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Releasing polysaccharide and protein from yeast cells by ultrasound: Selectivity and effects of processing parameters

Li Zhang, Yuan Jin, Yajuan Xie, Xiaofang Wu, Tao Wu *

College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

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ABSTRACT

A 20 kHz high-intensity ultrasound was employed for the selective release of polysaccharide and protein from yeast cells. While the release of polysaccharide and protein was affected by most of the processing parameters, the release selectivity, which is the ratio of the amount of polysaccharide released to that of protein, designated as *T*/*P* value, was only influenced by sonication time, temperature and ionic strength, among which temperature had the greatest influence. The *T*/*P* value at 85 °C was a factor of 9.3 of the one at 25 °C. The underlying mechanism of this selectivity is speculated to be thermal denaturation and aggregation of protein within yeast cells at elevated temperatures leading to the decrease of protein release by ultrasound. This finding may be useful in exploring a novel selective process for producing polysaccharide and protein fractions from yeast biomass.

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1. Introduction

Yeast biomass, a by-product of ethanol fuel production and brewing industries available in tremendous amount, is currently underutilized, mainly as low-value animal feed [1]. It is rich, however, in nutritional protein and bioactive polysaccharide, e.g. β-glucan, which are of great interests to the food industry for applications as functional food ingredients [1–3]. The amino acid scores of whole yeast biomass and yeast protein concentrate are 98.1% and 87.2%, respectively, presenting a well-balanced amino acid profile for human consumption [3]. Besides, the thermal and surface properties of yeast protein exhibit a great potential for applications as gelling and emulsifying agents [4,5]. Of the total yeast polysaccharide, β-glucans with β-(1 → 6) or β-(1 → 3) linkage can lower serum cholesterol level and reduce the risk of heart diseases [2,6]. They are also effective biological response modifiers with immunostimulation effect, and are using clinically as adjuvants in cancer therapy [7]. These health benefits are typically connected with β-glucans from oat or barley. However, yeast biomass is a more concentrated source of β-glucan than oat and barley products [6]. A full exploration of the nutritive and bioactive values of yeast biomass demands a cost-effective way to isolate protein and polysaccharide fractions from yeast cells with acceptable purities [8].

Conventional chemical or enzymatic methods to isolate yeast protein and β-glucan are accomplished by hot alkali, acid or enzymatic treatments. Such methods are either harsh enough to alter amino acid profiles of protein or to degrade chains of β-glucan [8], or too expensive to be used in mass scale production due to the high cost of chemicals and enzymes [9]. Mechanical methods such as bead mill, high pressure homogenizer, and high-intensity ultrasound are frequently researched for cell disruption to release intracellular products prior to the downstream isolation and purification operations. The disadvantages of using bead mill and homogenizer are poor selectivity in product release (i.e. the co-release of contaminants) and micronization of the cell debris [9], either of which can substantially increase the costs of subsequent downstream operation.

High-intensity ultrasound refers to sound waves with frequencies beyond 20 kHz and intensity above 1–2 W/cm^2. The propagation of ultrasound in medium results in the formation of cavitation bubbles. Collapse of cavitation bubble produces extreme temperature and pressure gradients, strong shear force, shock wave, as well as free radicals (e.g. $\cdot$OH, $HO_2^{\cdot}$ and O$\cdot$) [10]. These physical and chemical effects are known to disrupt cellular structures and render cell death [10,11]. For this reason, ultrasound has been studied in the food industry for many years as a non-thermal food preservation technology [10,12]. It is effective against a number of microorganisms including yeasts [10]. The mechanism of microbial inactivation is the subject of much conjecture with many hypotheses [13], among which, damage of cell structure, e.g. pitting and erosion of cell surface by the above physical and chemical effects,

* Corresponding author. Tel.: +86 25 84396907.
E-mail address: twu@njau.edu.cn (T. Wu).

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followed by disintegration of whole microbial cells is most likely [14]. The effectiveness of ultrasound is influenced by many factors, including sonication intensity, shape and probe depth of sonoreactor, cell concentration and volume of processed sample, solution pH, ionic strength, and presence of such solutes as surface active compounds, as well as processing temperature and external pressure [10,12,14–16]. Combination of ultrasound with heat (thermosonication), pressure (manosonication), or both (manothermosonication) can substantially increase the lethality of treatments, reducing the processing temperature and time needed for microorganism inactivation [10,17].

In biotechnology, ultrasound has been studied as a cell disruption technique in harvesting of intracellular biological products [9,18]. Compared to other cell disruption methods, the release of product by ultrasound has certain selectivity, which makes ultrasound more attractive in bioprocess design [9,19]. For example, compared with high pressure homogenization at 5000 psi, sonication is more selective in releasing of periplasmic penicillin acylase from *Escherichia coli* [20]. This selectivity is also varied with ultrasound parameter. A more selective release of β-galactosidase from *Lactobacillus acidophilus* was found with an increase of ultrasound power from 10 to 50 W [21]. Although the underlying mechanism is not fully understood in details, the localized heat generated by cavitation was believed to cause the translocation of β-galactosidase from cytoplasm to periplasm space [21]. The release of periplasm enzymes is faster than that of cytoplasm enzymes [9], leading to the increased selectivity as observed [21].

Besides individual enzymes, release of total protein from yeast cells by ultrasound was also studied, and the release kinetics was found to be first order [22]. Interestingly, release of protein from yeast cells was used to evaluate the physical effects in the acoustic fields [23]. To the best of our knowledge, release of polysaccharide by ultrasound has seldom been studied either in the fields of food science or biotechnology. As stated earlier, this information is commercially important in exploring the added-value of yeast biomass and in the cost control of downstream processing in biotechnology. Therefore in this research we monitored the release of polysaccharide from yeast cells during an ultrasonic disruption process and compared it with the release of protein. An attempt was also made to enhance the release selectivity, i.e. the ratio of the amount of polysaccharide released to that of protein released, by manipulation of the processing parameters.

2. Experimental details

2.1. Materials

Sugar tolerate baker's yeast (*Saccharomyces cerevisiae*), usually used for bread making, was purchased in the form of compressed cake from Angel Yeast Corporation (Yichang, China), stored at 4 °C and used within the 45 days of shelf life. The moisture content of the product was determined gravimetrically as 64.47 ± 0.16%. All water used was 18 MΩ de-ionized (D.I.) water unless indicated otherwise. BCA reagent was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were purchased from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China).

2.2. High-intensity ultrasound treatment

Yeast cells were dispersed in D.I. water or phosphate buffers in a double wall jacket beaker and stirred to reach a homogeneous state. The temperature of the beaker was maintained by a circulating water bath as set. Sonication was conducted by a 20 kHz horn-type sonicator (Sonics and Materials VC-750, Newton, CT) with a 1/2 in. titanium probe in the continuous mode. The probe was usually penetrated into the liquid surface for 1 cm. In a typical experiment set, 4.0 g yeast, 40 ml D.I. water, ultrasound intensity of 24 W/cm^2, temperature of 5 °C, and sonication time of 5 min were used.

The effect of pH was studied in D.I. water (pH was determined as 5.7) and 0.05 M phosphate buffers with pH adjusted to 5.5, 6.5 and 7.5 with hydrochloric acid. The effect of ultrasound intensity was studied at five levels, as 10, 17, 24, 32 and 39 W/cm^2, which were determined calorimetrically, and correspond to 20%, 30%, 40%, 50% and 60% of the maximum power level that can be delivered by the sonicator, respectively. Influence of sonication time was studied in the duration of 5–30 min. Temperature effect was studied at 5, 25, 45, 65 and 85 °C. Yeast concentrations (Conc.) were studied at 1.25%, 2.5%, 5%, 10% and 20% (w/v). Mechanical stirring magnetic stirrer at 500 rpm; three processed volumes, 40, 100 and 200 ml; two probe depths (the distance between the probe surface and the bottom of double wall jacket beaker), 1.7 and 3.3 cm, which were half ($\lambda/2$) and one wavelength (λ) of the ultrasound wave, respectively; three ionic strengths at 0.05, 0.55 and 1.05 M adjusted with sodium chloride; three levels of ethanol addition at concentrations of 10, 50 and 100 mM, were also investigated to observe their potential influences on protein and polysaccharide release.

After each sonication treatment, 1 ml yeast suspension was drawn immediately, diluted with 9.0 ml water, and centrifuged at 3000*g* for 10 min before measurement of the amount of protein and polysaccharide released.

2.3. Anthrone assay for total polysaccharide determination

0.5 ml supernatant was drawn, followed by the addition of 4.5 ml Anthrone reagent, mixed by a vortex, incubated at 90 °C for 10 min, and then cooled in an iced bath to room temperature. A proper dilution was made if required. The absorbance at 620 nm was then recorded to calculate the amount of total polysaccharide released by ultrasound on the yeast cell dry weight basis (D.B.) using glucose as standard.

2.4. BCA protein assay

0.1 ml above supernatant was drawn, followed by the addition of 2.0 ml BCA reagent, mixed by a vortex, incubated at 60 °C for 15 min, and then cooled in an iced bath to room temperature. The absorbance at 562 nm was then recorded by a spectrophotometer (LabTech, UV Bluestar A, Beijing) to calculate the amount of protein released by ultrasound on yeast cell dry weight basis using bovine serum albumin as standard.

2.5. T/P (Total polysaccharide released/Protein released) value calculation

The T/P value was calculated by taking a ratio of the amount of polysaccharide released (T) to the amount of protein released (P), where T and P were in percentage ratios against yeast cell dry weight. A higher T/P value would be expected when the release of polysaccharide is favored; thereby more polysaccharide in comparison with protein is released from cells into the solution, while a fewer polysaccharide remains in the insoluble cell residues. We proposed this value to be used as an index to evaluate the selectivity in releasing since a higher T/P value would enable the harvesting of polysaccharide with higher purity.

2.6. Statics analysis

All experiments were triple replicated and the data was analyzed using one-way ANOVA, followed by Duncan's new multiple range tests. All statistical analysis were performed with SAS

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