Methionine;
767 Cys – Cysteine; Val – Valine; Ile - Isoleucine; Leu – Leucine; Nva – Norleucine; Tyr – Tyrosine; Phe – Phenylalanine; Lys – Lysine; His – Histidine; Trp –
768 Tryptophan

4.3. Protein size

The determination of protein MW is a routine procedure in many research laboratories since it allows to identify specific proteins, oligomers and monomers [119]. In the characterization of *S. cerevisiae* protein, it has been described for the identification of invertase and peptides with antimicrobial, antidementia or antihypertensive properties (**Table 5**). Furthermore, MW analysis has also been described for the evaluation of protein extraction extent from brewer's yeast and characterization of yeast extracts produced for functional food (**Table 5**).

4.3.1. Mass spectrometry

Mass spectrometry (MS) has been used for characterization of the higher order structure of protein therapeutics as early as mid-1990s. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are complementary MS ionization techniques that allow MW determination of large biomolecules based on mass and charge (*m/z*) ratio. ESI-MS produces multiple charged ions with a mass range up to m/z 3000 with a mass limitation around 100 kDa, which allow a correct determination of protein MW[119]. As seen in **Table 5**, the MW of different bioactive peptides from *S. cerevisiae* was determined by ESI-MS.

MALDI in combination with time-of-flight (TOF) mass analyser has also been reported for protein size characterization of *S. cerevisiae* extracts (**Table 5**). This technique is highly sensitive, enabling the identification of unknown proteins at very low concentrations [119]. In comparison with ESI, MALDI generates ions with low charge states (≤ 3) which limits the identification of proteins with high MW [127]. Podpora and Swiderski [80] produced yeast autolysates for food supplements with 74-96% of 1000-2000 Da peptides (about 10-20 amino acid residues) while Podpora *et al.* [84] obtained yeast extracts contain large amounts of free amino acids and only trace amounts of peptides (1740Da).

4.3.2. SDS-PAGE

Although protein size characterization techniques are developing at a fast pace, the current standard method is still denaturing SDS-PAGE, which includes a number of laborious and time-consuming manual steps [119]. This technique separates proteins according to their size as they are forced through a gel by an electrical current [104]. Generally, proteins are denatured by binding to SDS anionic detergent, where the amount

of bound SDS is proportional to their size. The treatment with reducing agents as 2-mercaptoethanol or dithiothreitol is usually necessary to reduce protein disulphide bridges before the proteins adopt the random-coil configuration necessary for separation by size. Sizing accuracy depends on other protein characteristics since particular proteins are not truly migrating according to their MW, as is the case of glycosylated proteins [119]. As can be seen in **Table 5**, SDS-PAGE has been described for peptides and protein size evaluation in *S. cerevisiae.* Gddoa Al-sahlany *et al.* [7] obtained a single band in SDS-PAGE that corresponded to a purified antibacterial peptide with approximately 9770 Da which might match the antibacterial activity produced by *S. cerevisiae*. Albergaria *et al.* [100] obtained three small bands in SDS-PAGE (6.0, 4.5 and 4.0 kDa) produced by *S. cerevisiae* during alcoholic fermentation which might correspond to antimicrobial compounds that are active against some non-*Saccharomyces* wine-related strains. Estimating MW from specific proteins by SDS-PAGE, Akardere *et al.* [96] obtained a invertase with 52 kDa by TPP extraction since MW of invertases vary according to their source and the applied method. *S. cerevisiae* provides internal and external invertase with MW from 50 to 300 kDa [96].

In addition to estimate size of specific peptides or proteins, SDS-PAGE has also been reported as a characterization technique to evaluate the extent of protein extraction in *S. cerevisiae* using different methods. In fact, most the chemical approaches to extract protein from brewer's yeast, listed at **Table 2**, are followed by MW evaluation. Kushnirov [75] used SDS-PAGE to optimize the introduction of a mild alkali treatment in yeast protein extraction protocol where the maximum extraction was obtained at 5 min. After 2 min, the reaction was almost complete except for proteins exceeding 100 kDa [75]. Zhang *et al.* [76] evaluate the extent of yeast protein extraction with LiAc, followed by NaOH and SDS-PAGE buffer treatment using SDS-PAGE technique. Mukherjee *et al.* [77] also used SDS-PAGE to evaluate protein pattern of different approaches to extract protein from *S. cerevisiae* at different growth phases. The hot-SDS method showed a better size distribution of protein bands with a good yield of proteins with MW higher than 80 kDa. The alkali pre-treatment allowed an apparent complete spectrum of proteins across a range of MW and the modified alkali pre-treatment [75] resulted in maximum yield with sharp and distinct bands in SDS-PAGE. However, loss of enzymatic activity may be of concern using these alkali protocols.

Some physical approaches for *S. cerevisiae* protein extraction have also used SDS-PAGE. Geneva *et al.* [70] applied an electrical treatment to yeast crude extracts and supernatants obtaining the most of the bands above 29 kDa. Shynkaryk *et al.* [128] compared protein patterns for untreated and PEF, HVED and HPH treated yeast suspensions. The more powerful physical cell disintegration allowed an effective extraction of high MW proteins. Electric treatments (PEF and HVED) can produce effective electroporation and accelerate release of the low MW components, but it was not sufficient for release of high MW intracellular components.

Using the principle of electrophoresis, commercial kits coupled to protein analyser equipment have recently been used for protein size fractionation. Protein 80 kit was used by Jacob *et al.* [44] in order to compare the protein profiles of different industrial methods to produce yeast extracts. Autolysis yeast extract only presented protein below 4 kDa in opposition to physical methods (cell mill and sonotrode) that yielded the most protein in the range from 3.5 to 63 kDa.

849 **Table 5.** Size characterization methods for evaluation of protein from *S. cerevisiae*.

Methods	Extract characterization	Extraction and purification	Size	Reference
LC-ESI-MS	ACE inhibitory peptide	Enzymatic hydrolysis (pepsin, trypsin, protease; 12h) followed by 5kDa ultrafiltration and SEC and RP-HPLC purification	1.178 kDa	[99]
	Antidementia β-secretase inhibitor peptide	Bead milling followed by 1 kDa ultrafiltration and SEC and RP-HPLC purification	697 Da	[103]
	Antimicrobial peptides	10 and 2 kDa ultrafiltration followed by SEC and IEC purification	1.638 and 1.622 kDa	[101]
Maldi-TOF	Yeast autolysates	Autolysis (2-48h; 47ºC)	1000-2000 Da	[80]
	Yeast extracts	Enzymatic hydrolysis (Papain; 24h; 50-60ºC)	703-1740 Da	[84]
SDS-PAGE	Peptide fraction	Enzymatic hydrolysis (trypsin, alkaline protease mixture; 72h; 37ºC) followed by 10 kDa and 2kDa ultrafiltration	6.0, 4.5 and 4.0 kDa	[100]
	Invertase	Sonication followed by TPP with ammonium sulfate and t-butanol	52 kDa	[96]
	Antimicrobial peptide	10 kDa and 2 kDa ultrafiltration followed by SEC purification	9770 Da	[7]

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4.3.3. SEC

As described at **Section 3.2.4**, SEC is a chromatographic technique that allow to separate and isolate proteins based on their size and shape. One of the most widely SEC techniques used for protein size characterization is the Fast Protein Liquid Chromatography (FPLC). For that reason, this technique has also been used for protein size estimation of nucleoprotein complexes [93] and hydrolysates [81] from *S. cerevisiae.* They are determined by calibrating SEC column retention times or elution volumes with an appropriate series of macromolecular standards, as FPLC, or by employing molecular mass sensitive detection methods such as viscosimetry or light scattering [119]. Butylina *et al.* [93] estimated MW distribution of yeast nucleoprotein complexes obtained three different fractions: firstly, a tightly bound proteins fraction; secondly, a 200–6 kg/mol nucleic acids fraction and the last only included single nucleotides (MM below 6 kg/mol). Xie *et al.* [81] evaluate the MW distribution of hydrolysates acquired after 24 h of enzymatic hydrolysis at different solid concentrations and observed almost identical MW distribution in the Papain and Alcalase hydrolysates with MW peptides below 3 kDa reaching 90%. However, Papain, at high solid concentrations, produced hydrolysates with high fractions of low MW peptides (below 1 kDa) while Alcalase fractions at different solid concentration were almost identical. Also, *Marson et al.* [85] observed that 2h of enzymatic hydrolysis with proteases cocktail (Brauzyn, Protamex and Alcalase) (pH 7.0, 50ºC) cleaved the yeast original protein into peptides from 7000 to 1000 g/mol (43%) and 1000-100 g/mol (25%) since non-treated yeast presented higher molecules with MW above 7000 g/mol.

5. Conclusions

Since spent brewer's yeast is one of the main brewing by-products with 309,400 to 418,600 tonnes production per year, several strategies for its valorisation have been developed in a circular economy concept. Its nutritional composition, with more than 50% of protein, as well as being an inexpensive source of bioactive ingredients, are pointed out as some of the reasons for the yeast grow market.

S. cerevisiae has been commercialized for years as yeast extracts, but the production of protein-rich extracts is still a challenge because of the necessary increase of protein rate in the final product. For that, different protein extraction processes have been optimized, attempting to achieve efficient and cost-effective release of proteins from

yeast cells. The *S. cerevisiae* extraction methods for protein release described in literature can be classified, according to their main operation mode, in physical (either using pressure or waves), chemical (using chemical substances) or enzymatic methods (autolysis and/or hydrolysis). However, a combination of methods is also possible and often desirable.

Among the abovementioned methods, ultrasonication seems to be quite effective, although limited by operational and economical constraints, thus leading the way to bead milling and HPH as favoured physical extraction processes at industrial scale. However, since they both present the downside of poor selectivity, enzymatic hydrolysis may be preferred when specificity is the key parameter. Chemical methods are also relatively specific, although the experimental conditions may provoke alteration on final protein content or peptide and amino acid profile. Finally, autolysis has the main advantage of only using yeast's own enzymes for the process, but the temperature and long-time treatment can lead to bacterial contamination on yeast suspension, turning the final product unfeasible. In conclusion, process cost and selectively are inversely related, although it must be kept in mind that a clean extract will decrease the downstream isolation and purification costs. Nevertheless, the selection of the extraction method will be dictated by the final use of the protein extract.

In general, several procedures of protein isolation and purification are coupled to extraction since they are required to obtain the final protein-rich extracts with the desired performance according to economic sector to be applied. Protein precipitation, dialysis, membrane filtration and chromatography have been applied individually or combined, in order to concentrate the protein, isolate specific ones or remove unwanted contaminants. However, many of them are difficult to scale-up due to economic constrains, such as chromatography, or can lead to low protein yields, as dialysis. On the other hand, membrane filtration has been widely used since is considered a gentle, being quite fast and relatively economical, which makes it a potential tool to protein-rich extracts production.

Characterization techniques applied to protein-rich extracts produced are extremely useful to evaluate the efficiency of extraction and the subsequent purification and isolation processes. Kjeldahl is the reference method for protein analysis, followed by spectrophotometric assays; within these methodologies an over or underestimation of

protein content can occur since the readings are indirect and/or due to the interference of other chemical substances present. A direct and more precise protein determination is obtained when the amino acid residues are quantified by chromatographic techniques. Some of these protocols are also capable of identify peptides and their MW, which is particularly important concerning bioactive peptides.

Currently, one of the main challenges related to protein extraction from spent yeast remain in establishing a scalable, low cost, efficient and reproducible process in order to produce large amounts of bioactive peptides, due to their growing interest for nutraceutical and cosmetic sectors. Therefore, the advances within all the above-mentioned processes and techniques are also providing benefits to increasing areas of application for *S cerevisiae.*

Author contributions

A.S. Oliveira drafted the work, being in charge of conceptualization, literature search and writing; C. Ferreira conduced the conceptualization, revision and edition; J.O. Pereira contributed for revision and edition; M. E. Pintado leaded the supervision and project administration; A. P. Carvalho was responsible for final revision and edition, and supervision.

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Declarations

Competing of interest

The authors declare no competing interests.

References

1. Hayes M (2018) Food Proteins and Bioactive Peptides: New and Novel Sources, Characterisation Strategies and Applications. Foods 7:38. https://doi.org/10.3390/foods7030038

2. Food and Agriculture Organization of the United Nations (2018) The future of food and agriculture – Alternative pathways to 2050. http://www.fao.org/global-perspectives-studies/resources/detail/en/c/1157082/. Accessed 26 Nov 2021

3. Henchion M, Hayes M, Mullen A, et al (2017) Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. Foods 6:53. https://doi.org/10.3390/foods6070053

4. Okolie CL, Akanbi TO, Mason B, et al (2019) Influence of conventional and recent extraction technologies on physicochemical properties of bioactive macromolecules from natural sources: A review. Food Res Int 116:827–839. https://doi.org/10.1016/j.foodres.2018.09.018

5. Transparency Market Research (2020) Peptide Therapeutics Market- Global Industry Analysis, Size, Share, Growth, Trends, and Forecast 2019 - 2027. https://www.transparencymarketresearch.com/peptide-therapeutics-market.html. Accessed 26 Nov 2021

6. Mirzaei M, Mirdamadi S, Safavi M (2019) Antioxidant activity and protective effects of Saccharomyces cerevisiae peptide fractions against - H 2 O 2 - induced oxidative stress in Caco - 2 cells. J Food Meas Charact 13:2654–2662. https://doi.org/10.1007/s11694-019-00186-5

7. Gddoa Al-sahlany ST, Altemimi AB, Abd Al-Manhel AJ, et al (2020) Purification of Bioactive Peptide with Antimicrobial Properties Produced by *Saccharomyces cerevisiae*. Foods 9:1–11. https://doi.org/10.3390/foods9030324

8. Jung EY, Lee HS, Choi JW, et al (2011) Glucose Tolerance and Antioxidant Activity of Spent Brewer's Yeast Hydrolysate with a High Content of Cyclo-His-Pro (CHP). J Food Sci 76:272–278. https://doi.org/10.1111/j.1750-3841.2010.01997.x

9. Kim KM, Chang UJ, Kang DH, et al (2004) Yeast Hydrolysate Reduces Body Fat of Dietary Obese Rats. Phytother Res 18:950–953. https://doi.org/10.1002/ptr.1582

10. Indumathi P, Mehta A (2016) A novel anticoagulant peptide from the Nori hydrolysate. J Funct Foods 20:606–617. https://doi.org/10.1016/j.jff.2015.11.016

11. Amorim M, Marques C, Pereira JO, et al (2019) Antihypertensive effect of spent brewer yeast peptide. Process Biochem 76:213–218. https://doi.org/10.1016/j.procbio.2018.10.004

12. de la Hoz L, Ponezi AN, Milani RF, et al (2014) Iron-binding properties of sugar cane yeast peptides. Food Chem 142:166–169. https://doi.org/10.1016/j.foodchem.2013.06.133

13. Marson GV, de Castro RJS, Belleville MP, Hubinger MD (2020) Spent brewer's yeast as a source of high added value molecules: a systematic review on its characteristics, processing and potential applications. World J Microbiol Biotechnol 36:1–22. https://doi.org/10.1007/s11274-020-02866-7

14. Food Agricultural Organization of the United Nations (2019) Food balance sheets: Protein supply quantity (g/capita/day). http://www.fao.org/faostat/en/#data/FBS. Accessed 26 Jan 2022

15. Sá AGA, Moreno YMF, Carciofi BAM (2020) Plant proteins as high-quality nutritional source for human diet. Trends Food Sci Technol 97:170–184. https://doi.org/10.1016/j.tifs.2020.01.011

16. Hayes M, Mora L, Hussey K, Aluko RE (2016) Boarfish protein recovery using the pH-shift process and generation of protein hydrolysates with ACE-I and antihypertensive bioactivities in spontaneously hypertensive rats. Innov Food Sci Emerg Technol 37:253–260. https://doi.org/10.1016/j.ifset.2016.03.014

17. Akhtar Y, Isman MB (2018) Insects as an Alternative Protein Source. In: Proteins in Food Processing. Elsevier, pp 263–288

18. Ritala A, Häkkinen ST, Toivari M, Wiebe MG (2017) Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. Front Microbiol 8:.

https://doi.org/10.3389/fmicb.2017.02009

19. Nasseri AT, Rasoul-Ami S, Morowvat MH, Ghasemi Y (2011) Single Cell Protein: Production and Process. Am J Food Technol 6:103–116. https://doi.org/10.3923/ajft.2011.103.116

20. Jones SW, Karpol A, Friedman S, et al (2020) Recent advances in single cell protein use as a feed ingredient in aquaculture. Curr Opin Biotechnol 61:189–197. https://doi.org/10.1016/j.copbio.2019.12.026

21. Tibbetts SM (2018) The Potential for 'Next-Generation', Microalgae-Based Feed Ingredients for Salmonid Aquaculture in Context of the Blue Revolution. In: Microalgal Biotechnology. InTech

22. World Health Organization (2007) Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation

23. Kuhad RC, Singh A, Tripathi KK, et al (1997) Microorganisms as an Alternative Source of Protein. Nutr Rev 55:65–75

24. Rudravaram R, Chandel AK, Rao LV, et al (2009) Bio (Single Cell) Protein: Issues of Production, Toxins and Commercialisation Status. In: Agricultural Wastes. pp 129–153

25. Fleet GH (2007) Yeasts in foods and beverages: impact on product quality and safety. Curr Opin Biotechnol 18:170–175. https://doi.org/10.1016/j.copbio.2007.01.010

26. Pereira PR, Freitas CS, Paschoalin VMF (2021) Saccharomyces cerevisiae biomass as a source of next-generation food preservatives: Evaluating potential proteins as a source of antimicrobial peptides. Compr Rev Food Sci Food Saf 20:4450–4479. https://doi.org/10.1111/1541-4337.12798

27. Fărcaş AC, Socaci SA, Mudura E, et al (2017) Exploitation of Brewing Industry Wastes to Produce Functional Ingredients. Brew Technol. https://doi.org/10.5772/intechopen.69231

28. García-Garibay M, Gómez-Ruiz L, Cruz-Guerrero AE, Bárzana E (2014) Single cell protein: Yeasts and Bacteria. In: Encyclopedia of Food Microbiology.

Elsevier, pp 431–438

29. Kurcz A, Błażejak S, Kot AM, et al (2018) Application of Industrial Wastes for the Production of Microbial Single-Cell Protein by Fodder Yeast Candida utilis. Waste and Biomass Valorization 9:57–64. https://doi.org/10.1007/s12649-016-9782-z

30. Bombe K (2019) Specialty Yeast Market by Type (Yeast Extract, Yeast Autolysate, Yeast Beta - Glucan), Application (Bakery Production, Flavoring, Biofuels), Species (*Saccharomyces Cerevisiae*, *Kluyveromyces*), and Industry – Global Forecast to 2025. https://www.meticulousresearch.com/product/specialty-yeast-market-5032/?utm_source=Globnewswire.com&utm_medium=PressRelease&utm_campaign=Paid

31. Jaeger A, Arendt EK, Zannini E (2020) Brewer ' s Spent Yeast (BSY), an Underutilized Brewing By-Product. 1–23. https://doi.org/10.3390/fermentation6040123

32. Rakowska R, Sadowska A, Dybkowska E, Świderski F (2017) Spent yeast as natural source of functional food additives. Rocz Panstw Zakl Hig 68:115–121

33. Payen C, Thompson D (2019) The renaissance of yeasts as microbial factories in the modern age of biomanufacturing. Yeast 36:685–700. https://doi.org/10.1002/yea.3439

34. Stewart GG (2016) Saccharomyces species in the production of beer. Beverages 2:. https://doi.org/10.3390/beverages2040034

35. Conway J (2021) Beer production worldwide from 1998 to 2020. https://www.statista.com/statistics/270275/worldwide-beer-production/. Accessed 26 Nov 2021

36. Cooray ST, Lee JJL, Chen WN (2017) Evaluation of brewers' spent grain as a novel media for yeast growth. AMB Express 7:117. https://doi.org/10.1186/s13568-017-0414-1

37. Vieira EF, Cunha SC, Ferreira IMPLVO (2019) Characterization of a Potential

Bioactive Food Ingredient from Inner Cellular Content of Brewer's Spent Yeast. Waste and Biomass Valorization 10:3235–3242. https://doi.org/10.1007/s12649-018-0368-9

38. Feldmann H (2012) Yeast Cell Architecture and Functions. In: Yeast: Molecular and Cell Biology, Second Edi. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 5–24

39. Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 26:239–256. https://doi.org/10.1111/j.1574-6976.2002.tb00613.x

40. Faustino M, Durão J, Pereira CF, et al (2021) Mannans and mannan oligosaccharides (MOS) from Saccharomyces cerevisiae – A sustainable source of functional ingredients. Carbohydr Polym 272:. https://doi.org/10.1016/j.carbpol.2021.118467

41. Wang J, Li M, Zheng F, et al (2018) Cell wall polysaccharides: before and after autolysis of brewer's yeast. World J Microbiol Biotechnol 34:137. https://doi.org/10.1007/s11274-018-2508-6

42. Orlean P (2012) Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics 192:775–818. https://doi.org/10.1534/genetics.112.144485

43. Liu D, Ding L, Sun J, et al (2016) Yeast cell disruption strategies for recovery of intracellular bio-active compounds — A review. Innov Food Sci Emerg Technol 36:181–192. https://doi.org/10.1016/j.ifset.2016.06.017

44. Jacob FF, Hutzler M, Methner F-J (2019) Comparison of various industrially applicable disruption methods to produce yeast extract using spent yeast from top-fermenting beer production: influence on amino acid and protein content. Eur Food Res Technol 245:95–109. https://doi.org/10.1007/s00217-018-3143-z

45. Jamshad M, Darby RAJ (2012) Disruption of Yeast Cells to Isolate Recombinant Proteins. In: Methods in Molecular Biology. pp 237–246

46. Bzducha-Wróbel A, Błażejak S, Kawarska A, et al (2014) Evaluation of the Efficiency of Different Disruption Methods on Yeast Cell Wall Preparation for β-

Glucan Isolation. Molecules 19:20941–20961. https://doi.org/10.3390/molecules191220941

47. Contreras M del M, Lama-Muñoz A, Manuel Gutiérrez-Pérez J, et al (2019) Protein extraction from agri-food residues for integration in biorefinery: Potential techniques and current status. Bioresour Technol 280:459–477. https://doi.org/10.1016/j.biortech.2019.02.040

48. Ekpeni LEN, Benyounis KY, Nkem-Ekpeni FF, et al (2015) Underlying factors to consider in improving energy yield from biomass source through yeast use on high-pressure homogenizer (hph). Energy 81:74–83. https://doi.org/10.1016/j.energy.2014.11.038

49. Gaver D, Huyghebaert A (1991) Optimization of yeast cell disruption with a newly designed bead mill. Enzyme Microb Technol 13:665–671. https://doi.org/10.1016/0141-0229(91)90082-L

50. Middelberg APJ (1995) Process-scale disruption of microorganisms. Biotechnol Adv 13:491–551. https://doi.org/10.1016/0734-9750(95)02007-P

51. Currie JA, Dunnill P, Lilly MD (1972) Release of protein from Bakers' yeast (*Saccharomyces cerevisiae*) by disruption in an industrial agitator mill. Biotechnol Bioeng 14:725–736. https://doi.org/10.1002/bit.260140504

52. Jacob FF, Striegel L, Rychlik M, et al (2019) Yeast extract production using spent yeast from beer manufacture: influence of industrially applicable disruption methods on selected substance groups with biotechnological relevance. Eur Food Res Technol 245:1169–1182. https://doi.org/10.1007/s00217-019-03237-9

53. Hedenskog G, Mogren H (1973) Some methods for processing of single-cell protein. Biotechnol Bioeng 15:129–142. https://doi.org/10.1002/bit.260150110

54. Koubaa M, Imatoukene N, Drévillon L, Vorobiev E (2020) Current insights in yeast cell disruption technologies for oil recovery: A review. Chem Eng Process - Process Intensif 150:107868. https://doi.org/10.1016/j.cep.2020.107868

55. Bystryak S, Santockyte R, Peshkovsky AS (2015) Cell disruption of *S. cerevisiae* by scalable high-intensity ultrasound. Biochem Eng J 99:99–106.

https://doi.org/10.1016/j.bej.2015.03.014

56. Ekpeni LEN, Benyounis KY, Stokes J, Olabi AG (2016) Improving and optimizing protein concentration yield from homogenized baker's yeast at different ratios of buffer solution. Int J Hydrogen Energy 41:16415–16427. https://doi.org/10.1016/j.ijhydene.2016.05.243

57. Balasundaram B, Harrison STL (2008) Influence of the extent of disruption of Bakers' yeast on protein adsorption in expanded beds. J Biotechnol 133:360–369. https://doi.org/10.1016/j.jbiotec.2007.07.724

58. Siddiqi SF, Titchener-Hooker NJ, Shamlou PA (1997) High pressure disruption of yeast cells: The use of scale down operations for the prediction of protein release and cell debris size distribution. Biotechnol Bioeng 55:642–649. https://doi.org/10.1002/(SICI)1097-0290(19970820)55:4<642::AID-BIT6>3.0.CO;2-H

59. Liu D, Lebovka NI, Vorobiev E (2013) Impact of Electric Pulse Treatment on Selective Extraction of Intracellular Compounds from *Saccharomyces cerevisiae* Yeasts. Food Bioprocess Technol 6:576–584. https://doi.org/10.1007/s11947-011-0703-7

60. Lin HM, Chan EC, Chen C, Chen LF (1991) Disintegration of yeast cells by pressurized carbon dioxide. Biotechnol Prog 7:201–204. https://doi.org/10.1021/bp00009a001

61. Lin HM, Yang Z, Chen LF (1992) An improved method for disruption of microbial cells with pressurized carbon dioxide. Biotechnol Prog 8:165–166. https://doi.org/10.1021/bp00014a012

62. Kadam SU, Tiwari BK, Álvarez C, O'Donnell CP (2015) Ultrasound applications for the extraction, identification and delivery of food proteins and bioactive peptides. Trends Food Sci Technol 46:60–67. https://doi.org/10.1016/j.tifs.2015.07.012

63. Zhang L, Jin Y, Xie Y, et al (2014) Releasing polysaccharide and protein from yeast cells by ultrasound: Selectivity and effects of processing parameters. Ultrason Sonochem 21:576–581. https://doi.org/10.1016/j.ultsonch.2013.10.016

64. Wu T, Yu X, Hu A, et al (2015) Ultrasonic disruption of yeast cells: Underlying mechanism and effects of processing parameters. Innov Food Sci Emerg Technol 28:59–65. https://doi.org/10.1016/j.ifset.2015.01.005

65. James CJ, Coakley WT, Hughes DE (1972) Kinetics of protein release from yeast sonicated in batch and flow systems at 20 kHz. Biotechnol Bioeng 14:33–42. https://doi.org/10.1002/bit.260140105

66. Apar DK, Özbek B (2008) Protein releasing kinetics of bakers' yeast cells by ultrasound. Chem Biochem Eng Q 22:113–118

67. Iida Y, Tuziuti T, Yasui K, et al (2008) Protein release from yeast cells as an evaluation method of physical effects in ultrasonic field. Ultrason Sonochem 15:995–1000. https://doi.org/10.1016/j.ultsonch.2008.02.013

68. Liu D, Zeng X-AA, Sun D-WW, Han Z (2013) Disruption and protein release by ultrasonication of yeast cells. Innov Food Sci Emerg Technol 18:132–137. https://doi.org/10.1016/j.ifset.2013.02.006

69. Agrawal PB, Pandit AB (2003) Isolation of α-glucosidase from *Saccharomyces cerevisiae*: cell disruption and adsorption. Biochem Eng J 15:37–45. https://doi.org/10.1016/S1369-703X(02)00178-X

70. Ganeva V, Galutzov B, Teissié J (2003) High yield electroextraction of proteins from yeast by a flow process. Anal Biochem 315:77–84. https://doi.org/10.1016/S0003-2697(02)00699-1

71. Ganeva V, Galutzov B (1999) Electropulsation as an alternative method for protein extraction from yeast. FEMS Microbiol Lett 174:279–284. https://doi.org/10.1111/j.1574-6968.1999.tb13580.x

72. Ohshima T, Sato M, Saito M (1995) Selective release of intracellular protein using pulsed electric field. J Electrostat 35:103–112. https://doi.org/10.1016/0304-3886(95)00014-2

73. Kim SK (2016) Marine glycobiology: Principles and applications, First ed. CRC Press

74. Klimek-Ochab M, Brzezińska-Rodak M, Zymańczyk-Duda E, et al (2011)

Comparative study of fungal cell disruption-scope and limitations of the methods. Folia Microbiol (Praha) 56:469–475. https://doi.org/10.1007/s12223-011-0069-2

75. Kushnirov V V. (2000) Rapid and reliable protein extraction from yeast. Yeast 16:857–860. https://doi.org/10.1002/1097-0061(20000630)16:9<857::AID-YEA561>3.0.CO;2-B

76. Zhang T, Lei J, Yang H, et al (2011) An improved method for whole protein extraction from yeast *Saccharomyces cerevisiae*. Yeast 28:795–798. https://doi.org/10.1002/yea.1905

77. Mukherjee M, Nandi A, Chandra K, et al (2020) Protein extraction from *Saccharomyces cerevisiae* at different growth phases. J Microbiol Methods 172:105906. https://doi.org/10.1016/j.mimet.2020.105906

78. Ge L, Wang XT, Tan SN, et al (2010) A novel method of protein extraction from yeast using ionic liquid solution. Talanta 81:1861–1864. https://doi.org/10.1016/j.talanta.2010.02.034

79. Takalloo Z, Nikkhah M, Nemati R, et al (2020) Autolysis, plasmolysis and enzymatic hydrolysis of baker's yeast (*Saccharomyces cerevisiae*): a comparative study. World J Microbiol Biotechnol 36:1–14. https://doi.org/10.1007/s11274-020-02840-3

80. Podpora B, Swiderski F (2015) Spent Brewer's Yeast Autolysates as a New and Valuable Component of Functional Food and Dietary Supplements. J Food Process Technol 6:. https://doi.org/10.4172/2157-7110.1000526

81. Xie J, Cui C, Ren J, et al (2017) High solid concentrations facilitate enzymatic hydrolysis of yeast cells. Food Bioprod Process 103:114–121. https://doi.org/10.1016/j.fbp.2017.03.004

82. Chae HJ, Joo H, In M (2001) Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresour Technol 76:253–258. https://doi.org/10.1016/S0960-8524(00)00102-4

83. Celus I, Brijs K, Delcour JA (2007) Enzymatic hydrolysis of brewers' spent grain

proteins and technofunctional properties of the resulting hydrolysates. J Agric Food Chem 55:8703–8710. https://doi.org/10.1021/jf071793c

84. Podpora B, Swiderski F, Sadowska A, et al (2016) Spent brewer's yeast extracts as a new component of functional food. Czech J Food Sci 34:554–563. https://doi.org/10.17221/419/2015-CJFS

85. Marson GV, Lacour S, Hubinger MD, Belleville MP (2022) Serial fractionation of spent brewer's yeast protein hydrolysate by ultrafiltration: A peptide-rich product with low RNA content. J Food Eng 312:110737. https://doi.org/10.1016/j.jfoodeng.2021.110737

86. Hobson J (1991) A co-hydrolytic process for the production of novel extracts from yeast and non-yeast proteins

87. Kortes J (2020) Process flavours with low acrylamide

88. Jolly R (1978) Modified protein

89. Ason K (2019) Effective use of yeast and yeast extract residue

90. Farra CD (2015) Cosmetic and/or pharmaceutical composition comprising a yeast peptide hydrolysate and use of the yeast peptide hydrolysate as an active agent for strengthening hair

91. Hedhammar M, Karlström AE, Hober S (2006) Chromatographic methods for protein purification, Royal Institute of Technology, Stockholm, Sweden. Stockholm: Royal Institute of Technology

92. Liu D, Savoire R, Vorobiev E, Lanoisellé JL (2010) Effect of disruption methods on the dead-end microfiltration behavior of yeast suspension. Sep Sci Technol 45:1042–1050. https://doi.org/10.1080/01496391003727890

93. Butylina S, Shataeva LK, Nyström M (2007) Separation of nucleoprotein complexes with antioxidant activity from yeast *Saccharomyces cerevisiae*. Sep Purif Technol 53:64–70. https://doi.org/10.1016/j.seppur.2006.06.014

94. Caballero-Córdoba GM, Sgarbieri VC (2000) Nutritional and toxicological evaluation of yeast (*Saccharomyces cerevisiae*) biomass and a yeast protein

concentrate. J Sci Food Agric 80:341–351. https://doi.org/10.1002/1097-0010(200002)80:3<341::AID-JSFA533>3.3.CO;2-D

95. Yamada EA, Sgarbieri VC (2005) Yeast (*Saccharomyces cerevisiae*) protein concentrate: Preparation, chemical composition, and nutritional and functional properties. J Agric Food Chem 53:3931–3936. https://doi.org/10.1021/jf0400821

96. Akardere E, Özer B, Çelem EB, Önal S (2010) Three-phase partitioning of invertase from Baker's yeast. Sep Purif Technol 72:335–339. https://doi.org/10.1016/j.seppur.2010.02.025

97. Mohammad AW, Ng CY, Lim YP, Ng GH (2012) Ultrafiltration in food processing industry: review on application, membrane fouling, and fouling control. Food Bioprocess Technol 5:1143–1156. https://doi.org/10.1007/s11947-012-0806-9

98. Vollet Marson G, Belleville M, Lacour S, Dupas Hubinger M (2020) Membrane Fractionation of Protein Hydrolysates from By-Products: Recovery of Valuable Compounds from Spent Yeasts. Membranes (Basel) 11:23. https://doi.org/10.3390/membranes11010023

99. Kim J, Dae-Hyoung L, Jong-Soo L, et al (2004) Characterization of antihypertensive angiotensin I-converting enzyme inhibitor from *Saccharomyces cerevisiae* . J Microbiol Biotechnol 14:1318–1323

100. Albergaria H, Francisco D, Gori K, et al (2010) *Saccharomyces cerevisiae* CCMI 885 secretes peptides that inhibit the growth of some non-Saccharomyces wine-related strains. Appl Microbiol Biotechnol 86:965–972. https://doi.org/10.1007/s00253-009-2409-6

101. Branco P, Francisco D, Chambon C, et al (2014) Identification of novel GAPDH-derived antimicrobial peptides secreted by *Saccharomyces cerevisiae* and involved in wine microbial interactions. Appl Microbiol Biotechnol 98:843–853. https://doi.org/10.1007/s00253-013-5411-y

102. Mirzaei M, Mirdamadi S, Ehsani MR, et al (2015) Purification and identification of antioxidant and ACE-inhibitory peptide from *Saccharomyces cerevisiae* protein hydrolysate. J Funct Foods 19:259–268

103. Lee DH, Lee DH, Lee JS (2007) Characterization of a new antidementia β-secretase inhibitory peptide from *Saccharomyces cerevisiae*. Enzyme Microb Technol 42:83–88. https://doi.org/10.1016/j.enzmictec.2007.08.003

104. Nehete J, Narkhede M, Bhambar R, et al (2013) Natural proteins: Sources, isolation, characterization and applications. Pharmacogn Rev 7:107. https://doi.org/10.4103/0973-7847.120508

105. Clark EDB (2001) Protein refolding for industrial processes. Curr Opin Biotechnol 12:202–207. https://doi.org/10.1016/S0958-1669(00)00200-7

106. Sui H, Zhou J, Ma G, et al (2018) Removal of ionic liquids from oil sands processing solution by ion-exchange resin. Appl Sci 8:1611. https://doi.org/10.3390/app8091611

107. Dick K, Molan P, Eschenbruch R (1992) The isolation from *Saccharomyces cerevisiae* of two antibacterial cationic proteins that inhibit malolactic bacteria. Vitis 31:105–116

108. Grönberg A (2018) Ion exchange chromatography. In: Biopharmaceutical Processing: Development, Design, and Implementation of Manufacturing Processes. Elsevier, pp 379–399

109. Lothe RR, Purohit SS, Shaikh SS, et al (1999) Purification of α-glucosidae and invertase from bakers ' yeast on modified polymeric supports. 293–306. https://doi.org/10.1023/A:1008126628635

110. Josic D, Kovac S (2010) Reversed-phase high performance liquid chromatography of proteins. Curr Protoc Protein Sci 2010:1–22. https://doi.org/10.1002/0471140864.ps0807s61

111. Shetty JK, Kinsella JE (1980) Lysinoalanine formation in yeast proteins isolated by alkaline methods. J Agric Food Chem 28:798–800. https://doi.org/10.1021/jf60230a019

112. Shetty JK, Kinsella JE (1980) Ready separation of proteins from nucleoprotein complexes by reversible modification of lysine residues. Biochem J 191:269–272. https://doi.org/10.1042/bj1910269

113. Lindblom M (1977) Properties of intracellular ribonuclease utilized for RNA reduction in disintegrated cells of Saccharomyces cerevisiae. Biotechnol Bioeng 19:199–210. https://doi.org/10.1002/bit.260190204

114. Shetty KJ, Kinsella JE (1979) Preparation of Yeast Protein Isolate With Low Nucleic Acid By Succinylation. J Food Sci 44:633–638. https://doi.org/10.1111/j.1365-2621.1979.tb08464.x

115. Kinsella JE, Damodaran S (1984) Dissociation of Yeast Nucleoprotein Complexes by Chemical Phosphorylation. J Agric Food Chem 32:1030–1032. https://doi.org/10.1021/jf00125a021

116. Huang Y -T, Kinsella JE (1986) Phosphorylation of yeast protein: Reduction of ribonucleic acid and isolation of yeast protein concentrate. Biotechnol Bioeng 28:1690–1698. https://doi.org/10.1002/bit.260281112

117. Oliveira AM, Oliva Neto P de (2011) Improvement in RNA extraction from S. cerevisie by optimization in the autolysis and NH3 hydrolysis. Brazilian Arch Biol Technol 54:1007–1018. https://doi.org/10.1590/S1516-89132011000500019

118. Sombutyanuchit P, Suphantharika M, Verduyn C (2001) Preparation of 5′-GMP-rich yeast extracts from spent brewer's yeast. World J Microbiol Biotechnol 17:163–168. https://doi.org/10.1023/A:1016686504154

119. Goetz H, Kuschel M, Wulff T, et al (2004) Comparison of selected analytical techniques for protein sizing, quantitation and molecular weight determination. J Biochem Biophys Methods 60:281–293. https://doi.org/10.1016/j.jbbm.2004.01.007

120. Mæhre HK, Dalheim L, Edvinsen GK, et al (2018) Protein determination—method matters. Foods 7:. https://doi.org/10.3390/foods7010005

121. AOAC (2005) Official Methods of Analysis of AOAC International

122. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem. https://doi.org/10.1016/0922-338X(96)89160-4

123. Smith PK, Krohn RI, Hermanson GT, et al (1985) Measurement of protein using

bicinchoninic acid. Anal Biochem 150:76–85. https://doi.org/10.1016/0003-2697(85)90442-7

124. Puligundla P, Mok C, Park S (2020) Advances in the valorization of spent brewer's yeast. Innov Food Sci Emerg Technol 62:102350. https://doi.org/10.1016/j.ifset.2020.102350

125. Rai AK, Pandey A, Sahoo D (2019) Biotechnological potential of yeasts in functional food industry. Trends Food Sci Technol 83:129–137. https://doi.org/10.1016/j.tifs.2018.11.016

126. Kaltashov IA, Bobst CE, Pawlowski J, Wang G (2020) Mass spectrometry-based methods in characterization of the higher order structure of protein therapeutics. J Pharm Biomed Anal 184:113169. https://doi.org/10.1016/j.jpba.2020.113169

127. Ryan DJ, Spraggins JM, Caprioli RM (2019) Protein identification strategies in MALDI imaging mass spectrometry: a brief review. Curr Opin Chem Biol 48:64–72. https://doi.org/10.1016/j.cbpa.2018.10.023

128. Shynkaryk M V., Lebovka NI, Lanoisellé JL, et al (2009) Electrically-assisted extraction of bio-products using high pressure disruption of yeast cells (*Saccharomyces cerevisiae*). J Food Eng 92:189–195. https://doi.org/10.1016/j.jfoodeng.2008.10.041

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Figure captions

Figure 1: Global protein supply in 2018 [14, 15]

Figure 2: Schematic longitudinal section of *S. cerevisiae* cell and their protein composition (cell structures and components are not in scale)

Figure 3: Classification of protein extraction methods from *S. cerevisiae* according to operation mode